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# Unravelling abiogenic and biogenic sources of methane in the Earth's deep subsurface

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## Abstract

At four underground sites in Precambrian Shield rocks in Canada and South Africa, hydrocarbon and hydrogen gases exsolving from saline fracture waters are analyzed for compositional and isotopic signatures. Dominated by reduced gases such as $CH_4$, $H_2$ and higher hydrocarbons (ethane, propane, butane), the most $^{13}C$-enriched methane end-members at all four sites show a pattern of carbon and hydrogen isotopic values similar to abiogenic gases produced by water–rock interaction that have been identified previously at one site on the Precambrian Shield in Canada. The abiogenic nature of these gases was not previously recognized due to mixing with a second methane component produced by microbial processes. The microbial methane end-member is identified based on carbon and hydrogen isotopic signatures, and DNA gene amplification (PCR) data that indicate the presence of methanogens. A framework is presented to estimate the relative contribution of abiogenic versus microbial hydrocarbon gases at these sites. This approach has important implications for evaluation of potential abiogenic hydrocarbon reservoirs in a wide range of geologic settings, including the longstanding controversy concerning the possible contribution of abiogenic gases to economic petroleum hydrocarbon reservoirs. The association of high concentrations of $H_2$ with $^{13}C$-enriched $CH_4$ end-members, and $H_2$ depletion in the $^{13}C$-depleted methanogenic end-members further suggests the possibility that abiogenic gases may support $H_2$ autotrophy linked to methanogenesis in the deep subsurface.
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_Keywords:_ Abiogenic; Methane; Hydrogen; Autotrophy; Deep biosphere; Mars

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## 1. Introduction

Recent reports of $CH_4$ in the Mars atmosphere focussed scientific and public attention on possible geological and biological sources of these hydrocarbons (Formisano et al., 2004; Kerr, 2004). Resolving the question of the origin of atmospheric $CH_4$ on Mars is even more challenging given that distinguishing abiogenic versus biogenic sources of $CH_4$ in the terrestrial subsurface is still controversial (Gold, 1979; Kenney et al., 2002; Shock, 1995). Deep subsurface fluids in Precambrian Shield rocks have been shown to be dominated by reduced gases such as $CH_4$ and locally, high concentrations of $H_2$ (up to 30% by volume) (Sherwood Lollar et al., 1993a,b). Sherwood Lollar et al. (2002) used stable isotope signatures to suggest that $CH_4$ and higher hydrocarbon gases (ethane, propane and butane) at Kidd Creek mine on the Canadian Shield are produced abiogenically by water–rock interaction such as surface-catalysed polymerization (Anderson, 1984; Foustoukos and Seyfried, 2004); metamorphism of graphite–carbonate bearing rocks (Giardini and Salotti, 1969; Holloway, 1984; Kenney et al., 2002); and other gas–water–rock alteration reactions such as serpentinization (Berndt et al., 1996; Charlou and Donval, 1993; Horita and Berndt, 1999; Kelley et al., 2001, 2005; McCollom and Seewald, 2001; Vanko and Stakes, 1991). In this paper, data from 4 new sites in Canada and South Africa suggests that abiogenic hydrocarbon gases are more globally pervasive than has been understood previously. This is due to the fact that at many sites, the distinct abiogenic signature of such hydrocarbons is obscured by mixing with microbial $CH_4$. Based on this data, we present a model for identification of abiogenic hydrocarbons through the resolution of microbial and abiogenic mixing — an approach that is applicable to a wide variety of crustal settings where potential abiogenic hydrocarbon reserves have been suggested (Gold, 1979; Kenney et al., 2002; Shock, 1995).

