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

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Abstract

The recent expansion of studies at hydrothermal submarine vents from investigation of abiotic methane formation to include abiotic production of organics such acetate and formate, and rising interest in processes of abiotic organic synthesis on the ocean-world moons of Saturn and Jupiter, have raised interest in potential Earth analogs for investigation of prebiotic/abiotic processes to an unprecedented level. The deep continental subsurface provides an attractive target to identify analog environments where the influence of abiotic carbon cycling may be investigated, particularly in hydrogeological isolated fracture fluids where the products of chemical water-rock reactions have been less overprinted by the biogeochemical signatures of the planet's surficial water and carbon cycles. Here we report, for the first time, a comprehensive set of concentration measurements and isotopic signatures for acetate and formate, as well as the dissolved inorganic and organic carbon pools, for saline fracture waters naturally flowing 2.4 km below surface in 2.7 billion year-old rocks on the Canadian Shield. These geologically ancient fluids at the Kidd Creek Observatory were the focus of previous investigations of fracture fluid geochemistry, microbiology and noble gas-derived residence times. Here we show the fracture waters of Kidd Creek contain high concentrations of both acetate and formate with concentrations from 1200 to 1900 µmol/L, and 480 to 1000 µmol/L, respectively. Acetate and formate alone account for more than 50% to 90% of the total DOC – providing a very simple “organic soup”. The unusually elevated concentrations and profoundly ^{13}C-enriched nature of the acetate and formate suggest an important role for abiotic organic synthesis in the deep carbon cycle at this hydrogeologically isolated site. A variety of potential abiotic production reactions are discussed, including a radiolytically driven H, S and C deep cycle that could provide a mechanism for sustaining deep subsurface habitability. Scientific discoveries are beginning to reveal that organic-producing reactions that would have prevailed on Earth before the rise of life, and that may persist today on planets and moons such as Enceladus, Europa and Titan, can be accessed in some specialized geologic settings on Earth that provide valuable natural analog environments for the investigation of abiotic organic chemistry outside the laboratory.

Introduction

The past ten years of research has identified large gaps in our understanding of the deep carbon cycle (Orcutt et al., 2020), the interconnection of reservoirs of carbon in the marine and continental lithosphere with the surface carbon cycle, and the implications therein for deep subsurface life on Earth (Hoehler and Jørgensen, 2013; Onstott, 2016; Trembath-Reichert, et al., 2017). Recent papers have significantly revised estimates of total subsurface biomass in both the ocean sediments and marine crust (Kallmeyer et al. 2012; Lipp et al., 2008; McMahon and Parnell 2014; Inagaki et al., 2015; D’Hondt et al., 2019 and references therein) and for the continental lithosphere (Magnabosco et al., 2018a). Major efforts to develop more quantitative estimates of CO_2 flux (Plank and Manning, 2019), to quantify reservoirs of modern groundwater (<50-100 years old; Gleeson et al. 2016), and to estimate the quantity and nature of deep groundwaters sequestered in the continental and marine crust (Fisher et al., 2005; Holland et al., 2013; Heard et al., 2018; Warr et al., 2018) are challenging preconceptions about reservoir volumes and flux in the deep water and deep carbon cycle on a global scale.

In parallel with these questions regarding reservoir volumes and flux, there have been discoveries of novel processes in the subsurface, processes whose roles had been previously underestimated, and in some cases entirely unforeseen. Abiotic CH_4 formation via Sabatier reactions (and related production of higher chain alkanes by Fischer-Tropsch type reactions) has been long investigated in the laboratory and the chemical engineering industry (McCollom and Seewald, 2007 and references therein). In the past 20 years, field studies have demonstrated that in specific geologic settings on both the continents (Sherwood Lollar et al., 2002; 2006; Kietäväinen et al., 2017), as well as in ophiolites and the ocean floor (Proskurowski et al. 2008; Schrenk et al., 2013; Lin et al., 2014; Klein et al., 2019 and references therein), an abiotic CH_4 cycle can be identified in natural settings if rates of abiotic organic synthesis are fast enough, long-term storage of the products of abiotic organic reactions occurs, and/or biologic cycling and mixing are limited, such that "abiotic" signatures remain discernable. Recently a role for abiotic organic production for other low molecular weight organic compounds such as formate (McDermott et al., 2015; Lang et al., 2010; 2018) and aromatic amino acids has been increasingly recognized (Menez et al., 2018). We are beginning to understand that organic-producing reactions that would have prevailed on Earth before the rise of life, and that persist today on planets and moons such as Enceladus and Titan, can be accessed in some specialized geologic settings on Earth that provide valuable natural analog environments for the investigation of abiotic organic chemistry outside the laboratory.

Processes of H_2 production in the deep Earth have also been a fertile area of discovery over the past decade – with identification of a variety of H_2-producing water-rock reactions contributing to subsurface H_2 production. The global contribution of H_2 as an electron donor for subsurface life has been estimated by several studies for the marine lithosphere (Bach and Edwards, 2003; Canfield et al., 2006; Sleep and Bird, 2007; Cannat et al., 2010; Lin et al. 2014), while recent studies for the continental lithosphere have demonstrated an equally important, but hitherto under-appreciated contribution to global H_2 production by water-rock reactions (Lin et al., 2005; Sherwood Lollar et al., 2007; 2014; Warr et al., 2019; Zgonnik, 2020 and references therein). Radiolysis-driven processes have been an important subset of the water-rock reactions under investigation, particularly as radiolysis has been shown to be not only a source of electron donors (H_2) to deep subsurface ecosystems (Lin et al., 2005a&b; D'Hondt et al., 2009; Dzaugis et al., 2016), but a novel process generating electron acceptors such as dissolved sulfate via indirect radiolytic oxidation of sulfide minerals (Lin et al., 2006; Li et al., 2016). Given the importance of H_2-utilizing metabolisms in general, and H_2-utilization coupled to SO_4^{2-} reduction in particular, as an important metabolic strategy in the deep subsurface biosphere (Moser et al., 2005; Lin et al., 2006; Chivian et al., 2008; Nyyssonen et al., 2012; Suzuki et al. 2014; Lollar et al., 2019), radiolytically-driven metabolic landscapes providing both potential electron donors and electron acceptors has become a major topic of research on Earth, Mars and for the ocean worlds such as Enceladus and Europa (Onstott et al., 2006; Vance et al., 2007; 2016; Hand et al., 2017; Tarnas et al., 2018). Similarly, the hydration of mafic and ultramafic rocks, including serpentinization, long recognized by the geochemical community (Barnes et al., 1967), has increasingly been investigated by microbiologists and even planetary scientists and astrobiologists striving to understand the role of water-rock reactions in sustaining subsurface habitability (NASEM 2018). The recent selection of the Dragonfly mission to Saturn's moon, Titan as a NASA New Frontiers mission to investigate the organic mineralogy (Maynard-Casely et al., 2018 has heightened interest in Earth analogs for prebiotic/abiotic organic geochemistry

Scientific opportunities to investigate the abiotic organic carbon cycle on Earth are necessarily limited by the undisputed fact that life, and the biologic carbon cycle, largely dominate and utterly overprint the geochemical signatures on the planet's surface. The subsurface provides an attractive target to identify environments where the influence of abiotic carbon cycling may be investigated. High temperature volcanic systems, hot springs and submarine vents, and subduction zones all provide opportunities, but at the extreme end are beyond the thermal limit to life. To investigate questions at the intersection of abiotic organic synthesis and life, we require environments that are not beyond the thermal (or other) limits to life, but where abiotic processes may still occur at significant enough rates, or even if slow rates prevail, where preservation of the products of such reactions is significant enough that "abiosignatures" can be unambiguously identified. Settings that provide a window into "abiotic organic factories" in the subsurface, as well as environments where the abiotic cycle and biological carbon cycle co-exist are key. Due to the great depths to which life penetrates the subsurface (Moser et al., 2005; Inagaki et al., 2015; Onstott, 2016; Nuppunen-Puputti et al., 2018; Lollar et al., 2019; Purkamo et al., 2020), and the dilution of deep subsurface geochemical environments by recharging groundwater from surface (or circulation and mixing of ocean fluids into the subseafloor), such target environments are challenging to identify and access. Even surface sites where serpentinization has been shown to contribute to H_2 production and related abiotic CH_4 production are typically heavily impacted by mixing with surface-derived fluids and the impacts of surface biology on the carbon and hydrogen cycle (Schrenk et al. 2013 and references therein; Suzuki et al., 2014; Miller et al., 2016; Meyer-Dombard et al., 2019). Mixing between surface and subsurface fluids and processes is the norm, and can extend even to kilometers depth below surface depending on the setting (Miettinen et al., 2015; Heard et al., 2018). Within such necessarily mixed systems, identification and interpretation of “abiotic” end-members is challenging (Etiope and Sherwood Lollar 2013 and references therein; Suzuki et al., 2014; McDermott et al., 2015; Lang et al., 2010; 2018; Kietäväinen and Purkamo 2015).

