

Clumped isotope signatures of abiotic methane: the role of combinatorial isotope effect

Jabrane Labidi^{1,*}, Thomas M McCollom¹, Thomas Giunta¹, Barbara Sherwood Lollar¹, EDWARD DONALD YOUNG¹, and will leavitt¹

¹Affiliation not available

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Labidi, J.,^{1,*}, McCollom, T. M.,²Giunta, T.,³ Sherwood Lollar, B.,^{1,4}Leavitt, W.,^{5,6} Young E.D.,⁷

¹ Université de Paris Cité, Institut de physique du globe de Paris, CNRS, Paris, France.

² Laboratory for Atmospheric and Space Physics, University of Colorado, Boulder, CO, USA

³ University of Brest, CNRS, Ifremer, Geo-Ocean, F-29280 Plouzané, France

⁴ Department of Earth Sciences, University of Toronto, Toronto, ON, CANADA

⁵ Department of Earth Sciences, Dartmouth College, Hanover, NH, USA

⁶ Department of Chemistry, Dartmouth College, Hanover, NH, USA

⁷ Department of Earth, Planetary, and Space Sciences, UCLA, Los Angeles, CA, USA.

*Correspondence: labidi@ipgp.fr

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Abstract

Methane clumped isotope signatures of abiogenesis may be diagnostic of the origin of methane on Earth and other planetary bodies. On Earth, identifying methane synthesis pathways has proven challenging because of the lack of unambiguous signatures of the provenance of methane molecules. The abundances of mass-18 isotopologues of methane, $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_2\text{D}_2$, could help identify occurrences of abiotic methane in nature. We performed synthesis of abiogenic methane in the laboratory and determined $\delta^{13}\text{C}$, δD , $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$. We carried out a set of experiments in hydrothermal conditions between 130 and 300 °C. The experiments were performed by heating water in the presence of Fe^0 powder and CO. The reduction of water on metallic iron led to the formation of H_2 . CO was reacted with both H_2 and H_2O , generating both CH_4 and CO_2 . We systematically extracted and quantified methane, and determined $\delta^{13}\text{C}$, δD , $\Delta^{12}\text{CH}_2\text{D}_2$, and $\Delta^{13}\text{CH}_3\text{D}$ of the methane using a Panorama gas-source mass spectrometer (Nu, Ametek). We observe $\delta^{13}\text{C}$ and δD of methane to be isotopically depleted relative to the starting materials. The $\delta^{13}\text{C}$ data indirectly suggest isotopic equilibrium may have been reached for carbon isotopes between methane, carbon monoxide and carbon dioxide in our experiments. In contrast, D/H ratios are inconsistent with equilibrium isotopic fractionation. This suggests that under our experimental conditions, hydrogen additions to carbon are governed by kinetics, and that subsequent D/H re-equilibration was limited. While $\Delta^{13}\text{CH}_3\text{D}$ values approximately track experimental temperature, $\Delta^{12}\text{CH}_2\text{D}_2$ values are displaced far from equilibrium. We find exclusively negative $\Delta^{12}\text{CH}_2\text{D}_2$ values, showing deficits down to 40‰ interpret the data as evidence for distinct, kinetically induced D/H pools contributing to methane assembly (a combinatorial effect). The cumulative D/H fractionations associated with CO (or CO_2 in nature) hydrogenation likely explain the direction

and magnitude of $\Delta^{12}\text{CH}_2\text{D}_2$ values during abiotic methane formation. We suggest that near equilibrium $\Delta^{13}\text{CH}_3\text{D}$ with negative $\Delta^{12}\text{CH}_2\text{D}_2$ signatures will help identify methane formed abiotically in nature.

Plain language summary

Methane is observed in various environmental settings on Earth, including but not limited to hydrothermal fluids and sedimentary systems. It is also found on other worlds, as it was reported to occur in Mars's rocks, in Enceladus's geysers, and as of recently, in an exoplanet's atmosphere. Methane may be formed by life, by the degradation of organic matter, or by abiotic chemistry. However, identifying synthesis pathways has proven challenging because of the lack of unambiguous signatures of the provenance of methane molecules. We performed synthesis of abiotic methane in the laboratory and determined its composition. We observe isotopic compositions that are consistent with carbon isotope equilibrium associated with hydrogen isotope disequilibrium. These isotope signatures are non-unique, in contrast with mass-18 isotopologues data, $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_2\text{D}_2$. While $\Delta^{13}\text{CH}_3\text{D}$ values approximately track experimental temperature, $\Delta^{12}\text{CH}_2\text{D}_2$ values are extremely depleted, far below equilibrium. The data are explained by kinetically fractionated D/H pools contributing to methane molecules, i.e., a combinatorial effect. We suggest that near equilibrium $\Delta^{13}\text{CH}_3\text{D}$ with negative $\Delta^{12}\text{CH}_2\text{D}_2$ signatures will help identify methane formed abiotically on Earth and on other worlds.

1. Introduction

Methane (CH_4) is observed in a variety of environments on Earth. Abiotic methane (i.e., synthesized by mechanisms that do not involve life or thermal decomposition of organic matter), is suspected to occur in various fluids also containing molecular hydrogen (H_2). These fluids are especially prominent in hydrothermal systems where olivine-rich ultramafic rocks are altered via serpentinization reactions. For instance, elevated concentrations of CH_4 and H_2 are observed in ultramafic-hosted seafloor hydrothermal vent fluids at temperatures typically exceeding 250 °C (Charlou et al., 2010; Welhan and Craig, 1979). In these environments, abiotic methane synthesis is thought to originate from abiotic reactions between CO_2 and H_2 :



At temperatures under 340 °C, thermodynamic equilibrium favors carbon reduction in fluids that contain H_2 (Klein et al., 2019). This is the Sabatier reaction, often lumped with a class of reactions referred to as Fischer-Tropsch-Type synthesis, or FTT, as reviewed elsewhere (McCollom, 2013; McCollom and Seewald, 2007). Methane is also observed with H_2 in seeps and fracture waters from the continental subsurface, at environmental temperatures typically [?] 100 degC. In fluids from the Kidd Creek mine (Canada), or in hyperalkaline fluids issuing from the underlying ophiolite from the Oman ophiolite, CH_4 and H_2 are present as major species. The environmental temperatures there allow microbial methane synthesis, but CH_4 has been suggested as being dominantly abiotic with only minor mixing with microbial methane on the basis of C and H stable isotopes of methane and associated gases in these sites (Etiope et al., 2015; Fritz et al., 1992; Sherwood Lollar et al., 2008, 2002, 1993). Contributions from microbial methane have also been identified in gas seeps from the sultanate of Oman on the basis of microbiological data (Miller et al., 2016).