## 2. Geological setting and samples

In gold and base-metal mines throughout the Precambrian Shield rocks of Canada, Finland and South Africa, flammable gases discharge from fractures and exploration boreholes (Nurmi and Kukkonen, 1986; Lahermo and Lampen, 1987; Cook, 1998; Sherwood Lollar et al., 1993a,b). Originally dissolved in saline groundwater (TDS levels range from several 1000 to tens of 1000s of ppm) in sealed fracture systems in the rocks, gases are released via depressurization into mine

workings at rates of 1 to >30 L gas/min/borehole (Sherwood Lollar et al., 1993a,b). The groundwaters are typically NaCaCl-rich or CaNaCl-rich with low levels of $SO_4$ (typically <15 ppm and always <100 ppm) with pH levels between 7–9. This study focuses on two sites in Canada (Kidd Creek and Copper Cliff South mines) and three in South Africa (Driefontein, Kloof and Mponeng mines). Kidd Creek mine, situated in the southern volcanic zone of the Abitibi greenstone belt (approximately 2700 Ma), 24 km N of Timmins, Ontario is one of the world's largest volcanogenic massive sulfide deposits. All samples were collected from 2–2.1 kmbls (kilometers below land surface). The Cu–Ni ore at Copper Cliff South mine (CCS) in Sudbury, Ontario is associated with a quartz diorite dike offset of the Sudbury Igneous Complex — a micropegmatite, norite and quartz diorite irruptive emplaced approximately 1840 Ma (Cochrane, 1984) and samples were from approximately 1.3 kmbls. In South Africa, Kloof, Driefontein and Mponeng mines are all located west of Johannesburg in the Witwatersrand Basin — a large Archean intracratonic basin composed of volcanosedimentary sequences that unconformably overlie 3.0 Ga granitic basement. The basin is divided chronologically into quartzite, shale and minor volcanic strata of the Witwatersrand Supergroup (2900 Ma), the basaltic lava sequence of the Ventersdorp Supergroup (2700 Ma) and the overlying clastic and dolomitic sediments of the Transvaal Supergroup (2400–2500 Ma) (Coward et al., 1995). Almost all the samples in this study are from the Ventersdorp Supergroup and are located between 2.7 and 3.4 kmbls. The exceptions are borehole DR938H3, which while drilled in the Ventersdorp, intersects the underlying Witwatersrand Supergroup at approximately half its 750 m length, and DR9IPC, which is drilled in the overlying Transvaal Supergroup and is located at 0.9 kmbls.

## 3. Methodology

### 3.1. Sampling methods

All gas and fracture water samples were collected at the borehole collar after the method of Sherwood Lollar et al. (2002) and Ward et al. (2004). A packer was placed into the opening of the borehole and sealed to the inner rock walls below water level to seal the borehole from the mine air and minimize air contamination. Gas and water were allowed to flow through the apparatus long enough to displace any air remaining in the borehole or the apparatus before sampling. Plastic tubing was attached to the end of the packer and the

flow of gas and/or water from the borehole was directed into an inverted graduated funnel. Gases collected in the inverted funnel were transferred directly into evacuated vials through a needle that was attached to the top of the funnel. The gas sampling vials were pre-evacuated 130 ml borosilicate vials sealed with butyl blue rubber stoppers prepared after the method of Oremland and Des Marais (1983). Vials were pre-fixed with 50 μl of a saturated $HgCl_2$ solution to kill any microbes contained in the sample so microbial activity post-sampling would not alter the gas composition and isotopic signatures. Previous studies, comparing the isotopic values of gases taken at the borehole collars to values determined for gases in solution at depth in the same boreholes, showed that exsolution of the hydrocarbon gases from solution does not alter their isotopic signatures (Sherwood Lollar et al., 1993a,b, 1994).

For the South African sites, the packer was sterilized by autoclaving before insertion into the borehole in order to reduce the possibility of introducing surface microorganisms by that means. In mining environments, aseptic drilling techniques were not possible. Microbial populations were characterized and compared however between the saline fracture waters from these sites, the low salinity mine service water, and ventilation air. Comparison of 16S rRNA gene clone libraries from these possible sources of contamination and from the fracture waters yielded no commonalities (Kieft et al., 1999; Onstott et al., 2003; Takai et al., 2001). In addition the fact that dissimilatory sulphate reductase (DSR) and 16S ribosomal (rRNA) genes amplified from the fracture water DNA yield sequences that are most similar to those reported for sulphate-reducing bacteria isolated from other subsurface sites is consistent with the fracture water microbial populations being in situ subsurface communities and not surface contaminants (Baker et al., 2003).