Investigations of deep subsurface fluids and life in Precambrian continental settings have, over the past decade, demonstrated the critical opportunity such settings provide to address this challenge. Given the thickness and age of the Precambrian cratons (30-50 km and up to 4 Ga, versus a maximum of 5-10 km and less than 200 Ma in age for marine crust), the Precambrian continents provide an opportunity to investigate potential abiotic organic synthesis at much greater depths and timescales (Sherwood Lollar et al., 2014). In the absence of modern tectonic activity, the cratons preserve fluids and habitable environments on a range of timescales from thousands, to millions, to even billions of years (Lippmann-Pipke et al. 2011; Holland et al., 2013; Kietäväinen et al., 2014; Heard et al., 2018; Warr et al., 2018). Finally, the low porosity and permeability of these settings means products of water-rock reaction can be preserved and less diluted by mixing with younger fluids than in continental or ocean sediments, providing a critical locale for investigation of abiotic processes and water-rock reactions on a planet otherwise dominated by the surface hydrosphere and biologically dominated carbon cycle.

Kidd Creek mine in Timmins, Ontario on the Canadian Shield provides one such archetypical deep craton setting for investigation of the preservation of deep fluids, abiotic organic synthesis, water-rock reaction driven habitability, and subsurface microbial life (Lollar et al., 2019). Over the past 2 decades a series of studies have demonstrated the important role played by abiotic organic synthesis in the production of CH_4 and higher alkanes (ethane, propane, butane) (Sherwood Lollar et al., 2002; 2006; 2008), and H_2 production by serpentinization (Sherwood Lollar et al., 1993; 2014). Further, radiolytic processes related to the highly radiogenic environment have been shown to not only produce additional H_2 from radiolytic decomposition of water, but to produce dissolved SO_4^{2-} by indirect radiolytic oxidation of the pyrite and reduced sulfur minerals (Li et al., 2016). Cell counts and culture-based methods (Most Probable Number analysis) have recently demonstrated a response from H_2- utilizing and alkane-oxidizing SO_4^{2-}-reducing bacteria in the Kidd Creek fluids (Lollar et al., 2019), but the low cell concentrations suggest rates of microbial activity are low, consistent with observations in other deep subsurface communities in both deep continental (Lin et al., 2006; Magnabosco et al., 2018a) and oceanic subsurface ecosystems (Trembath-Reichert et al., 2017; NASEM, 2018). The low biomass and low rates of activity may help explain why the bulk geochemical signatures of the gases and fluids in the Kidd Creek systems retain a predominantly abiotic character. This chemical landscape simplifies the components of the carbon cycle, enabling investigation of habitability driven primarily by water-rock reactions, and of the chemolithotrophic microbial communities therein. This setting provides an invaluable analog system for astrobiology and planetary sciences, and may additionally provide insight into what the breadth of reactions driving abiotic organic synthesis in a prebiotic Earth might have been. Relevant analogs for prebiotic chemistry outside the laboratory are a challenge due to the geologic reprocessing of the Earth's surface and crust over its 4.5 billion year history. Recent high profile studies on the emergence of an RNA world have pointed to Mars as a “planet frozen in time” providing the best physical and chemical analog for an RNA world (Mojarro et al., 2020). It is thought provoking to consider whether kilometers below the Earth’s surface, the > 2.5 to 3 billion year old rocks of the Precambrian cratons might, in some locations such as Kidd Creek, provide a more accessible window into abiotic chemistry relevant to early Earth history and the prebiotic to biotic transition.

In addition to the CH_4 and higher hydrocarbons (ethane, propane, butane) described previously in the abiotic organic factory at Kidd Creek, the fracture waters at this site contain dissolved acetate and formate, but to date the composition and isotopic characteristics of these organic compounds have not been investigated. Volatile fatty acids (VFA) are nearly ubiquitous in oil field brines, and are also commonly found in sedimentary basins, marine sediments, saline springs, and hydrothermal systems (Heuer et al., 2009, 2010 and references therein). VFA can be central to microbial nutrient cycling in both surface and deep subsurface microbial ecosystems, and VFA pools are typically the combined result of competing production and consumption pathways (Onstott et al., 2006; Heuer et al., 2006). VFA production can be from either organic or inorganic precursors and occur via microbial, thermal, or metal-catalyzed reactions. Organic precursor reactions generally produce VFA by cleaving carbon-carbon bonds, releasing shorter carbon chains from more complex starting material. For instance, much of the VFA associated with oil field brines is attributed to thermal maturation and break down of kerogen deposits, as demonstrated through laboratory based hydrolysis experiments (Barth and Neilsen, 1993; Knauss et al., 1997; Dias et al., 2002a). In systems without kerogen, hydrocarbons can be broken down through a series of oxidation reactions to yield VFA (Seewald 2001; McCollom and Seewald 2003a&b). Microorganism-mediated fermentations also break down organic precursors to VFA from starting compounds that include sugars, alcohols, alkanes, or larger organic acids (Madigan et al., 2000). VFA production from inorganic precursors can follow either biogenic/microbial or abiogenic pathways. Microbial autotrophic acetogens, for instance, use H_2 to reduce CO_2 to acetate (Madigan et al., 2000). Archaeal methanogens and sulfate reducers have been shown to produce acetate and formate under growth conditions with carbon monoxide CO (Rother and Metcalf, 2004; Henstra et al., 2007). Huber and Wächtershäuser (1997) demonstrated that metal catalysts also can be used to produce acetate from CO and methyl sulfide precursors, independent of microbial activity. Cody et al. (2004) further demonstrated the ability of metal sulfides to form carboxylic acids (including VFAs) by a similar abiogenic mechanism, and Fu et al. (2008) demonstrated carboxylic acid elongation as a product of formate reaction on a mineral catalyst. Finally, it should be noted that formate is the hydrated form of CO, a characteristic that sets it apart from other VFAs, and this paper will consider both acetate and formate. Under hydrothermal conditions (Seewald et al., 2006) or during olivine serpentinization (McCollom and Seewald, 2003a), formate is produced by reversible CO_2 reduction and hydration which can lead to equilibrium between CO_2 and CO with formate as an intermediate between the two. Laboratory experiments demonstrate that equilibrium between CO_2 and formate can be indicative of *in situ* conditions with higher formate concentrations characteristic of reducing conditions (McCollom and Seewald, 2003a).

Here we present the first data for acetate and formate in the Kidd Creek fluids from the same long-term observatory boreholes at 2.4 km below surface that were the focus of previous investigations of fracture fluid geochemistry, microbiology and noble gas-derived residence times (Holland et al., 2013; Li et al., 2016; Warr et al., 2018; Lollar et al., 2019). This dataset is the first to provide a full characterization of the carbon isotope signatures for both acetate and formate as well as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) from the deep continental subsurface. Previous studies of deep terrestrial systems in South Africa and Fennoscandia published only concentration data (to date only one carbon isotope value to our knowledge has been published for a single site) (Onstott et al., 2006; Lin et al, 2006; Simkus et al., 2016; Kietäväinen, 2017; Kieft et al., 2018). Even in submarine hydrothermal vent studies, the number of carbon isotope values published to date for both acetate and formate remain few (McDermott et al. 2015; Lang et al. 2010; 2018). This study addresses whether the production and fate of actetate and formate in the Kidd Creek fracture fluids is predominantly controlled by microbial activity, as is the case in surface ecosystems and most marine settings, or whether, as recently identified by McDermott et al. (2015) and Lang et al. (2010, 2018) for the hydrothermal vents, novel abiotic processes might play a role in the subsurface cycling of acetate and formate at Kidd Creek, as they have been demonstrated to do for the alkane hydrocarbons, and H_2 dissolved in these fluids.