The abundances of mass-18 methane isotopologues, $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_2\text{D}_2$, may be tracers of methane origin in nature (Stolper et al., 2014; Wang et al., 2015; Young et al., 2017). The potential for $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ to demonstrate abiogenicity of natural methane on Earth and other worlds is illustrated by preliminary experimental work. So far, two Sabatier-type experimental runs have been investigated for $\Delta^{13}\text{CH}_3\text{D}$ versus $\Delta^{12}\text{CH}_2\text{D}_2$ (Young et al., 2017). Those methane aliquots were generated by CO_2 reduction (reaction 1) catalyzed by ruthenium at 70 and 90 °C following the method of Etiope and Ionescu (2015). The experiments yielded near-equilibrium $\Delta^{13}\text{CH}_3\text{D}$ values of $\sim +4$ large $^{12}\text{CH}_2\text{D}_2$ depletions of approximately 70 values have been thought to reflect a signature acquired by methane during the abiotic reduction of oxidized carbon in the laboratory and potentially in nature (Young et al., 2017).

At Kidd Creek, methane samples have compositions that appear consistent with the Sabatier experimental runs. In the deepest levels of the mine, CH_4 shows $\Delta^{13}\text{CH}_3\text{D}$ values of 5.2 ± 0.5 $\Delta^{12}\text{CH}_2\text{D}_2$ values are 10

to 30 direct evidence for an abiotic origin of methane (data in Young et al., 2017; Warr et al. 2021). In contrast, methane from Oman shows negative $\Delta^{12}\text{CH}_2\text{D}_2$ value and near-zero $\Delta^{13}\text{CH}_3\text{D}$. The near-zero was considered inconsistent with abiotic signatures (Nothhaft et al., 2021). In deep-sea high temperature vents, concordant $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ data suggested apparent equilibrium temperatures of $\sim 340^\circ\text{C}$ (Labidi et al., 2020). Equilibrium signatures could be acquired during methane synthesis at high temperature, or may reflect bond reordering after synthesis, before or during transport.

The Sabatier experiments reported in Young et al., (2017) were performed at temperatures below 100°C and in the absence of water. These experimental conditions are challenging to extrapolate to deep-sea hydrothermal systems or continental environments for which $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ data are available. Consequently, it is not clear how the experimental data should be applied to the interpretation of natural systems. To constrain the $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ signatures produced by abiotic FTT reactions at variable temperatures, we performed controlled FTT synthesis at hydrothermal conditions in the laboratory at temperatures varying between 130°C and 300°C .

2. Experiments

The experimental methods employed follow those used previously to study abiotic methane and other organic syntheses by Fischer-Tropsch-type (FTT) reactions under hydrothermal conditions (McCollom et al., 2010; McCollom and Seewald, 2006). Briefly, a flexible-cell reaction cell composed of a gold bag with titanium fittings is contained inside a stainless-steel pressure housing (Seyfried Jr et al., 1987). An external valve connected to the reaction cell with titanium tubing allowed for collection of volatile gases at the end of the experiments. Prior to the experiments, the reaction cell components were heated for two hours at 450°C in air to remove organic contaminants and to create an inert TiO_2 layer on the surface of the titanium fittings. Previous experiments with the same reaction system have demonstrated that the reactor itself is not catalytic for organic synthesis under the experimental conditions (McCollom and Seewald, 2006; McCollom et al., 2010).

The experiments were performed by heating carbon monoxide (CO) and native iron (Fe^0) in the presence of water at a range of temperatures. The experiments were initiated by placing 1.5 g of Fe^0 (Fe powder, -22 mesh; Alfa Aesar) and ~ 25 g of DI water in a 55 mL gold reaction cell, so the $\text{H}_2\text{O}/\text{Fe}$ molar ratio was ~ 50 . The native Fe is included both as catalyst and as a source of molecular hydrogen (H_2) since it rapidly reacts with water at elevated temperatures to form magnetite (Fe_3O_4) and H_2 according to the reaction:



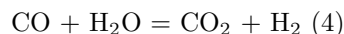
To promote reaction of Fe^0 , the water was acidified to a pH of about 2 with HCl. Individual experiments were conducted over a range of temperatures from 130°C to 300°C . The target pressure for the experiments was 15 MPa, but in practice the pressures varied up to 3 MPa from this value during some experiments.

After sealing the reaction cell with the Ti closure piece, it was placed in the pressure containment vessel, pressurized at room temperature, and the entire apparatus was placed horizontally and heated in a furnace. Once the target temperature was attained, CO was injected through the sampling valve using the following procedure. First, a loop of stainless-steel tubing (12.6 mL) was filled with CO at a pressure of 0.93 MPa (the maximum of the pressure regulator on the CO tank) and attached to the sampling valve. A solution with the same composition as that in the reaction cell was then pumped into the tubing until pressure was equal to that within the heated pressure vessel. The valve was then opened and the CO along with 13 mL of solution was pumped into the reaction cell, with an equal amount of water bled from the pressure containment vessel to maintain constant pressure during injection. In practice, some amount of CO remained in the injection loop after this procedure, so it is not possible to determine exactly how much CO was contained in the reaction cell following injection. However, assuming 90% injection efficiency, the amount of CO injected would have been about 4.3 millimoles. Considering that 38 mL of water (25 mL initial + 13 mL added during injection) corresponds to ~ 2.1 moles H_2O , the starting CO/ H_2O molar ratios of the experiments are estimated to be approximately 2×10^{-3} .

The tank of CO used in the experiments was the same as that used in McCollom et al. (2010) and has a $\delta^{13}\text{C}$ of -28 . The water used in this study is deionized water from Univ. Colorado tap water. The δD of DI water sampled in April 2022 and April 2023 was -119 . Hydrogen (H_2) generated from reaction 2 subsequently reacts with CO to form methane, according to the net reaction:



Reaction 3 is a Fischer-Tropsch reaction that utilizes CO for oxidized carbon rather than CO_2 . It is distinct from the Sabatier reaction (reaction 1) that may be similar to reactions in natural hydrothermal systems where CO_2 is available in large amounts. Carbon monoxide was used here because it is more active in hydrothermal FTT reactions than CO_2 (McCollom et al., 2010), which allowed for obtaining measurable CH_4 quantities under our experimental timescales. In addition to CO reduction, the so-called “water-gas shift reaction” (McCollom and Seewald, 2006) was prominent under the experimental conditions; reaction between CO and water led to the oxidation of a fraction of the CO to form CO_2 :



Through a competition between reaction 3 and 4, CO is rapidly and predominantly converted to CH_4 and CO_2 , with much of the CO_2 precipitating as siderite (FeCO_3). Reaction 4 is anticipated to be dominant over reaction 3 (McCollom et al., 2010).