### 3.2. Compositional gas analysis

Compositional analyses of gas samples were performed at the Stable Isotope Laboratory at the University of Toronto. A Varian 3400 GC equipped with a flame ionization detector (FID) was used to determine concentrations of $CH_4$, $C_2H_6$, $C_3H_8$ and $C_4H_{10}$. The hydrocarbons were separated on a J and W Scientific GS-Q column (30 m × 0.32 mm ID) with a helium gas flow and temperature program: initial 60 °C hold 2.5 min, increase to 120 °C at 5 °C/min. A Varian 3800 GC equipped with a micro-thermal conductivity detector (μTCD) and a Varian Molecular Sieve 5A PLOT column (25 m × 0.53 mm ID) was used to determine concentrations of the inorganic gas components ($H_2$, He, Ar, $O_2$, $CO_2$ and $N_2$). To determine concentrations of Ar, $O_2$ and $N_2$ the helium gas flow rate was 3 ml/min and the temperature program was: initial 30 °C hold 6 min, increase to 80 °C at 15 °C/min, hold 4 min. To determine $CO_2$ concentrations, the helium gas flow rate was 50 ml/min and temperature program was: initial 60 °C, increase to 250 °C at 20 °C/min, hold 6 min. To determine concentrations of $H_2$ and He, the argon carrier gas flow rate was 2 ml/min and temperature program was: initial 10 °C hold 10 min, increase to 80 °C at 25 °C/min, hold 7 min. All analyses were run in triplicate and mean values are reported in Table 1. Reproducibility for triplicate analyses was ± 5%.

### 3.3. Isotopic analysis

Stable carbon and hydrogen isotopic analysis for all hydrocarbons were performed at the University of Toronto (Table 2). Analyses for $\delta^{13}C$ values were performed by continuous flow compound specific carbon isotope ratio mass spectrometry with a Finnigan MAT 252 mass spectrometer interfaced with a Varian 3400 capillary GC. Hydrocarbons were separated by a Poraplot $Q^{TM}$ column (25 m × 0.32 mm ID) with temperature program: initial 40 °C hold 1 min, increase to 190 °C at 5 °C/min, hold 5 min. To separate $CO_2$ from $CH_4$ the program was started at 10 °C (hold 2 min) increased to 190 °C at 5 °C/min (hold 5 min). Total error incorporating both accuracy and reproducibility is ± 0.5‰ with respect to V-PDB standard.

The $\delta^2H$ analysis was performed on a continuous flow compound specific hydrogen isotope mass spectrometer which consists of an HP 6890 gas chromatograph (GC) interfaced with a micropyrolysis furnace (1465 °C) in line with a Finnigan MAT Delta$^+$-XL isotope ratio mass spectrometer. The hydrocarbon gases were separated on a Poraplot $Q^{TM}$ column (25 m × 0.32 mm ID) with a helium carrier at 2.2 ml/min and temperature program: initial 35 °C hold 3 min, increase to 180 °C at 15 °C/min. Total error incorporating both accuracy and reproducibility is ± 5‰ with respect to V-SMOW.

### 3.4. DNA gene amplification (PCR)

Methyl coenzyme M reductase α-subunit genes (*mcrA*) were amplified from community DNA extracts according to the methods of Hales et al. (1996). Archaeal 16S rRNA gene was amplified using a nested PCR reaction in which PCR product generated using the primers 21F (Moyer et al., 1998), and 1492R (Rey-