Geologic setting

Underground research laboratories (URLs) and mine networks provide access to deep subsurface environments on the continents, permitting sample collection and observation in some of the deepest ecosystems ever studied, including those in the Witwatersrand basin of South Africa, and the Canadian, and Fennoscandian Shields (Lin et al., 2006; Sherwood Lollar et al., 2014; Onstott, 2016; Kietäväinen et al., 2017; Lollar et al., 2019). Observed metabolisms in these environments include methanogenesis (Ward et al., 2004; Moser et al., 2005; Gihring et al., 2006; Lau et al. 2016), sulfate reduction (Kieft et al., 2005; Lin et al. 2006; Chivian et al., 2008; Nyyssonen et al., 2012; Lollar et al., 2019), and metal reduction (Pedersen, 1997; Moser et al., 2003). Biomass in these systems is low (Gihring et al., 2006; Lin et al. 2006; Lollar et al., 2019) but species diversity can range from very low (Lin et al., 2006; Chivian et al., 2008) to more recent indications of the complex intersectionality between diverse phylogenetic organisms in these subsurface lithotrophic microbial ecosystems (SLiMEs) (Lau et al., 2016; Magnabosco et al., 2018b; Nuppunen-Puputti et al., 2018). Extreme salinities in the saline fluids and brines in these systems can increase microbial energy requirements, providing one limitation to species diversity (Oren, 2002; Telling et al., 2018; Payler et al., 2019). The low number of potential energy yielding redox pairings can also result in reduced microbial diversity compared to surface environments (Onstott et al., 2006; Gihring et al., 2006; Lau et al., 2014, Magnabosco et al. 2018b).

Located some 20 km north of Timmins, Ontario (Figure 1) Kidd Creek Mine is located in southern Volcanic Zone of the Abitibi greenstone belt in the Superior Province of the Canadian Shield north of Toronto and the Great Lakes (Bleeker et al., 1999). The mine is hosted in a series of steeply dipping interlayered felsic, mafic, ultramafic and metasedimentary deposits and is the deepest base metal (Cu-Ag-Zn) mine in North America. Ore deposition was associated with massive hydrothermal vent activity starting ~2.7 Ga ago, as a result of silica and metal-rich hydrothermal fluid circulation below the Archean seafloor. Stringer ore deposits and banded and massive sulphide ores initially deposited as inorganic precipitates formed where metal-rich hydrothermal solutions mixed with seawater in a system analogous to modern day “black smokers” (Thurston et al., 2008). During periods of volcanic quiescence, proximal seafloor sediments accumulated, forming argillite to chert carbonaceous horizons within the assemblage, the latter forming graphite-rich horizons (Thurston et al., 2008). The entire formation was metamorphosed to greenschist facies during the last major regional metamorphic event at 2.67-2.69 Ga, followed by low-grade metamorphic hydrothermal events at 2.64 Ga with peak temperatures of between

300 ºC and ~220 ºC (Smith et al., 1993; Bleeker et al., 1999). The system has been continuously cooling since that time with estimated temperatures below 100°C for the past two billion years (Li et al., 2016).

The Kidd Creek Deep Fluid and Deep Life Observatory is located at 2.4 km below surface where a series of flowing fracture water systems have been monitored for ten years, making this observatory to our knowledge, the longest time series investigation of subsurface fluids available for the scientific community at such a profound depth. From this location, studies of dissolved gases (Sherwood Lollar et al., 2002; 2006; 2008; Young et al., 2017); noble gas residence times (Holland et al., 2013; Warr et al., 2018; 2019); and habitability studies (Li et al., 2016; Lollar et al., 2019) have all been conducted. In this study, samples were collected and analyzed from the boreholes on the 7850 level (2.4 km below surface) between 2007 and 2014.

Methods

Sample collection and analysis

Mining operations at Kidd Creek involve lateral coring via multiple boreholes at each level as described in detail in Li et al. (2016). Exploration boreholes can intersect pockets of fracture fluids trapped within the host rock which, once pierced, flow for periods of months to years. Flow of the anoxic fracture fluids through the boreholes sampled here has been continuous since their drilling in 2007 - between 10-300 mL/minute depending on the borehole – a factor that also contributes to contamination control as the fractures remain well flushed at all times (Warr et al., 2018; Lollar et al., 2019). In addition, to exclude mine air and possible contamination, at borehole 12299, a sterile stainless steel manifold was installed, analogous to the “CORKs” (Circulation

Obviation Retrofit Kits; Cowen et al., 2003) in deep-sea boreholes. Before installation, the manifold was combusted at 400 °C for 8 hours and autoclaved. Before sampling from the main valve, four side valves were opened to let the fracture water flow under natural high pressures for several minutes. This process flushed out any water that might have been oxygenated during the initial contact with mine air after opening the valve, and also flushed air out of the sterile manifold.

Samples were collected into sterile, sealed serum bottles containing 100 µL saturated $HgCl_2$ solution to prevent any microbial activity in the sampling vials post sample collection (after the methods of Ward et al., 2004; Hunkeler et al., 2008). For dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) samples, fluids were filtered through 0.45-µm filters into glass vials also fixed with $HgCl_2$, leaving no headspace. Water samples for isotopic analysis were collected in 60-mL Nalgene bottles with no remaining headspace. Methods for collection of DOC, DIC, formate, and acetate were previously published in McDermott et al. (2015). All samples were refrigerated at ~4 °C until analysis.

One fracture water sample from Kidd Creek (borehole 12262) was collected to qualitatively test for the presence of volatile methyl sulfides. An evacuated glass serum vial containing $HgCl_2$ (as above) was filled with fracture water, stored in the dark, and analyzed within 4 days of collection. For this sample, 1.0 µL was injected onto an Agilent 6890N GC with a GS-Q column and quadrupole mass spectrometer detector. The oven temperature was held at 35 °C for five minutes then increased at 5 °C per minute to 200 °C and held for 20 minutes.

Temperature and pH were measured with hand-held probes (Hanna Instruments, Woonsocket, RI, USA, or Extech Instruments, Nashua, NH, USA). DOC and DIC were analyzed by the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa (after the methods of St-

Jean, 2003; Lalonde et al., 2014). Reproducibility was ± 20% for DOC and DIC concentrations based on duplicates. Reproducibility of $\delta^{13}C$ values for DIC and DOC was ±2.5‰ with variation likely attributable to high salinity of these samples. Hydrogen and oxygen isotopic compositions of water samples were determined at the Environmental Isotope Laboratory at the University of Waterloo after the methods of Ward et al. (2004). Errors for individual measurements are ±0.2 and ±0.8‰ for $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ respectively (Figure 2).

Quantitative and isotopic analyses acetate and formate were performed on an isotope ratio monitoring liquid chromatography/mass spectrometry system (irm-LC/MS) equipped with a Finnigan^{TM} LC IsoLink at the University of Bremen. The methods of Heuer et al. (2006; 2009) were followed with slight modification. Briefly, the analysis involved separation of compounds by high performance liquid chromatography (ThermoFinnigan Surveyor HPLC) combined with chemical oxidation of the effluents using the ThermoFinnigan LC IsoLink and subsequent online transfer of the resulting CO_2 into an irm-MS (ThermoFinnigan Delta Plus XP). The HPLC system was equipped with a VA 300/7.8 Nucleogel Sugar 810 H column (300 mm length: 7.8 mm i.d.) and a guard column (CC30/4 Nucleogel Sugar 810H: 30 mm length from Macherey-Nagel, which were kept at room temperature. As a mobile phase 135 µL of 85% H_3PO_4 in 500 mL of MilliQ water was used with a flow rate of 300 µL min^{-1}. The sample injection volume via autosampler was 50 µl. The oxidation reagent for the LC IsoLink interface was composed of 6.0 g $Na_2S_2O_8$, 10 ml of 85% H_3PO_4 and 300 ml of MilliQ water, and had a flow rate of 60 µl min^{-1}. The oxidation reactor temperature was set to 99.9°C. Isotope-ratio-monitoring was conducted on a ThermoFinnigan Delta Plus XP, to which the interface was connected with helium as carrier gas. Concentrations were determined from the peak area of mass 44 via a five-point standard calibration curve (0.5-20 mg C/l) with a relative error of ±10%. All $\delta^{13}C$ analyses for acetate and formate were run in at least duplicate and typically triplicate. Mean values are reported here as the reproducibility on replicates was always between 0.1 to 0.5‰. In house isotopic standards of known $\delta^{13}C$ value were run in between every 5-6 samples to ensure accuracy throughout. $\delta^{13}C$ values are reported by comparison to V-PDB with total analytical uncertainties of ± 1‰.