The experiments were heated for 18 to 138 hours, with longer incubation times used at lower temperatures to allow for the synthesis reactions to proceed. Following reaction, the reactors were gradually cooled to room temperature while pumping water into the containment vessel to maintain elevated pressure. The pressure was then reduced to ~ 5 MPa by releasing water from the pressure containment vessel in order to allow volatile gases to exsolve and accumulate at the top of the reaction cell. Within 24 h of experiment termination, the gas phase was vented through the sampling valve and transferred to 300 mL stainless steel cylinders (Swagelok) for isotope analysis.

3. Isotope Measurements

Methane was extracted from the stainless-steel storage cylinders on a vacuum system at UCLA and purified by gas chromatography using procedures described previously by Young et al. (2017). Gas aliquots of a given sample were opened to a silica gel-filled stainless-steel U-trap cooled with liquid nitrogen. Helium carrier gas was used to flush the sample to the gas chromatograph. Separation was accomplished with a 3m long, 1/8 in. OD stainless steel column packed with 5 A molecular sieve, followed in series by a 2m long [?] 1/8 in. OD stainless steel column packed with HayeSep D porous polymer. Peaks from the gas chromatograph were detected with an in-line thermal conductivity detector (TCD). Because total pressures were > 1 bar in the reaction vessels, and because samples were dominated by H_2 , purification on the vacuum line had to be performed in sequence of multiple aliquots. Once collection of a purified aliquot of methane was complete, this methane was transferred to a glass vial filled with silica-gel at liquid nitrogen temperature. Another sample aliquot would then be purified and added to the same glass vial, until a given reaction vessel had been entirely processed. For the cylinders to be emptied, 6 to 8 aliquots were processed in this way on the vacuum line. Samples were then transferred to the inlet of the mass spectrometer where they were warmed and expanded into the mass spectrometer using a silica-gel filled cold finger.

Mass spectrometry measurements were done on a Nu Instruments Panorama with a mass resolving power that allows the simultaneous measurement of ion currents for resolved $^{12}\text{CH}_4^+$, $^{13}\text{CH}_4^+$, $^{12}\text{CH}_3\text{D}^+$, $^{13}\text{CH}_3\text{D}^+$ and $^{12}\text{CH}_2\text{D}_2^+$ ion beams, as described in Young et al., (2016). The analyte peaks are in most cases baseline resolved from surrounding interferences, and where there is the potential for a tail effect (in the case of $^{13}\text{CH}_5^+$ tailing into $^{12}\text{CH}_2\text{D}_2^+$), previous work shows that the effects on our final results are less than quoted uncertainties (Young et al., 2016). The measured ratios of these ion currents yield values for both $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ as well as for bulk $^{13}\text{C}/^{12}\text{C}$ and D/H. The methane quantities available for isotopic measurements were relatively low. For standards and samples available in quantities > 80 μmol , relative abundances of $^{13}\text{CH}_3\text{D}^+$ and $^{12}\text{CH}_2\text{D}_2^+$ are measured for up to 10 and 20 hours, respectively. In these

cases, typical propagated internal uncertainties for the calculation of $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ are generally $\pm 0.1\text{‰}$ were available (Table 1), and uncertainties are larger, reflecting less favorable counting statistics. The internal uncertainties range from $0.2\Delta^{13}\text{CH}_3\text{D}$, and between $1.1\Delta^{12}\text{CH}_2\text{D}_2$.

4. Results

For nine experiments conducted at temperatures between 130 and 300°C, between ~5 and ~80 micromoles of abiotic CH_4 were obtained. All results may be found in Table 1. Previous experimental studies of abiotic synthesis have shown that background sources can contribute significant amounts of methane, which can be misleading if not carefully quantified (McCollom et al., 2016 and references therein). In previous experiments employing the same methodology as used here but without added CO indicated that background sources contribute $< 3 \mu\text{moles CH}_4$ (McCollom et al., 2010). Our experiments for which we have isotope results have resulted in the accumulation of 35 to 80 micromoles of methane, suggesting our sample are dominated by genuinely abiotic CH_4 , synthesized by CO reduction. A simple mass balance suggests that the near-complete conversion of the Fe^0 powder to magnetite (reaction 2) produced the H_2 -rich vapor phase, as shown in McCollom et al., (2010). This is an essential condition to ensure reaction 3 proceeds efficiently, allowing the abiotic synthesis of methane (McCollom, 2016).

An inverse correlation between CH_4 quantities and temperatures is observed (Fig. 1), indicating that experiments at lowest temperatures allowed the highest accumulations of methane from CO reduction. The experiments at 275 °C and 300°C yielded 5 and 10 micromoles of CH_4 respectively, which was insufficient to characterize isotopologue abundances; the low yields at these temperatures likely reflect rapid conversion of CO to CO_2 by the water-gas shift reaction (reaction 4), short-circuiting reduction to methane. Seven experiments conducted at temperatures between 130 and 250 °C yielded at least 35 micromoles of CH_4 , allowing measurements of rare isotopologue ratios. We interpret the relationship between methane amount and experimental temperatures (Fig. 1) as a reflection of the lifetime of the injected CO: upon CO injection, conversion to CO_2 through reaction 4 begins, precluding methane synthesis. Equilibrium thermodynamics suggest that reaction 4 goes to completion, hence CH_4 synthesis occurs in a short time window between injection of CO and conversion to CO_2 . Reaction 4 is faster at higher temperatures. Evidently, in our experiments conducted at $T > 250^\circ\text{C}$, CO conversion to CO_2 happens so rapidly that very little FTT-derived methane is generated.

The δD of product methane in our experiments vary between -583 – -608 (–119 with temperatures (Fig. 2). At the highest temperatures, the hydrogen isotope composition of methane tends to be the most elevated. A clear outlier to this trend is FT18-4, the experiment conducted at the lowest temperature (130°C), which shows the highest δD of the series (–582). The δD values for the methane synthesized in this study are different from those obtained in McCollom et al., (2010) (with values of ~ –550). The δD values were similar. We attribute the small differences as the simple reflection of variable starting water for the experiments: in the study of McCollom et al., (2010), the reactant water was purchased from Fischer®. The measured $\delta^{13}\text{C}$ of methane varies between -44 and -62 here are similar to previous results obtained using the same experimental procedures and starting CO (McCollom et al., 2010). The $\delta^{13}\text{C}$ values are weakly correlated with temperature but interpretations of the correlation should be regarded with caution, considering the importance of FT18-4, the one outlier on Fig. 2b.