Table 1
Gas compositional data (in %)

| Site | Borehole No. | Ar | $H_2$ | He | $O_2$ | $N_2$ | $CH_4$ | $C_2H_6$ | $C_3H_8$ | $C_4H_{10}$ |
|------|--------------|-----|-------|------|-------|-------|--------|----------|----------|-------------|
| Copper Cliff | CCS4577 | 0.06 | 54.0 | 3.46 | 0.11 | 1.92 | 34.4 | 5.31 | 0.49 | 0.11 |
| Copper Cliff | CCS4547 | 0.07 | 43.0 | 3.38 | 0.12 | 2.20 | 38.1 | 5.28 | 0.56 | 0.12 |
| Copper Cliff | CCS4546 | 0.17 | 9.94 | 4.37 | 0.52 | 6.38 | 69.5 | 7.14 | 0.78 | 0.17 |
| Copper Cliff | CCS4572 | 0.05 | 57.8 | 2.62 | 0.15 | 1.72 | 25.3 | 4.16 | 0.38 | 0.09 |
| Copper Cliff | CCS4880 | 0.27 | 19.7 | 6.42 | 0.10 | 4.54 | 59.3 | 7.39 | 1.11 | 0.29 |
| Mponeng | MPA | NA | 3.30 | 9.06 | 4.77 | 28.1 | 53.8 | 2.47 | 0.36 | 0.08 |
| Mponeng | MP104 | NA | 11.5 | 12.3 | 3.65 | 21.1 | 49.6 | 3.99 | 0.60 | 0.13 |
| Driefontein | DR548 | NA | 10.3 | 3.05 | 6.18 | 23.6 | 50.7 | 3.85 | 0.52 | 0.11 |
| Driefontein | DR938H1 | NA | <0.01 | 0.90 | 16.5 | 72.5 | 5.45 | 0.14 | 0.02 | <0.01 |
| Driefontein | DR938H3-0m | NA | 0.74 | 5.98 | 0.55 | 14.0 | 76.0 | 3.15 | 0.32 | 0.06 |
| Driefontein | DR938H3'-0m* | NA | 0.32 | 4.64 | 4.95 | 28.6 | 61.4 | 2.45 | 0.26 | 0.04 |
| Driefontein | DR938H3-648 m | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Driefontein | DR938CH1 | NA | <0.05 | 0.21 | 23.4 | 89.9 | 1.19 | <0.05 | <0.05 | <0.05 |
| Driefontein | DR9IPC | NA | <0.05 | 2.75 | 3.77 | 81.9 | 11.6 | <0.05 | <0.05 | <0.05 |
| Kloof | KL1GH | NA | 9.15 | 3.42 | 14.5 | 56.4 | 10.7 | 0.49 | <0.01 | <0.01 |
| Kloof | KL441H3 | NA | <0.01 | 15.9 | 0.43 | 45.1 | 33.0 | 0.66 | 0.07 | 0.02 |
| Kloof | KL739 | NA | 9.25 | 13.5 | <0.04 | 7.72 | 64.9 | 2.86 | 0.41 | 0.08 |
| Kloof | KL441H2 | 1.68 | <0.01 | 15.6 | 6.55 | 61.9 | 23.4 | 0.51 | 0.05 | <0.01 |
| Kloof | KL443HWND1 | 1.39 | <0.01 | 18.2 | 1.24 | 13.3 | 57.3 | 2.03 | 0.28 | 0.05 |
| Kloof | KL443HWDN | 1.79 | <0.01 | 20.8 | 0.97 | 16.8 | 53.4 | 2.04 | 0.21 | 0.03 |

a. All $CO_2$ was below detection limit.

b. NA — not analyzed.

c. $C_4H_{10}$ incorporates both *n*-butane and *iso*-butane.

d. *DR938H3'-0 m is a sample from the same borehole as DR938H3-0 m taken 8 months afterwards. Such temporal sampling was not possible for the other boreholes due to the common practice of sealing them immediately after completion of drilling.

e. DR938H3-0 m and DR938H3-648 m were collected from the same borehole on the same day. The 0 m sample was taken at the borehole collar while the 648 m sample was taken at 648 m depth.

senbach et al., 2000) was used as template in PCR reactions with the "nested" primers 21F and 958R (Moyer et al., 1998). *McrA* and archaeal 16S rRNA gene PCR products were cloned using Invitrogen TOPO kits. For each library, 48 clones were screened by restriction fragment length polymorphism. Unique clones were sequenced, then analyzed using BLAST (Altschul et al., 1997) to identify sequences in the NCBI Genbank database having high similarity (Table 3). Sequence identities were calculated using BioEdit.