Assessment of Equilibrium Using Chemical Affinity

The equilibrium state of the following reactions:

$$CO_2 + H_2 \rightleftharpoons HCOO^- + H^+ \qquad (1);$$

$$2CO_2 + 4H_2 \rightleftharpoons C_2H_3O_2^- + H^+ + 2H_2O \qquad (2);$$

$$2CO + 4H_2 \rightleftharpoons C_2H_3O_2^- + H^+ \qquad (3)$$

were assessed by calculating the chemistry affinity for reaction (*A*) defined by:

$$A = -\Delta_r G = -RT \ln (Q_r/K_{eq}) \qquad (4)$$

where R is the universal gas constant, T is equal to the measured fluid temperature of 25°C (298.15 Kelvin), Q_r is the activity product, and K_{eq} is the equilibrium constant at 25°C, 1 bar (McDermott et al., 2015). Activities for H_2O, CO_2, formate ion ($HCOO^-$) and acetate ion ($C_2H_3O_2^-$) were determined at 25°C and 1 bar using Geochemist's Workbench software and the Pitzer equation-based Harvie–Møller–Weare activity model (Plummer et al., 1988). It was assumed that all measured total formate ($HCOO^- + HCOOH$) and total acetate ($C_2H_3O_2^- + C_2H_4O_2$) was present as $HCOO^-$ and $C_2H_3O_2^-$ species, respectively, at the near-neutral brine conditions (pH 6.1 to 6.5). The Pitzer approach is necessary to appropriately model the high ionic strength of the fracture waters (Plummer et al., 1988; Pitzer, 1991). These calculations were performed at 1 bar due to a lack of pressure-dependent Pitzer coefficients in the literature; however, at estimated in situ lithostatic pressures of ~500 bar, the change in dissolved species distribution would not contribute significant effects to these calculations. The Pitzer model does not include data for $HCOO^-$ or $C_2H_3O_2^-$, therefore an activity coefficient of 2.4, equal to that of HCO_3^- was used after the method of McDermott et al. (2015), as HCO_3^- has the same ionic charge and similar radius to $HCOO^-$ and $C_2H_3O_2^-$. Values for H_2 gas abundance and fluid pH are previously published (Li et al., 2016 for boreholes 12299, 12287A, and 12262; Holland et al., 2013, Lollar et al., 2019 for borehole 12261). Aqueous H_2 molalities were determined using gas to water flow rate ratios given in these publications. An activity coefficient of 1.6 was used for H_2 based on calculations of the brine activity coefficient for H_2S, a similarly uncharged dissolved gas, in Barrett et al. (1998). Aqueous CO was constrained by equilibrium with OH^- and $HCOO^-$ as described by Abelson (1966) and van Trump and Miller (1973):

$$CO + OH^- \rightleftharpoons HCOO^- \qquad (5).$$

Results

Figure 2 shows the $\delta^{18}O$ and δ^2H values for fracture fluids in this study (open squares), compared to values from the literature for other selected sites on the Canadian Shield. The Kidd Creek service water (SW) samples (black squares) fall along the GMWL (Global Meteoric Water Line), consistent with the fact that mine service waters are of surface origin drawn from a lake near the property, pumped to depth and recirculated for mine operations. The SW values define a slope slightly lower than that of the GMWL, consistent with classic evaporation effects seen for lake waters. The shaded triangle shows where fracture waters would lie if mixing of service waters was occurring in the discharging fracture waters, thus providing a means of ruling out such contamination. Instead all the fracture waters lie well above the GMWL, a feature characteristic of saline waters and brines from deep Precambrian settings throughout the world and attributed to the effects of a variety of low temperature water-rock reactions (WRI) (Frape et al., 1984; Onstott et al., 2006; Li et al., 2016). Notably, these fracture waters are the most elevated above the GMWL seen to date – consistent with the billion to hundreds of millions of year residence times calculated for these fluids from noble gas studies (Holland et al., 2013; Warr et al., 2018) and consistent with the $\delta^{18}O$ and $\delta^{2}H$ measurements in past studies for the system at 2.4 km below surface (Li et al., 2016; Lollar et al., 2019). The large amounts of water that have discharged from these boreholes since the time of drilling completion (estimated to be >1.3x10^6 L, and >4.3x10^5 L from 12299 and 12261 respectively; Lollar et al., 2019) also confirm any contaminating fluid has been flushed out, and hence these fluids represent indigenous uncontaminated groundwater end-members.

Concentrations and $\delta^{13}C$ results for dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and the major components of the DOC (acetate and formate) are provided in Table 1 for four boreholes on the 2.4 km level. Table 2 provides the results for long-term monitoring of fracture water from 12299, demonstrating that the characteristics of this fracture water's geochemistry and isotopic composition are consistent over time within the uncertaintyof the methodology. Results for the organic components of the Kidd Creek fracture waters show several distinctive characteristics compared to most of the systems described in the current literature on DOC, acetate and formate. Total DOC at Kidd Creek is composed primarily of just acetate and formate (Figure 3), making this a very simple organic "soup" compared to other sites reported in the literature, either for marine and lacustrine waters (Figure 4) (Heuer et al., 2006, 2009, 2010), for ocean water and hydrothermal vents (McDermott et al. 2015; Lang et al. 2010; 2018), or for other sites in the Precambrian continental systems (Lin et al., 2005a; 2006; Onstott et al., 2006;

Simkus et al., 2016; Kieft et al., 2018). The fracture waters of Kidd Creek contain high concentrations of both acetate and formate with concentrations from 1200 to 1900 µmol/L, and 480 to 1000 µmol/L, respectively (Tables 1 and 2). Acetate and formate alone account for more than 50% to 90% of the total DOC. This picture of a very simple, acetate and formate dominated DOC load is further supported by Figure 3 that demonstrates the isotopic mass balance, where the $\delta^{13}C$ value of the total bulk DOC is also within uncertainty of the weighted sum of the $\delta^{13}C$ values of acetate and formate.

Concentrations of acetate in the Kidd Creek fracture waters are consistently > 1000 µmol/L, among the highest reported from environmental settings (Figure 4) and in the range of those found at > 500 m depth in sediments at the Blake Ridge (Wellsbury et al., 1997; Egeberg and Barth 1998). Even fracture waters in other Precambrian continental settings such as the gold mines in the Witwatersrand Basin, South Africa show levels of acetate and formate that are an order of magnitude lower than those observed at Kidd Creek. Dissolved acetate concentrations reported for the Witwatersrand gold mines are < 1-437 µmol/L (and typically <100 µmol/L); and <1-88 µmol/L (typically <10 µmol/L), respectively, as reported in Lin et al., 2005a; Onstott et al., 2006; Simkus et al., 2016; Kieft et al., 2018). Intriguingly the Kidd Creek waters also show consistently higher concentrations of acetate (1200 to 1900 µmol/L) compared to formate (480 to 1000 µmol/L). McDermott et al. (2015) reported formate concentrations between 82-669 µmol/L at submarine hydrothermal vents, which they attributed to abiotic production of formate, while suggesting kinetic inhibition might account for the absence of any detectable acetate. Lang et al. (2018) values for the Lost City Hydrothermal field showed typically higher levels of formate (36-158 µmol/L) relative to acetate (1-35 µmol/L) – with both acetate and formate present at levels significantly lower than those found at Kidd Creek.