We find $\Delta^{13}\text{CH}_3\text{D}$ values between 1.7 ± 0.4 experiments except one outlier, FT18-3, with a $\Delta^{13}\text{CH}_3\text{D}$ of 5.8 ± 0.2 . Most of the $\Delta^{13}\text{CH}_3\text{D}$ values track the temperature of abiotic methane synthesis within 1 contrast with $\Delta^{13}\text{CH}_3\text{D}$, $\Delta^{12}\text{CH}_2\text{D}_2$ values are exclusively negative, ranging from -3.0 ± 2.5 – -32.0 – -1.6 . $\Delta^{12}\text{CH}_2\text{D}_2$ values indicate that the relative amount of $^{12}\text{CH}_2\text{D}_2$ among methane isotopologues is lower than predicted for a stochastic gas equilibrated at $T > 1000^\circ\text{C}$. The $\Delta^{12}\text{CH}_2\text{D}_2$ values are positively correlated with peak experimental temperatures (Fig. 3b); the magnitude of the disequilibrium relative to stochastic is greater at the lowest temperatures. The data are shown in the $\Delta^{13}\text{CH}_3\text{D}$ - $\Delta^{12}\text{CH}_2\text{D}_2$ space on Fig. 4, with natural samples from deep-sea vents (Labidi et al., 2020), methane from the Kidd Creek mine (Young et al., 2017) and Oman fluids (Nothhaft et al., 2021), for which methane is potentially abiotic in origin.

5. Discussion

5.1 The carbon isotope balance in our experiments

The $\delta^{13}\text{C}$ signatures of methane vary around a value of -55‰ compared to the starting CO (-28‰ isotopic difference between CH_4 and the starting CO of $\sim 25\text{‰}$ reflects $^{13}\text{C}/^{12}\text{C}$ fractionation during methane synthesis. Kueter et al., (2019) determined the equilibrium $^{13}\text{C}/^{12}\text{C}$ fractionations between CO, CO_2 and CH_4 at various temperatures between 1200°C and 300°C . At 300°C , the equilibrium $^{13}\text{C}/^{12}\text{C}$ fractionation between CO and CH_4 is -3.1‰ . CH_4 should be enriched in ^{13}C relative to CO by 3.1‰ in $^{13}\text{C}/^{12}\text{C}$ in CH_4 relative to the initial reactant CO. Instead methane $\delta^{13}\text{C}$ values are broadly consistent with an approach to $^{13}\text{C}/^{12}\text{C}$ equilibrium between methane and CO_2 . This implies that the synthesis of CO_2 from CO oxidation via reaction 4 was nearly quantitative so that CO_2 had nearly the same $^{13}\text{C}/^{12}\text{C}$ as the initial CO reactant (-28‰ equilibration between CH_4 and this CO_2 would yield methane with a 25‰ depletion relative to CO_2 at 250°C , and roughly 40‰ corresponding to predicted methane $\delta^{13}\text{C}$ values of $\sim -53\text{‰}$ respectively. This data supports near-complete conversion of CO to CO_2 and complete equilibration between CH_4 and CO_2 . While imperfect, the magnitude of the shift in $\delta^{13}\text{C}$ from the initial CO reactant values to the product methane are generally consistent with these CH_4 - CO_2 equilibrium values. In this context, the scatter in the $\delta^{13}\text{C}$ values, and imperfect correlation with T (Fig. 2a) would reflect various degrees of departure from full isotopic equilibrium and/or conversion of CO to CO_2 . The FT18-4 outlier would reflect the furthest removal from CH_4 - CO_2 equilibrium, and/or incomplete conversion of CO to CO_2 , consistent with this experiment being conducted at the lowest temperature. The alternative interpretation is that the $\text{CH}_4\delta^{13}\text{C}$ values reflect a kinetic carbon isotope effect involving the reduction of CO. In this case the correlation with temperature, albeit imperfect, would perhaps reflect a reservoir effect with a single kinetic fractionation factor, or a temperature-dependent kinetic fractionation factor.

5.2 The hydrogen isotope balance in our experiments

The δD signatures of methane range from -610 to -580‰ methane is in D/H equilibrium with experimental water (-119‰ the methane δD would fall between -250 and -350‰ conducted at temperatures between 250 and 130°C (Horibe and Craig, 1995). Thus, the observed δD signatures of methane of roughly -600‰ reflect unambiguous D/H disequilibrium with water. During FTT synthesis, methane molecules form via a catalytic surface reaction of sequential hydrogen additions, presumably sourced directly from H_2 . It is expected, therefore, that it is the D/H ratio of H_2 that is a dominant factor in controlling the D/H of methane in our experiments, not that of water. No D/H measurements of H_2 are available in this study, but at equilibrium, CH_4 should be $\sim 540\text{‰}$ coexisting H_2 at $\sim 200^\circ\text{C}$ (Horibe and Craig, 1995). In lieu of D/H for H_2 in our experiments, we may make use of three FTT experimental studies that have reported D/H of both of CH_4 and H_2 to infer the likely extent of isotopic equilibrium between these species. All of these studies report D/H ratios in CH_4 and H_2 are within 150 (2010), far from the $\sim 500\text{‰}$ equilibrium. This suggests that abiotic methane synthesized in the laboratory is not in isotopic equilibrium with H_2 . We can explore this further if we assume H_2 formed in D/H equilibrium with water in our experiments. In this case δD for H_2 is predicted to be approximately -560‰ at 200°C , in equilibrium with the liquid water δD of -119‰ methane has δD values of approximately -600‰ the CH_4 has D/H similar to that of the reactant H_2 , as in previous experiments. Under strict isotopic equilibrium between methane and an infinite pool of H_2 , CH_4 should be $\sim 540\text{‰}$ coexisting H_2 at $\sim 200^\circ\text{C}$. Similar D/H for CH_4 and H_2 would suggest that either the methane consumed most or all of the H_2 available locally, or that there is a D/H kinetic fractionation that offsets the equilibrium fractionation by nearly 500‰ fractionation between H_2O and product H_2 , followed by synthesis of methane with similar D/H than reactant H_2 explains the methane δD values of approximately -600‰ .

The predicted range in δD values relative to VSMOW for H_2 formed in equilibrium with the effectively infinite reservoir of liquid H_2O from ~ 170 to 250°C is -588 to -513‰ slope of $0.44\text{‰}/^\circ\text{C}$ similar to the observed positive correlation between methane δD values with T in our experiments, with a slope of $\sim 0.38\text{‰}/^\circ\text{C}$ the inheritance of D/H from H_2 equilibrated with water, with the exclusion of the 130°C outlier FT18-4 (Fig. 2). This interpretation of inheritance of D/H from H_2 convolved with a kinetic isotope effect is consistent with previous similar published experiments in which the D/H values of CH_4 and H_2 are within a few tens of

permut of one another (e.g., McCollom et al., 2010).