## 4. Results and discussion

### 4.1. Isotopic patterns suggest an abiogenic origin

Mantle-derived abiogenic hydrocarbons are typically identified based on three criteria: a $\delta^{13}C$ value for $CH_4$ more enriched than $-25‰$; a "carbon isotopic reversal" trend of increasing isotopic depletion in $^{13}C$ with increasing molecular weight for $CH_4$–ethane–propane–butane; and a $^3He/^4He$ ratio indicative of mantle-derived helium ($R/Ra > 0.1$) (Jenden et al., 1993). Based on these criteria, coal bed or thermogenic hydrocarbon gases with anomalously enriched $\delta^{13}C$ values and/or anomalously depleted $\delta^2H$ values for methane could be

distinguished from mantle-derived abiogenic gases (Jenden et al., 1988; Jenden and Kaplan, 1989; Poreda et al., 1986, 1988; Lyon and Hulston, 1984; Rigby and Smith, 1981). Over the past decade however, there has been a growing body of literature that indicates that not all abiogenic gases are mantle-derived. A variety of low temperature water–rock interactions have been shown to produce both $CH_4$ and higher hydrocarbons such as ethane, propane, and butane — including surface-catalyzed polymerization from reduction of CO or $CO_2$ in a Fischer–Tropsch synthesis; heating or metamorphism of graphite or carbonate bearing rocks; or other vapor–water–rock alteration reactions such as serpentinization (Holloway, 1984; Yuen et al., 1990; Berndt et al., 1996; Hu et al., 1998; Horita and Berndt, 1999; McCollom and Seewald, 2001; Foustoukos and Seyfried, 2004). Significantly, several of these studies have demonstrated that production of abiogenic $CH_4$ by these water–rock interactions can result in $\delta^{13}C$ values as depleted as $-57‰$ (Yuen et al., 1990; Hu et al., 1998; Horita and Berndt, 1999). In a crustal-dominated geologic setting, these processes will produce abiogenic hydrocarbons whose $\delta^{13}C$ values reflect local crustal carbon sources and will not have either $^{13}C$-enriched $\delta^{13}C$ values or $R/Ra$ values >0.1. In addition, it has

Table 2
Carbon and hydrogen isotopic values (in ‰) for $CH_4$–$C_4$ hydrocarbon gases

| Site | Borehole no. | $\delta^{13}C_{CH_4}$ | $\delta^2H_{CH_4}$ | $\delta^{13}C_{C_2}$ | $\delta^2H_{C_2}$ | $\delta^{13}C_{C_3}$ | $\delta^2H_{C_3}$ | $\delta^{13}C_{nC_4}$ | $\delta^2H_{nC_4}$ |
|---|---|---|---|---|---|---|---|---|---|
| Copper Cliff | CCS4577 | −36.1 | −413 | −24.6 | −266 | ND | ND | ND | ND |
| Copper Cliff | CCS4547 | −34.4 | −424 | −28.1 | −290 | ND | ND | ND | ND |
| Copper Cliff | CCS4546 | −32.0 | −452 | −34.6 | −348 | −33.4 | ND | ND | ND |
| Copper Cliff | CCS4572 | −36.7 | −401 | −24.4 | −269 | ND | ND | ND | ND |
| Copper Cliff | CCS4880 | −37.3 | −372 | −31.6 | −301 | ND | ND | ND | ND |
| Mponeng | MPA | −27.8 | −349 | −35.0 | −267 | −33.3 | −212 | ND | ND |
| Mponeng | MP104 | −32.8 | −366 | −37.6 | −270 | −34.8 | −193 | −35.3 | −88 |
| Driefontein | DR548 | −46.5 | −403 | −50.5 | −285 | −47.2 | −192 | −45.6 | −72 |
| Driefontein | DR938H1 | −53.6 | −295 | −42.9 | −326 | −42.3 | ND | ND | ND |
| Driefontein | DR938H3-0 m | −40.2 | −368 | −43.1 | −290 | −42.0 | ND | ND | ND |
| Driefontein | DR938H3′-0 m* | −42.3 | −369 | ND | ND | ND | ND | ND | ND |
| Driefontein | DR938H3-648 m | −40.9 | −371 | −44.0 | −234 | −42.9 | −179 | ND | ND |
| Dreifontein | DR938CH1 | −47.5 | −329 | −43.2 | ND | ND | ND | ND | ND |
| Driefontein | DR9IPC | −55.5 | −218 | ND | ND | ND | ND | ND | ND |
| Kloof | KL1GH | −29.2 | −312 | −33.2 | −230 | ND | ND | ND | ND |
| Kloof | KL441H3 | −40.0 | −253 | −30.9 | −241 | −25.2 | ND | ND | ND |
| Kloof | KL739 | −28.7 | −300 | −30.7 | −230 | −27.2 | −142 | ND | ND |
| Kloof | KL441H2 | −37.8 | −257 | −32.9 | −198 | −34.0 | −105 | ND | ND |
| Kloof | KL443HWND1 | −34.4 | −327 | −35.6 | −251 | −33.7 | −208 | ND | ND |
| Kloof | KL443HWDN | −34.3 | −329 | −34.3 | −269 | −33.5 | −206 | −33.6 | −207 |