Discussion:

While the levels of dissolved acetate at Kidd Creek are comparable to those reported for oil field brines in an absolute sense (Carothers and Kharaka, 1978), the Kidd Creek fluids differ significantly from those sedimentary basin brines in the high concentrations of formate (rarely found in oil field waters) and the absence of the suite of related organic acids that typically make up a large component of the organic acid pool in oil field brines (e.g. propionate, butyrate). McCollom and Seewald (2003a) summarized the two major patterns of VFA distribution in fluids studied to that point. One pattern consists of high concentrations of acetate, propionate and butyrate (with little to no formate) in sedimentary basin-hosted oil field waters (Carothers and Kharaka 1978). A different pattern was observed in waters from hydrothermal fluids, pore fluids from serpentinites, and products of hydrous pyrolysis of kerogens, which all show a pattern of formate levels typically greater than associated acetate (Dias et al., 2002a&b). The latter pattern had typically been attributed at one time to preferential acetate decomposition related to sampling artifacts, but McCollom and Seewald (2003a) argued the dominance of formate is in fact a primary characteristic of hydrothermal fluids and serpentinization pore fluids due to the thermodynamic stability of formate under strongly reducing and moderately alkaline pH conditions.

The Kidd Creek fluids may offer a third category of fluids – one where both acetate and formate are both present in high concentrations, but where acetate dominates over formate for all fracture fluids sampled and all time points. The carbon isotope values for acetate in the Kidd Creek fracture water are notable as well as they are among the most ^{13}C-enriched ever reported for natural samples (with a range of -4.9 to -7.3‰) (Tables 1 and 2; Figure 4). While carbon isotopic studies of acetate and formate in what might be termed “deep biosphere” settings are still relatively rare, Lang et al. (2018) published $\delta^{13}C$ values for acetate values in the range of -20.3 to -26.6‰ for the Lost City Hydrothermal field, and the one value to date from the Witwatersrand Basin for acetate is also relatively ^{13}C-depleted (-27.5‰ for KL445; Kieft et al., 2018). Carbon isotope values for formate of -6.8 to -12‰ were invoked by Lang et al. (2018) in support of an abiotic source for formate production at Lost City Hydrothermal field, with this range of values at their sites attributed to the mantle-carbon source at the vents. They explained this range of $\delta^{13}C$ values as a result of mixing between this mantle source of carbon and DIC in seawater, as well as the effects of microbial cycling by sulfate reducers. Such processes of abiotic production of formate, coupled with biological cycling, had been previously suggested by McDermott et al. (2015) for other hydrothermal fields, but the lines of evidence did not include isotopic values for acetate or formate. The range of $\delta^{13}C$ values for formate at Kidd Creek (-9.7 to -17.7‰) are not dissimilar to the Lost City Hydrothermal field – but the absence of any mantle derived input in the tectonically quiescent, ancient cratonic setting of Kidd Creek established by past noble gas studies (Holland et al., 2013; Warr et al, 2018) rules out a mantle-derived component contributing to the carbon source in this setting. At Kidd Creek, the combination of very high concentrations of acetate and formate, predominance of acetate over formate, and the highly ^{13}C enriched values for acetate, are unprecedented in the literature. Deciphering the origin of acetate and formate, and their participation in either a biologic or abiotic carbon cycle, or both, requires integration of information on the geochemical and geologic setting, on conventional biologically driven processes of acetate and formate cycles in the subsurface, and consideration of possible novel processes of abiotic production.

The bulk of the studies on acetate and formate have focused on terrestrial and marine systems within which biogeochemical cycling of these compounds were naturally assumed to be the major controlling processes on the production, formation of the accumulated pool of DOC, and their isotopic compositions. Understanding the relative controls on acetate and formate production and fate typically focuses on production of acetate by microbially mediated processes such as acetogenesis, production via fermentation or decomposition of kerogen or high molecular weight DOC precursors, and consumption via aceticlastic methanogenesis or sulfate reduction (Heuer et al., 2009 and references therein). The complex interplay between these processes requires careful interpretation of potential carbon sources, the balance between possible consumption processes in addition to production pathways, as well as consideration of the effects of extensive recycling of small substrate pools. To a first approximation then, the isotopic composition of acetate and formate in environmental settings is controlled by the relative contribution of production (e.g. fermentation of organic matter; acetogenesis) and consumption reactions (e.g. acetoclastic methanogenesis, sulfate reduction) and the isotopic fractionation associated with these processes.

In most surface environments and subsurface studies to date, production of acetate and formate relates to the associated dissolved organic carbon pool and organic phases present as solids (kerogens) or oil/gas. DOC pools typically have bulk δ^{13}C values in the range of -20 to -30‰, reflecting the dominance of organic material linked to the photosynthetic cycle (Orphan et al., 2001; Lang et al., 2010 and references therein). Table 1 shows that due to the dominance of acetate and formate in the DOC pool, the δ^{13}C values for DOC at Kidd Creek are, like the acetate and formate, remarkably enriched in ^{13}C, with values from -5.1 to -8.9‰. Few studies of DOC have been carried out for deep fracture fluids in Precambrian rocks, but the δ^{13}C of the local carbon-rich rocks of the Proterozoic and Archean rocks of the Canadian Shield have been extensively studied. Carbonaceous metasediments in Precambrian rocks show a wide range of $\delta^{13}C$ values, typically between -15‰ and -35‰ but with some values as depleted as -45‰ (Strauss, 1986; 1989; Schoell and Willmer, 1981; Ventura et al., 2007; 2008; Wellmer et al., 1999). Values for carbonaceaous argillites at Kidd Creek in particular occupy a more narrow range from -19.3 to -26.4‰ (Wellmer et al., 1999). Regardless, assuming as suggested by Lang et al. (2010) that either desorption of or decomposition of organic materials from these rocks to produce DOC would not be associated with isotopic fractionation, then it is unlikely that the ^{13}C-enriched DOC in the Kidd Creek fracture fluids can be derived from the significantly more ^{13}C-depleted carbon characteristic of the organic carbon in the host rocks (Figure 5).

This observation of highly ^{13}C-enriched acetate and formate is unusual even for the relatively under-investigated deep subsurface fracture waters reported to date in the literature. As noted above, the one $\delta^{13}C$ value reported to date for acetate in the gold mines of the Witwatersrand Basin is isotopically depleted (-27.5‰ for 27.9 µmol/L acetate at KL445; Kieft et al., 2018), and consistent with ^{13}C depleted dissolved organic matter in the fluids (ranging from -23.9‰ to -57‰ depending on the sites in the Witwatersrand basin). While Simkus et al. (2016) contained no carbon isotope data for DOM, acetate or formate, it confirms an overall picture for the fracture fluids of the South African gold mines quite different from the observations at Kidd Creek – with a dominance of formate (between 0.4 µmol/L to a maximum of 34 µmol/L) and much lower concentrations of acetate (0.07 to 2.2 µmol/L). Only one sample from South Africa at Kloof mine (KL 445) had acetate as high as a maximum of 27.9 µmol/L (Simkus et al., 2016). An earlier study reported higher values for acetate and formate (typically < 100 µmol/L; and < 10 µmol/L, respectively; Onstott et al., 2006) but as noted provided no carbon isotope measurements.

Elsewhere on the Canadian Shield, at the only two other sites investigated to date for acetate and formate (a set of co-located mines on the Canadian Shield at Thompson Manitoba), Telling et al. (2018) reported concentrations of acetate typically between 53 to 82 μmol/L for fracture waters at Thompson mine (with one sample as high as 496 μmol/L), and a similar range (44.5 to 268.3 μmol/L) for waters from the co-located Birchtree mine. In contrast to the pattern at Kidd Creek, the more conventional $\delta^{13}C$ values for dissolved acetate at the Thompson, Manitoba site (-24.8 to -27.5‰) are quite consistent with $\delta^{13}C$ values determined for high molecular weight carbon characteristic in the local host rocks (Figure 5). At both Thompson and Birchtree, formate levels were below detection.