5.3 Methane clumped signatures: does $\Delta^{13}\text{CH}_3\text{D}$ reflect peak temperature of methane synthesis?

We find $\Delta^{13}\text{CH}_3\text{D}$ values to roughly track with the expected equilibrium values within 1 except FT18-3 (170 °C - see below). Data from the Sabatier experiments reported in Young et al., (2017) are also included in Figure 3. Although run under different conditions, the Sabatier experiments also show equilibrium signatures for $\Delta^{13}\text{CH}_3\text{D}$. Overall, the data suggest that FTT processes generate $\Delta^{13}\text{CH}_3\text{D}$ values that are near equilibrium at the temperature of synthesis. The equilibrium signatures suggest that despite D/H kinetic isotope effects associated with hydrogen addition during the assembly of methane molecules, the $\Delta^{13}\text{CH}_3\text{D}$ bond-ordering remains controlled by synthesis temperature. This is an important perspective for the interpretation of natural methane where abiogenesis is suspected: unless substantial mixing is involved, one may use $\Delta^{13}\text{CH}_3\text{D}$ values to constrain the approximate synthesis temperature of abiotic methane.

For FT18-3, the measured $\Delta^{13}\text{CH}_3\text{D}$ of 5.8 ± 0.42 the measured $\Delta^{13}\text{CH}_3\text{D}$ value for FT18-3 is 25_{-8}^{+5} °C, far from the peak experimental temperatures, but similar to room temperature (Fig. 3a). We argue against the possibility of $\Delta^{13}\text{CH}_3\text{D}$ re-equilibration towards low temperature in FT18-3 since there is no physical reason why $\Delta^{13}\text{CH}_3\text{D}$ reordering to room temperature would occur only in this experiment. Like every other experiment, gases were extracted from the gold reaction cells within 24 hours of cooling the reactors, stored in stainless steel containers, and measured for $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ at UCLA within two weeks upon synthesis. On this basis, we argue that the elevated $\Delta^{13}\text{CH}_3\text{D}$ value for FT 18-3 must reflect an unidentified mass-dependent fractionation of up to 3 rather than re-equilibration.

5.4 Large $\Delta^{12}\text{CH}_2\text{D}_2$ deficits in abiotic methane

The $\Delta^{12}\text{CH}_2\text{D}_2$ values here exhibit pronounced disequilibrium, in sharp contrast with those of $\Delta^{13}\text{CH}_3\text{D}$. The $\Delta^{12}\text{CH}_2\text{D}_2$ values are exclusively of negative sign, ranging from -3.0 ± 2.5 - 32.0 ± 1.6 expected equilibrium values of 9.2 respectively. We argue that the $\Delta^{12}\text{CH}_2\text{D}_2$ deficits are apparent disequilibrium signatures resulting from combinatorial effects. In general, combinatorial effects arise when a molecule contains indistinguishable atoms of the same element, and that these atoms come from pools with distinct isotope ratios, as has been predicted and shown for methane previously both from theory and in the laboratory (Röckmann et al., 2016; Taenzler et al., 2020; Yeung, 2016). Among the two mass-18 isotopologues of methane, only $\Delta^{12}\text{CH}_2\text{D}_2$ can be affected by combinatorial effects, because it is the isotopologue with two indistinguishable deuterium substitutions for hydrogen.

The root of the combinatorial effect comes from the notation convention used with clumped isotopes. As an example, consider a sample of methane gas with $\delta\text{D} = -100$ $1.40184[?]10^{-4}$ and a $^{13}\text{C}/^{12}\text{C}$ ratio of 0.01112483. In this example we will use a measured $^{12}\text{CH}_2\text{D}_2/^{12}\text{CH}_4$ ratio of 1.11600×10^{-7} . To calculate the value for $\Delta^{12}\text{CH}_2\text{D}_2$, we compute the stochastic ratio from the measured bulk carbon and hydrogen isotope ratios. Isotope-specific mole fractions for singly-substituted isotopologues are closely approximated as:

$$x_D = \frac{R_D}{1 + R_D};$$

$$x_H = \frac{1}{1 + R_D};$$

$$x_{^{12}\text{C}} = \frac{1}{1 + R_{^{13}\text{C}}}$$

(5)

where R_D is the bulk D/H ratio and $R_{^{13}\text{C}}$ is the bulk $^{13}\text{C}/^{12}\text{C}$ ratio. From the properties of probabilities, the stochastic isotope-specific mole fraction of $^{12}\text{CH}_2\text{D}_2$, where isotopes are distributed randomly across all isotopologues, is:

$$x_{^{12}\text{CH}_2\text{D}_2} = 6x_D^2 x_H x_{^{12}\text{C}}$$

, (6)

where 6 refers to the six isotopic isomers for this isotopologue ($^{12}\text{CHDHD}$, $^{12}\text{CHHDD}$, $^{12}\text{CDHHDH}$, etc.). In this example, the stochastic value for $x_{^{12}\text{CH}_2\text{D}_2}$ is 1.16547×10^{-7} and the stochastic value for $x_{^{12}\text{CH}_4} = x_H^4 x_{^{12}\text{C}}$ is 0.9884432. With a measured $^{12}\text{CH}_2\text{D}_2/^{12}\text{CH}_4$ ratio of 1.16000×10^{-7} , we have:

$$\delta D = 10^3 \left(\frac{\frac{(^{12}\text{CH}_2\text{D}_2)}{(^{12}\text{CH}_4)_{\text{measured}}}}{\frac{(^{12}\text{CH}_2\text{D}_2)}{(^{12}\text{CH}_4)_{\text{stochastic}}}} - 1 \right) = -16.2$$

Now let us consider the possibility that in the same gas, the methane molecules were in fact constructed from two pools of hydrogen with distinct D/H ratios, with the average D/H being the measured bulk value in our methane, i.e., $\delta D = -100$ factor relating the D/H ratios for the two pools, then:

$$\frac{(R_1 + \alpha R_1)}{2} = R_D$$

(8)

where R_1 is the D/H for one of the reservoirs, R_2 is the D/H for the other reservoir, and R_D is the measured bulk ratio. We take $a = 1.5$, meaning two hydrogens have a D/H ratio fractionated by +500‰, given *a priori* knowledge of equal contributions from the two pools, is given by the corresponding mole fractions for the two pools, $x_{D(1)}$, $x_{D(2)}$ etc., so Equation (6) becomes:

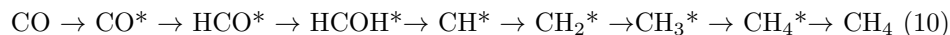
$$x^{12}\text{CH}_2\text{D}_2 = 6x_{D(1)} x_{D(2)} x_{H(1)} x_{H(2)} x^{12}\text{C}$$

(9)

Similarly, the stochastic mole fraction for $^{12}\text{CH}_4$ is $x^{12}\text{CH}_4 = x_{H(1)}^2 x_{H(2)}^2 x^{12}\text{C}$. In our example, the true stochastic value for $x^{12}\text{CH}_2\text{D}_2$ is 1.11885×10^{-7} . This is lower by 42% than the value from equation (6). Using the stochastic estimate from equation (9), the example gas has a true $\Delta^{12}\text{CH}_2\text{D}_2$ of +25‰.