One common feature of the sites described above, the gold mines of the Witwatersand basin and the Thompson mines, is that unlike Kidd Creek, these sites have been shown to have well–established and relatively active microbial communities. The gold mine sites described in the Witwatersrand basin by Kieft et al. (2018) and Simkus et al (2016) have been previously shown to have well–established microbial ecosystems with metabolisms including autotrophic sulfate reducers, H_2-utlizing methanogens, anaerobic methane oxidizers, and denitrifiers, among others (Ward et al., 2004; Lau et al., 2014, 2016; Magnabosco et al., 2018b). At Thompson, via MPN and cell counts, Telling et al (2018) demonstrated an active microbial population of aerobic H_2 oxidizers, Fe^{3+} reducers, SO_4^{2-} reducers, acetogens, and smaller numbers of acetate or formate-utilizing methanogens, with overall viable cells counts between 10,000 to 50,000 cells/mL. While microbial methanogenesis at most deep subsurface Precambrian sites has been shown to be consistent with methane production via CO_2 reduction from a DIC carbon source (Ward et al., 2004; Sherwood Lollar et al., 2006); Thompson is one of the few sites for which a contribution from aceticlastic methanogenesis is suggested. The evidence of diverse microbial metabolic activities at Thompson may account for the observation that acetate levels here are significantly lower than at Kidd Creek (39-273 µmol/L versus 1292-1921 µmol/L), as are overall DOC concentrations (maximum 727 µmol/L, well below the 2440-4996 µmol/L levels at Kidd Creek). As can be seen in Table 3 and Figure 5, $\delta^{13}C$ values for the DOC and acetate in fracture waters from Thompson support this hypothesis of active biological cycling of acetate, as both $\delta^{13}C$ acetate values (-24.8‰ to -27.5‰) and $\delta^{13}C$ values for DOC (-23.6‰ to – 34.3‰) are quite negative compared to the observations at Kidd Creek. Figure 5 suggests the DOC pool at Thompson is likely derived from local metasediments, based on the similarity of $\delta^{13}C$ values for DOC (-23.6‰ to – 34.3‰) to those of the $\delta^{13}C$ values for the Proterozoic host rocks at this locality (shown in grey on the histogram) (Strauss, 1986). As noted this contrasts with the $\delta^{13}C$ values for DOC and acetate at Kidd Creek, both significantly more enriched than any of the local host rocks sources (Figure 5).

While a recent study demonstrated the presence of extant microbial communities at Kidd Creek, cells counts were in the range of only 10^3-10^4 cells/mL, and the MPN culture based results were negative for methanogens and acetogens (Lollar et al., 2019). While false negatives are inherent in culture-based approaches, nonetheless, in marked contrast to the results from Thompson, the Kidd Creek system to date showed positive results only for sulfate-reducing metabolisms (Lollar et al., 2019), consistent with a previous sulfur isotope study (Li et al., 2016). Similarly a predominantly abiotic methane source was previously identified in the Kidd Creek fracture waters (Sherwood Lollar et al. 2002; 2006; 2008), with only a minor contribution from microbial activity (Young et al., 2017).

Different trends are also apparent in $\delta^{13}C$ values for acetate and DIC for Thompson, versus Kidd Creek (Figure 6). Some of the largest known fractionation effects in acetate biogeochemistry are associated with microbial acetogenesis, whereby DIC is reduced with H_2 to form acetic acid via the reaction (Madigan et al., 2000):

$$4H_2 + H^+ + 2HCO_3^- \rightleftharpoons CH_3COO^- + 4H_2O \qquad (6).$$

Pure cultures of acetogenic microorganisms have been shown to produce acetate depleted in ^{13}C by 39.7‰ to 58.6‰ relative to the inorganic carbon source (Gelwicks et al., 1989). H_2 is present in particularly high concentrations (3-12.7% by volume of the free gas phase; Sherwood Lollar et al., 2014; Lollar et al., 2019) at Kidd Creek. However, ^{13}C offsets between DIC and acetate here are small, with only a few ‰ difference (1.6 to 6.4‰; Table 1; Figure 6), not approaching the large (40-60‰) offsets associated with microbial acetogenesis. While it is possible for extensive acetate consumption to isotopically enrich a vanishingly small remaining acetate pool, the small fractionation factor associated with anaerobic acetate consuming metabolisms such as acetoclastic methanogenesis (Krzycki et al.,1987; Gelwicks et al.,1994; Valentine et al., 2004; Penning et al., 2006; Londry et al., 2008) or sulfate reduction (Londry and Des Marais, 2003) make it unlikely that microbial consumption of the acetate pool could enrich acetate from the levels of isotopic offset between DIC and acetate associated with acetogenesis (40-60‰) to the very small offsets observed in the Kidd Creek samples (Figure 6). Quantitative conversion of the DIC pool via Equation (1) could potentially result in isotopically enriched acetate, but there is no evidence of such reservoir effect at Kidd Creek where the DIC levels (26-61 µM) and DOC levels (2400-5000 µM) are in the same range as those of acetate. Similarly the low temperature (< 100°C) history of the Kidd Creek system for the past two billion years (Li et al., 2016), rules out a ^{13}C-enriched signature in the dissolved acetate due to carbon isotope equilibration with a ^{13}C-enriched DIC pool (Franks et al., 2001).

Processes of degradation and consumption may produce an isotopic enrichment in ^{13}C in a small residual pool of DOC, but as noted above, given the high concentrations of acetate and formate at Kidd Creek, this is not a system in which reservoir effects (extreme δ^{13}C values resulting from close to 100% conversion of the DOC pool) can be invoked to account for the ^{13}C enriched acetate observed at Kidd Creek (Figure 4). If consumption of the pool of acetate were occurring at Kidd Creek, either via aceticlastic methanogenesis, sulfate-reducing bacteria, or via thermal decomposition reactions such as decarboxylation or oxidation, a correlation with the δ^{13}C values of the DIC pool might be expected. Certainly, at Thompson, where aceticlastic methanogens have been identified (Telling et al., 2018), such a correlation is seen (Figure 6). In contrast, no such pattern emerges for the Kidd Creek data.

Alternative generation processes

Thermodynamic modelling and laboratory studies have, for some time, explored the potential for abiotic organic synthesis of acetate and formate (Shock, 1992; Shock and Schulte, 1999; McCollom and Seewald, 2003a&b; 2006). Recently abiotic production of formate has been identified at hydrothermal vents, based on concentrations of formate (36-669 µmol/L), and ^{13}C and ^{14}C evidence (Lang et al., 2010, 2018; McDermott et al 2015). Biologically mediated reactions between DIC and formate was suggested as the required mechanism to drive the system to equilibrium (Lang et al., 2018), as hydrothermal laboratory experiments suggest abiotic equilibration may otherwise be kinetically inhibited at $T < 175^{o}C$ (McCollom and Seewald 2003a, McCollom and Seewald, 2006). Lang et al. (2018) suggested microbial processes play an even larger role in acetate turnover cycle at Lost City, attributing the lower levels of acetate (1-35 μmol/L) found there to microbial cycling and decomposition of higher molecular weight organic matter, based on $\delta^{13}C$ values of -26.6 to -20.3‰ (Lang et al., 2010, 2018). The clear demarcation between near zero Mg^{2+} levels in vents fluids and high concentrations in ocean waters provide conservative mixing model constraints for identifying the end-members and resolving the mixing contributions in oceanic systems.

McCollom and Seewald (2001) observed rapid achievement of metastable equilibration in experiments between dissolved CO_2 and formate at elevated temperatures > 300°C. While such elevated temperatures have not been experienced by the Kidd Creek system in the past 2 billion years (Li et al., 2016); the highly reducing conditions in these fluids might favor formation of formate under equilibrium conditions (McCollom and Seewald, 2003a). To explore this possibility, the affinity for the production of formate in the Kidd Creek fracture waters from H_2 and CO_2 (reaction 1) is calculated here and found to be within 5 kJ/mol of 0 kJ/mol, indicating that reaction 1 is at equilibrium within reasonable error limits for thermodynamic calculation (Figure 7a). This calculation suggests that Kidd Creek fracture waters may be another site where abiotic formate production should be considered, although the demonstration of equilibrium cannot by itself parse abiotic versus biological processes. To date, the small concentrations of acetate (relative to formate) found at the hydrothermal vents meant that while those studies could not rule out an abiotic source for some of the acetate, neither the low concentrations nor the rather negative $\delta^{13}C$ values (Lang et al., 2018) required abiotic production. Rather these studies attributed acetate production to biogeochemical processes. As has been shown at Kidd Creek, the high concentrations and ^{13}C-enriched nature of acetate cannot be similarly attributed to local organic sources, nor to biologically mediated processes. Further the high $\delta^{13}C_{D1C}$ values at Kidd Creek (-

0.5 to -8.8‰) provide a plausible ^{13}C-enriched carbon source for the observed δ^{13}C values of formate. Given the longstanding evidence for abiotic production of methane and high hydrocarbons such as ethane, propane, and butane at this site, the role of a variety of possible abiotic production mechanism for acetate, as well as formate, are considered here.

Acetate has been shown to be produced abiotically at near-neutral pH (6.5), moderate temperature (60°C, 2 bar) conditions in the presence of a greigite (Fe_3S_4) catalyst (Preiner et al., 2020) via the reduction of CO_2 by H_2 in reaction 2. The reduction of CO by H_2 via reaction 3 is an equally plausible pathway for acetate synthesis from an inorganic precursor. Both acetate synthesis reactions have positive affinity at sampled conditions at Kidd Creek (Figure 7b and 7c), demonstrating that the reactions are thermodynamically favorable in the present day. However, the involvement of a catalyst is likely necessary to overcome kinetic inhibitions and form a C-C bond at low temperatures. Greigite is a demonstrated catalyst for acetate synthesis (Preiner et al., 2020) that can be produced by sulfate-reducing bacteria (Picard et al., 2016 and refs. therein), the dominant form of life inhabiting modern-day Kidd Creek borehole fluids (Lollar et al., 2019). Furthermore, the ancient seafloor hydrothermal deposits preserved at Kidd Creek contain ^{34}S-depleted pyrite that typifies pyrite precipitated as a result of bacterial sulfate reduction by chemosynthetic life in hydrothermal mixing zones (δ^{34}S values $\geq$ -4.26‰, Jamieson et al., 2013; Li et al., 2016). The pervasive presence of sulfate reducing bacteria over modern and geologic timescales at Kidd Creek could produce greigite in addition to pyrite, thereby providing possible catalyst to support the abiotic generation of acetate via reactions 2 and 3.

In other studies, acetate has also been shown to be produced abiotically under reducing conditions from methanthiol and CO via the reaction (Huber and Wächtershäuser, 1997):

$$CH_3SH + CO + H_2O \rightleftharpoons CH_3COOH + H_2S \qquad (7).$$

Heinen and Lauwers (1996) demonstrated CO_2 reduction in the presence of an iron sulfide catalyst to a series of alkythiols – possible precursors for the reactions described by Huber and Wächtershäuser (1997). A pilot experiment for the Kidd Creek fluids showed small amounts of multiple sulfides including dimethyl sulfide and diethyl sulfide in the headspace of waters collected from Kidd Creek mine (Fig. 8). These compounds could be used as starting materials in a Huber and Wächtershäuser (1997) type reaction leading to various organic acids via a generalized reaction such as:

$$R_1\text{-}S\text{-}R_2 + CO + H_2O \rightleftharpoons R_1\text{-}COOH + HSR_2 \qquad (8);$$

where R_1 and R_2 are hydrogen or various alkyl moieties. CO would also be expected to be in equilibrium with formate through reaction (5).

To date, no experiments have been carried out to understand the isotopic fractionation factors associated with these processes of abiotic organic synthesis, making it a challenge to quantitatively evaluate their potential contribution to fluid systems such as Kidd Creek or the hydrothermal vents. The increasing number of sites globally at which such processes are being investigated in the light of abiotic organic synthesis argues for the importance of such future experiments to better constrain specific mechanisms of abiotic organic synthesis producing acetate and formate in the deep subsurface.

Radiolysis and the deep carbon cycle

While the presence of high (up to mM) levels of dissolved H_2 has long been expected in geologic settings dominated by ultramafic rocks due to serpentinization; investigation of H_2

production from water-rock reactions in other geologic settings including the South Pacific Gyre (D'Hondt et al. 2009; Dzaugis et al., 2016) and the more felsic sites in South Africa (Lin et al., 2005a, 2006) first brought the importance of radiolytically driven reactions to the fore. Briefly, radiogenic reactions are driven by α, β, γ particles produced by the decay of elements such as U, Th and K. Empirical studies and models have calculated the net production of H_2 (from radiolytic decomposition of water), and of radiogenic ^{4}He, ^{40}Ar in these crystalline rock (Sherwood Lollar et al., 2014; Warr et al., 2019). The dominance of these radiogenic reactions defines what we have termed a "hidden hydrogeosphere" where, in deep low-permeability regions of the crust at kilometers below surface, the products of the radiogenic reactions accumulate to levels that were completely unforeseen (Lippmann-Pipke et al, 2011; Holland et al., 2013; Warr et al., 2018). The deep hydrogeologically isolated fractures of these Precambrian Shield environments have now been shown to be as H_2 rich as the hydrothermal vents (Sherwood Lollar et al., 2014) and to host novel radiogenic noble signatures (Lippmann-Pipke et al., 2011; Kendrick et al. 2011; Holland et al., 2013) that are the highest ever recorded in free fluids on Earth. The documentation of, for instance ^{40}Ar/^{36}Ar values > 100,000 (Warr et al., 2018) highlights the fact that these Kidd Creek observatory fracture fluids provide an ideal environment to investigate the importance of radiolytic water-rock reactions.

The role of radiolysis as a key process controlling the habitability of the deep subsurface was further emphasized by studies that examined not only the production of the conservative noble gases, and H_2 as an important electron donor, but explored the source of a major electron acceptor, dissolved SO_4^{2-}, in the Kidd Creek system (Li et al., 2016). Radiolytic decomposition of H_2O to form the mM concentrations of H_2 found in these waters must necessarily produce a stoichiometric equivalent of oxidants, but their rapid consumption in these highly anoxic fluids meant empirical confirmation of the oxidant pool had been lacking in most cases. The presence of an Archean mass independent sulfur isotope signature in the dissolved SO_4^{2-} in the Kidd Creek fracture fluids (Li et al., 2016), and supporting experimental studies (Lefticariu et al. 2010), demonstrated that radiolysis was responsible not only for production of H_2 but of dissolved SO_4^{2-}. At the contact between the mineral surfaces and fracture water, reaction between sulfides and dissolved oxidants (e.g., $OH^{\bullet}$ and/or H_2O_2) resulting from dissociation of water by energy released from radiogenic decay of U, Th and K in the wall rocks oxidizes sulfide minerals and transfers SO_4^{2-} to the fluids in a process described as IROP (indirect radiolytic oxidation of pyrite) (Li et al., 2016). Radiolysis has hence been shown to be a major driver of the subsurface H and S cycle in the deep hydrosphere (Figure 9), but the role of this novel process in the deep carbon cycle has been neglected to date.

Recent investigations of radiolytic physical chemistry provide plausible mechanisms to extend our understanding of radiolytic-generated habitability to the carbon cycle. Detailed experimental and modelling studies that integrate natural sedimentary and mineral phases, and the implications for the rate of H_2 generation, have only just been undertaken (Dzaugis et al., 2015; 2016; Sauvage et al., 2019). To our knowledge to date, no studies on radiolytically generated habitability at deep subsurface field sites have considered the effect of carbonate mineral phases. This issue however has been a feature of physical chemistry experiments conducted in the context of nuclear waste disposal. Costagliola et al. (2017) in particular demonstrated that the presence of calcite mineral phases has a profound impact on the rates and product distributions of radiolysis of water under the effect of α and γ particles. Experiments at different dose rates demonstrated that biphasic calcite-water systems generated a higher yield of H_2, but less $OH\cdot$ (due to the scavenging of the radicals by carbonate ions), but most significantly, the generation of significant concentrations of acetate and formate. A number of recent studies have estimated radiolytic yields of H_2 for Precambrian hosted systems for both South Africa and Canada based on both modelling and empirical approaches (Lin et a., 2005a&b; Onstott et al., 2006; Sherwood Lollar et al., 2014; Warr et al., 2018) – with estimates ranging from 0.1 to 1 nM/year (Lin et al., 2005b). Since Costagliola et al. (2017) demonstrates organic acid formation by recombination of carbonate radicals with the products of radiolysis of water, applying a similar radiolytic production rate (0.1 nM/year, to be conservative) to acetate and formate could account for concentrations of 0.1-1 mM in fracture fluids with fluid residence times on the order of 1-10 Ma; and concentrations of 100 mM for fracture fluids with billion year residence times such as the Kidd Creek fluids. Observed concentrations should be certainly < 1mM and < 100 mM respectively, due to loss to chemical and biological sinks which are difficult to quantify. While radiolytically produced H_2 is not quantitatively converted to acetate and formate, and observed concentrations of H_2, acetate and formate are the net result of both production and loss, it is nonetheless intriguing that such a first order estimate of acetate and formate production via radiolysis is consistent with the differences in observed concentrations of these organic compounds in the fracture fluids of the Witwatersrand Basin (typically < 110 µmol/L; Onstott et al., 2006; Kieft et al., 2018) with residence times between 1-20 Ma; and the very high concentrations of 1680-2750 µmol/L observed in the Kidd Creek fluids with much longer residence times. Details of the radiolytic mechanisms and production rates as a function of α, β and γ radiation require elucidation to evaluate the degree to which such reactions may reflect thermodynamic or metastable equilibrium conditions. Further, isotopic experiments should be carried out to determine the stable isotope fractionation associated with proposed radiolytic processes of acetate and formate production. Nonetheless these results suggest that radiolytic processes might provide a plausible mechanism to produce the extremely high concentrations of acetate and formate found in the Kidd Creek fluids, and their remarkable ^{13}C-enriched signatures (Figure 9).