In practice, one would not have any prior knowledge of the actual distribution of the D/H ratios and would have to apply equation (6) rather than equation (9) for the calculation of $\Delta^{12}\text{CH}_2\text{D}_2$. The elevated $x^{12}\text{CH}_2\text{D}_2$ from equation (6) is the cause of the negative $\Delta^{12}\text{CH}_2\text{D}_2$ value. The reason for the discrepancy is a mathematical truism (Yeung et al., 2016, Rockmann et al., 2016, Taenzer et al., 2020). It is straightforward to show that the actual stochastic isotopologue ratio $^{12}\text{CH}_2\text{D}_2/^{12}\text{CH}_4$ is proportional to the square of the geometric mean for the two D/H ratios (to see this, divide Equation 9 by $x^{12}\text{CH}_4 = x_{H(1)}^2 x_{H(2)}^2 x^{12}\text{C}$) while the measured values, by necessity, yield the square of the arithmetic mean of the two ratios, i.e., the measured bulk D/H ratio (divide Equation 6 by $x^{12}\text{CH}_4 = x_{H(1)}^2 x_{H(2)}^2 x^{12}\text{C}$, mindful of Equation 8). The geometric mean is always less than the arithmetic mean, unless $R_1 = R_2$. Thus in general, where molecular positions with distinct isotope ratios are indistinguishable, and the average isotope ratios rather than the site-specific isotope ratios are all that is accessible by the data (the usual case), the calculated stochastic ratio of the multiply-substituted molecule to the major isotopologue ($x^{12}\text{CH}_2\text{D}_2$) is overestimated. This in turn means Δ values derived from these overestimated stochastic ratios are underestimated. This is the combinatorial effect.

The building of CH_4 molecules from CO (or CO_2) is thought to involve a sequence of steps of carbon reduction followed by additions of hydrogen molecules (Wang et al., 2011). The set of reactions can be represented by these steps:



where * corresponds to a surface-adsorbed species. Here H_2 is the source of electrons for reduction. This reaction network allows for the various hydrogens added to methane during sequential steps to have different D/H ratios. A 500‰ between two of the hydrogens relative to the other two can account for our observed $\Delta^{12}\text{CH}_2\text{D}_2$ deficits of approximately -40‰ ($\delta D_{(1)} = \delta D_{(2)} = -850$ ‰, $\delta D_{(3)} = \delta D_{(4)} = -350$ ‰) methane δD (~ -600‰). According to the theoretical treatments in (Cao et al., 2019) and (Young, 2019), D/H fractionations associated with the assembly of methane would plausibly cause such variations in D/H from step to step. For example, a mixture of early steps involving D/H equilibrium followed by later H addition steps with purely

kinetic isotope effects could produce the requisite large differences in D/H of > 400 among hydrogens (Young, 2019) that can reproduce the observed low $\Delta^{12}\text{CH}_2\text{D}_2$ values.

The question arises as to whether our interpretation of the source of low $\Delta^{12}\text{CH}_2\text{D}_2$ values is affected by the source of carbon, i.e. CO versus CO_2 . CO_2 methanation begins by reduction to CO (i.e., the reverse of reaction 4). That additional step occurs prior to the onset of CO reduction and does not involve hydrogen additions to carbon. There is no clear physical reason to anticipate variable $\Delta^{12}\text{CH}_2\text{D}_2$ signatures as the result of the additional step of CO_2 reduction to CO prior to further reduction by hydrogen. This likely explains why the Sabatier experiments (run with CO_2) versus the FTT experiments here (with CO) can be interpreted together (Fig. 3b). Indeed, our data suggests the two pathways, from CO to methane and from CO_2 to methane, may not be very different at all. Our carbon isotope data suggesting that CO_2 is the precursor for the $^{13}\text{C}/^{12}\text{C}$ ratios for product CH_4 in our experiments in which CO is the initial carrier of carbon attest to this. Together, the data indicate that it may be valid to extrapolate our experiments to natural systems, where CO_2 is likely the primary carbon source for abiotic methane synthesis.

5.5 Της τεμπερατυρε ρελαιονσηπ ωιτη $\Delta^{12}\text{H}_2\Delta_2$

Experiments with data at the highest temperatures, 210 and 250 °C, produced methane with $\Delta^{12}\text{CH}_2\text{D}_2$ values of between -3 and -12 experiments done at 180, 170 and 130 °C, which produced methane with $\Delta^{12}\text{CH}_2\text{D}_2$ values of between -32 and -27 (Young et al., 2017) yielded methane with $\Delta^{12}\text{CH}_2\text{D}_2$ values of roughly -55. $\Delta^{12}\text{CH}_2\text{D}_2$ data show a clear positive correlation with temperature (Fig. 3b). This may reflect the possibility that different D/H ratios among pools of hydrogen at each reaction step are dependent on temperature, with greater differences at lower temperatures. At $> 200^\circ\text{C}$, the observed $\Delta^{12}\text{CH}_2\text{D}_2$ values require pools of hydrogen atoms that comprise methane to be at most 200 $\Delta^{12}\text{CH}_2\text{D}_2$ deficits of the Sabatier experiments require at least 700 pools of hydrogen atoms.

The effect of bond reordering is unclear in this set of experiments. One might imagine that irrespective of experimental temperatures, all abiotic methane synthesized during FTT experiments is associated with $\Delta^{12}\text{CH}_2\text{D}_2$ deficits > 70 D/H fractionation factors among the four hydrogen addition steps. This would be the case if kinetic isotope effects for each step in the CH_4 -building reaction sequence were fully independent of temperature. After methane synthesis, $\Delta^{12}\text{CH}_2\text{D}_2$ values would approach equilibrium values > 0 . Bond reordering – here, homogenization of the D/H among the hydrogens comprising CH_4 molecules – would generate *an a-posteriori* correlation between temperature and $\Delta^{12}\text{CH}_2\text{D}_2$ as observed on Fig. 3b. This scenario may not be excluded. Nonetheless, we favor a temperature-dependent fractionation in D/H between H-addition steps during CH_4 construction as the source of negative $\Delta^{12}\text{CH}_2\text{D}_2$ values since these effects can be seen in the theoretical treatments in Cao et al., (2019) and Young (2019).

To summarize, the $\Delta^{12}\text{CH}_2\text{D}_2$ relationship with experimental temperature (Fig. 3b) may be explained by two different scenarios. First, kinetic isotope effects for different H-addition steps may vary as a function of temperature. This would cause the resulting combinatorial $\Delta^{12}\text{CH}_2\text{D}_2$ values to show a variability naturally correlated to temperature. Alternatively, an *a-posteriori* bond reordering may occur in the hydrothermal cells, from an initial $\Delta^{12}\text{CH}_2\text{D}_2$ value that is always ~ -70 re-ordering than those at lower temperatures, temperature and $\Delta^{12}\text{CH}_2\text{D}_2$ will display a positive correlation.

5.6 Hydrogen isotope homogenization in nature: the link with the laboratory

The data collected for this study are plotted on figure 4 with published methane isotopologue data from a variety of geological settings, including sedimentary basins, deep crystalline environments and serpentinization sites (Ash et al., 2019, Giunta et al 2019 2021, Young 2017 2019, Warr et al 2021, Lin et al 2023). Extreme signatures associated with active anaerobic oxidation of methane (Giunta et al 2022, Liu et al 2023) were omitted for clarity. Signatures observed in abiotic methane synthesized in the laboratory are rare in nature (Fig. 4). This might suggest that abiotic methane has been under-sampled so far, and/or that bond reordering of once-abiotic methane is at play in natural environments.

Equilibrium values for $\Delta^{12}\text{CH}_2\text{D}_2$ (and $\Delta^{13}\text{CH}_3\text{D}$) are seen in most high-temperature hydrothermal vents

with temperatures [?] 250°C to date (Fig. 4). Based on experiments, we would anticipate natural abiotic methane synthesized at > 250°C to have equilibrated $\Delta^{13}\text{CH}_3\text{D}$ values associated with $\sim 10\Delta^{12}\text{CH}_2\text{D}_2$ deficits relative to equilibrium (Fig. 3b). Based on the experiments, subsequent bond reordering would seem to be required in order to erase the low $\Delta^{12}\text{CH}_2\text{D}_2$ values associated with constructing CH_4 molecules and replace them with the equilibrium values exhibited by the natural high-temperature hydrothermal samples (vents) shown in Fig. 4. High-temperature hydrothermal systems may provide conditions conducive to bond reordering. Methane is thought to be formed abiotically in fluid inclusions of basement rocks at temperatures of 300 °C or higher (Früh-Green et al., 2022; Klein et al., 2019). Those fluid inclusions might be maintained at temperatures of 300 °C or above for durations that may be thousands of years or even far longer, allowing bond reordering to take place. Because homogenization evidently occurs, the deficits associated with initial methane synthesis are “cryptic”, i.e., they are not observable in nature, only in the laboratory where experimental durations are short, and reordering does not take place.

Abiotic methane formed and kept at low temperature might have a chance to preserve deficits in $\Delta^{12}\text{CH}_2\text{D}_2$, should it be restricted to environments where the kinetics of D/H homogenization within methane molecules are unfavorable or residence times are short. This offers a chance to use the combination of $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ to identify abiotic methane in nature, as it has been done with microbial methane (Ash et al., 2018; Giunta et al., 2022, 2019; Young et al., 2017). Abiogenesis may notably account for signatures observed in methane from $\sim 25^\circ\text{C}$ fluids from the Kidd Creek mine. There, methane samples have $\Delta^{13}\text{CH}_3\text{D}$ values of 5.2 ± 0.5 translating to temperatures of $\sim 45^\circ\text{C}$. The methane $\Delta^{12}\text{CH}_2\text{D}_2$ values from the deepest level in the mine are 10 to 30 (Young et al., 2017, also shown here on Fig. 4). These deficits are not as low as those seen in experiments, and would be compatible with FTT signatures affected by later bond reordering resulting from processing by anaerobic methane oxidation (Warr et al., 2021; Young et al., 2017).

Abiogenesis alone is more challenging to reconcile with the observations from serpentinizing systems in Oman. There, methane is vented to the surface in $\sim 35^\circ\text{C}$ hyperalkaline fluids issuing from the underlying ophiolite. It carries some of the highest $\delta^{13}\text{C}$ yet measured in natural methane (Miller et al., 2016). Whether microbial methanogenesis can explain the isotopic features of Oman methane is debated (Etiope, 2017; Miller et al., 2016). Recent measurements of $\Delta^{12}\text{CH}_2\text{D}_2$ and $\Delta^{13}\text{CH}_3\text{D}$ were made available on new gas aliquots from Oman, in Nothaft et al., (2021). The one sample with the highest $\delta^{13}\text{C}$ has a negative $\Delta^{12}\text{CH}_2\text{D}_2$ value and near-zero $\Delta^{13}\text{CH}_3\text{D}$ (Fig. 4). This is inconsistent with abiotic signatures reported here: no abiotic methane made in the laboratory, at any of the investigated temperatures, has yielded a near-zero $\Delta^{13}\text{CH}_3\text{D}$ (Fig. 3a). Conversely, microbial methane does exhibit near-zero or negative $\Delta^{13}\text{CH}_3\text{D}$ values (Young et al., 2017, Giunta et al., 2019) and the Oman data appear consistent with microbial signatures (Fig. 4). The simplest explanation for the data in Nothaft et al., (2021) remains that despite the elevated $\delta^{13}\text{C}$, the $\Delta^{12}\text{CH}_2\text{D}_2$ - $\Delta^{13}\text{CH}_3\text{D}$ signature reflect substantial contributions of microbial methane in the Oman ophiolite (details in Nothaft et al., 2021).

5.7 Methane clumped signatures in astrobiology

Our experiments suggest methane produced by abiogenesis on other worlds may yield apparent $\Delta^{12}\text{CH}_2\text{D}_2$ deficits. This is because the difference between equation 6 and 9 remains insensitive to the absolute value of the D/H ratio; only D/H *differences* between various hydrogen pools matter in causing combinatorial effects (Rockmann et al., 2016, Taenzer et al., 2020), not the absolute values of the source pools. Since D/H differences inevitably follow from lower-T hydrogen additions to carbon, we expect $\Delta^{12}\text{CH}_2\text{D}_2$ deficits to be common anywhere methane is synthesized from the reduction of CO or CO_2 . This means that on other worlds, where the D/H ratio of molecular hydrogen is unknown and could be extremely different from Earth, abiotic methane should still exhibit $\Delta^{12}\text{CH}_2\text{D}_2$ deficits comparable to the values observed here (Fig. 3). As on Earth, if this methane is entrained in a hydrothermal system with ambient temperatures approaching 300 °C, this signature may be replaced by isotopologue equilibrium, a situation that may be uncommon however given the low temperatures conditions prevailing on most other solar system bodies.