Water-rock reactions have been shown to provide mechanisms for subsurface ecosystems to be sustained on long time scales even in the absence of energy from the surface photosynthetic cycle (Lin et al., 2006; Chivian et al., 2008; Magnabosco et al., 2018b). Water-rock reactions such as radiolysis have been shown to provide the mechanism for a long-term source of a major electron acceptor (sulfate) for a major metabolism (microbial sulfate reduction) in many deep subsurface ecosystems. Regeneration of electron acceptors in the presence of reactions that produce electron donors such as H_2 implies that such subsurface ecosystems could exist over long timescales even if isolated from the surface. While some chemosynthesis driven ecosystems have been shown to derive their carbon source from the dissolved inorganic carbon associated with the fractures waters (Ward et al., 2006; Simkus et al., 2016; Kieft et al., 2018), the details of the carbon sources available to subsurface microbial communities and the interplay of abiotic chemistry and biological reactions remains in many cases unresolved (McDermott et al., 2015; Lang et al., 2018). Abiogenic hydrocarbon production is documented at both the Kidd Creek mine (Sherwood Lollar et al., 2002; Sherwood Lollar et al., 2008) and other deep subsurface systems (Sherwood Lollar et al., 1993b; Onstott et al., 2006; Proskurowski et al., 2008; McDermott et al., 2015) in both the marine and continental lithosphere, but the role of abiotic production of other low molecular weight hydrocarbons, in particular the volatile fatty acids has been much less well understood. Here we highlight the valuable window into abiotic organic synthesis available in the hydrogeologically isolated deep subsurface fracture fluids of the Precambrian continental lithosphere and discuss a variety of potential reactions that could account for the unusually elevated concentrations and profoundly ^{13}C-enriched nature of the acetate and formate in these saline fracture fluids. Discussions of habitability typically focus on the necessity for fluid mixing and/or spatial geochemical gradients, but this investigation suggests even thermally and spatially "stagnant" systems may still be habitable through radiolysis.

Conclusions

This study demonstrates that geochemical (abiotic) processes of water-rock reaction may be able to sustain not only a deep subsurface H and S cycle essential to sustaining planetary habitability in the absence of photosynthesis, but that processes of water-rock reaction may also sustain an abiotic carbon cycle capable of bridging the abiotic-biotic interface by generation of simple organic acids from calcite. This study points out that a radiolytically driven H, S and C deep cycle provides a mechanism for sustaining deep subsurface microorganisms in the absence of interaction with the surface photosphere, and suggests a model for planetary habitability capable of sustaining chemolithotrophic life on planets or moons where photosynthesis may never have arisen. These findings further highlight the need for an expanded effort to characterize the mechanisms, rates, and isotopic fractionation effects associated with abiotic organic synthesis of acetate and formate in order to quantitatively assess the details of the abiotic production mechanisms involved.

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Research Data Statement – all data are provided in the tables herein in numerical format, or where previously published, the original publications are provided and referenced for easy access. All methods are described in the Methods section and again any relevant previous publications referenced**.** Any calculations have all equations and inputs provided. No software, code, models, or algorithms are involved in this paper. All data in this submission are public and readily available with this submission.

List of Figures:

Figure 1. Map of Kidd Creek in Timmins, Ontario, Canada in the Superior Province of the Canadian Shield in crystalline rocks of ~2.7 billion year age (modified after Warr et al., 2018). Latitude and Longitude of Kidd Creek Observatory, located at 2.4 km depth below surface is 48° 41' 24" North, 81° 22' 0" West.

Figure 2: The $\delta^{18}O$ and $\delta^{2}H$ signatures for fracture waters from this study (open blue squares), and for service water sourced from a local surface lake (black filled squares), plotted with respect to the Global Meteoric Water Line (GMWL) (modified after Lollar et al., 2019). Shaded area shows where samples would fall if contamination due to mixing with service waters were occurring. For example, systems at other locations on the Canadian Shield (e.g. Yellowknife and Thompson) are shown where hatched lines show approximate mixing lines between service waters (on GMWL) and fracture waters (data from Frape et al., 1984). Error bars are smaller than the plotted symbols in all cases.

Figure 3. Concentrations (in µmol/L) and $\delta^{13}C$ values for DOC, acetate and formate demonstrating isotopic mass balance and confirming the dissolved organic carbon in the fluids is almost entirely accounted for by acetate and formate. For all 4 fracture fluids the acetate/formate ratio is remarkably consistent (1.6 to 2.7).

Figure 4. Spectrum of observed $\delta^{13}C$ values for dissolved acetate from marine porewaters (adapted after Heuer et al., 2006) compared to data for Kidd Creek from this study (circles). Kidd Creek fracture fluids among the highest concentrations measured to date for which $\delta^{13}C$ values have been

reported and show the unprecedented ^{13}C-enriched values compared to the known literature. In contrast concentrations and δ^{13}C values for acetate for Thompson, Manitoba (blue squares) fall within ranges typically observed for acetate in marine sediments (see text). High acetate concentrations (100-17,000 µM) found at > 500 m depth in sediments at the Blake Ridge were not isotopically characterized (Wellsbury et al., 1997; Egeberg and Barth 1998) and hence are not plotted here.

Figure 5. Distribution of published δ^{13}C values for solid carbon phases in metasedimentary units from the Canadian Shield (Strauss, 1986; 1989; Schoell and Wellmer, 1981; Wellmer et al., 1999; Ventura et al., 2007) compared to the δ^{13}C values for fracture fluids from Kidd Creek (Table 1, this study) and Thompson, Manitoba (Table 3, this study).

Figure 6. Isotopic co-variation between δ^{13}C values of dissolved organic carbon (DIC) and acetate for Kidd Creek (Table 1, this study) and Thompson, Manitoba (Table 3, this study). Trends can be obscured by the admittedly limited sub-set. Correlation coefficient (r^2) is 0.4 for the Thompson data.

Figure 7. Thermodynamic equilibrium predictions for abiotic generation of formate via CO_2 reduction with H_2 (a. reaction 1); and of acetate via reactions 2 and 3 (b-c, respectively) in Kidd Creek fluids.

Figure 8. Chromatogram of volatile organic sulfides observed in Kidd Creek fracture fluid 12262. Some of the identified compounds (including dimethyl sulfide, methyl thio ethane, diethyl sulfide, methyl thio propane and dimethyl disulfide could be precursors for acetate formation following a pathway similar to that described by Huber Wachtershauser (1997).

Figure 9. Schematic showing the production of H_2 and SO_4^{2-} from radiolysis and indirect radioltic oxidation of pyrite, respectively, both previously documented by Lin et al., 2005a&b; Sherwood Lollar et al. (2014) and Li et al. (2016). Potential abiotic production of acetate and formate due to the effects of radiolysis and indirect radiolytic decomposition of sulfide minerals and calcium carbonate in fracture minerals due to α, β, and γ radiation resulting from U, Th K radiogenic reactions in these high rock-water ratio environments is shown in this schematic (see text).

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