While biosignatures have been a long-term focus of investigation, both field and experimental consideration of potential abiosignatures has been flagged as a critical gap in the search for life beyond Earth (NASEM,

2022). As such, bond reordering of abiotic methane may be a useful tool. The in-situ discovery of methane gas in Mars's atmosphere by Curiosity (Webster et al., 2015) indicates that methane is produced on the planet, likely from an on-going source. Methane is also detected in the plumes of Saturn's moon Enceladus, together with molecular hydrogen (Waite et al., 2017). There, the origin of CH_4 is thought to be abiotic, derived from hydrothermal reactions occurring at depth in the oceans of Enceladus, but at unconstrained temperatures (Waite et al., 2017). A Bayesian analysis of the escape rates of Enceladus has recently challenged a hydrothermal origin of methane, and suggested it might potentially be biological (Affholder et al., 2021). It is not clear that conventional isotopic tools can yield unambiguous results as to the origin of methane on Mars and Enceladus, because sound interpretations of bulk isotope ratios by conventional means require contextual information: what is the meaning of methane D/H ratios with no constraints on the D/H of the co-existing molecular hydrogen? What is the overall $^{13}\text{C}/^{12}\text{C}$ of the carbon reservoirs from which methane might evolve? This is underscored by the complex interpretations of $^{13}\text{C}/^{12}\text{C}$ ratios measured on Mars rocks by Curiosity (House et al., 2022). On Mars and on Enceladus, if methane is synthesized abiotically at high temperature, we see no physical reason against the appearance of equilibrium $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ signatures resulting from reordering, as on Earth. Should $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ measurements ever be done for Enceladus methane, any equilibrium temperatures [?] 250°C would rule out a microbial origin of methane.

A complication in developing methane isotopologues as tools for identifying biogenicity to extraterrestrial methane is that combinatorial effects are also associated with microbial methane and under the right conditions, with thermogenic methane (i.e., produced from thermal decomposition of organic matter). All of these processes can yield large $\Delta^{12}\text{CH}_2\text{D}_2$ deficits in the absence of bond reordering because all of them involve multiple steps of hydrogen addition and draw upon pools of hydrogen with distinct D/H. Experiments verify this effect for hydrogens with variable D/H causing $\Delta^{12}\text{CH}_2\text{D}_2$ deficits in microbial methane (Taenzer et al., 2020). Current evidence suggests that microbial methane is unique in that it shows non-equilibrium $\Delta^{13}\text{CH}_3\text{D}$ values, together with markedly negative $\Delta^{12}\text{CH}_2\text{D}_2$ values, probably as the result of classical kinetics involving carbon. The release of methane during cracking of organic matter in the laboratory can result in large $\Delta^{12}\text{CH}_2\text{D}_2$ deficits with near equilibrium $\Delta^{13}\text{CH}_3\text{D}$ (Dong et al., 2021), and could thus be confused with purely abiotic methane. In any case, mass-18 methane isotopologues significantly aid us in differentiating microbial, thermogenic, and abiotic methane on other planets or icy moons independent of the unknown bulk $^{13}\text{C}/^{12}\text{C}$ and D/H of sources.

6. Conclusion

We performed synthesis of abiogenic methane in the laboratory and determined $\delta^{13}\text{C}$, δD , $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ using a Panorama gas-source mass spectrometer. We carried out a set of 9 experiments in hydrothermal conditions, at temperatures between 130 and 300 °C. The experiments were performed by heating an aqueous solution in the presence of Fe^0 powder and gaseous CO. We find $\delta^{13}\text{C}$ and δD of methane to be isotopically depleted relative to the starting materials, consistent with equilibrium carbon isotope fractionation between CO_2 produced in the experiment and the product CH_4 , and kinetic isotope D/H fractionations between H_2 and product CH_4 . We find most of the product methane $\Delta^{13}\text{CH}_3\text{D}$ values trace the temperature of abiotic methane synthesis within a permil. At the same time, we observe $\Delta^{12}\text{CH}_2\text{D}_2$ deficits of up to 40‰ occurring during the methane assembly. The combinatorial effect results from our inability to determine D/H ratios of distinctive pools of hydrogen that contribute to individual hydrogens on the methane molecule. The D/H fractionation associated with the various steps of hydrogen addition to carbon can explain the direction and the magnitude of the $\Delta^{12}\text{CH}_2\text{D}_2$ signatures of methane that would otherwise be at bond-ordering equilibrium. In instances of some potentially abiotic methane found on Earth, particularly under high T, $\Delta^{12}\text{CH}_2\text{D}_2$ deficits may have been generated during methane synthesis, but were subsequently erased by D/H reordering. In contrast, $\Delta^{12}\text{CH}_2\text{D}_2$ deficits generated in our experiments are seen on Earth at sites where the C-H bond activation was too slow to re-equilibrate $\Delta^{12}\text{CH}_2\text{D}_2$ to environmental temperatures. As such these dramatic deficits, when combined with near-equilibrium $\Delta^{13}\text{CH}_3\text{D}$ values, might be an avenue for the identification of abiogenesis on other worlds.

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Data availability statement

All the methane isotope data collected for this study will be available online via a DOI, on the EarthChem data repository.

Captions

Table 1 : Methane isotope data for synthesized abiotic methane. Bulk $^{13}\text{C}/^{12}\text{C}$ and D/H are reported alongside $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$. Analytical uncertainties are below $0.1\delta^{13}\text{C}$ and δD . Propagated uncertainties are listed for $\Delta^{13}\text{CH}_3\text{D}$ and $\Delta^{12}\text{CH}_2\text{D}_2$ in 1σ . They are highly variable and directly reflect counting statistics, i.e., CH_4 quantity.

Figure 1: Quantities of synthesized abiotic methane versus temperature. Data display a negative trend with experiment temperature. See text for details. Note: there are nine samples here, but only seven for isotope measurements. The two highest temperatures did not yield enough methane for mass spectrometry.

Figure 2: $\delta^{13}\text{C}$ (panel a) and δD (panel b) of synthesized abiotic methane versus temperature and observed methane concentrations. $\delta^{13}\text{C}$ data display a weak negative trend with experiment temperature. See text for details.

Figure 3: Relationships between the two mass-18 isotopologues of methane in experimental samples and the peak experimental temperatures. Data of other methane synthesized abiotically from Young et al., (2017) are also shown. Data and uncertainties are from Table 1.

Figure 4: The two mass-18 isotopologues of methane for relevant experimental and natural data. The FTT data are from this work. The Sabatier experiments are from Young et al., (2017). Hydrothermal gases are from the Rainbow, Von Damm and Lucky Strike sites from Labidi et al., (2020). The Kidd Creek data are only from the 2.9 km level in the mine, from Young et al., (2017). The Oman data are from various vents in the ophiolite, and the data can be found in Nothaft et al., (2021). Other natural data from various geological settings are from Ash et al., (2019), Giunta et al., (2019, 2021), Gonzalez et al., (2019), Lin et al., (2023), Warr et al., (2021), Young et al., (2017) and Young (2019).

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