a. ND — below detection limit for isotope analysis.

b. *DR938H3′-0m is a sample from the same borehole as DR938H3-0m taken 8 months afterwards. Such temporal sampling was not possible for the other boreholes due to the common practice of sealing them immediately after completion of drilling.

c. DR938H3-0m and DR938H3-648m were collected from the same borehole on the same day. The 0 m sample was taken at the borehole collar while the 648 m sample was taken at 648 m depth.

become clear that the "carbon isotopic reversal" trend alone is not sufficient evidence to support an abiogenic origin (Horita and Berndt, 1999).

Based on crustal $^3He/^4He$ ratios at all sites, no mantle-derived component for the Precambrian Shield hydrocarbon gases is indicated (Lippmann et al., 2003; Sherwood Lollar et al., 1993b). Sherwood Lollar et al. (2002) suggested a crustal abiogenic origin due to water–rock interactions for the hydrocarbon gases at Kidd Creek however, based on a new criteria — an inverse correlation of $^{13}C$-depletion and $^2H$-enrichment between $CH_4$ and ethane (Fig. 1A). Building on a hypothesis for carbon isotope distribution patterns first proposed by Des Marais et al. (1981), it was suggested that this pattern results from synthesis of higher molecular weight hydrocarbons from $CH_4$ during polymerization. In such a process $^{12}CH_4$ reacts faster than $^{13}CH_4$ to form chains, so that $^{12}C$ is more likely to be incorporated

into larger hydrocarbon chains in polymerization reactions, whereas, owing to preferential cleavage of the weaker $^{12}C$–$^1H$ bond versus the $^{12}C$–$^2H$ bond, the light ($^1H$) isotope will be preferentially eliminated (Sherwood Lollar et al., 2002). In contrast, the pattern for commercial thermogenic natural gas deposits consists of a positive correlation of $\delta^{13}C$ and $\delta^2H$ values between $CH_4$, ethane and the higher hydrocarbon gases propane and butane (Fig. 1A). This isotopic pattern of increasing isotopic enrichment in both $^{13}C$ and $^2H$ with increasing molecular weight for the $C_1$–$C_4$ homologues for thermogenic gases results from production of hydrocarbons by thermal cracking of a high molecular weight organic precursor (Des Marais et al., 1981; Schoell, 1988). At each of the 4 newly investigated Precambrian Shield sites in Canada and South Africa from this study, hydrocarbon gases were identified with the same pattern of $^{13}C$-depletion and $^2H$-enrichment between $CH_4$ and

Table 3
16S rRNA gene and *mcrA* clones from borehole DR9IPC having high relatedness to known methanogenic organisms

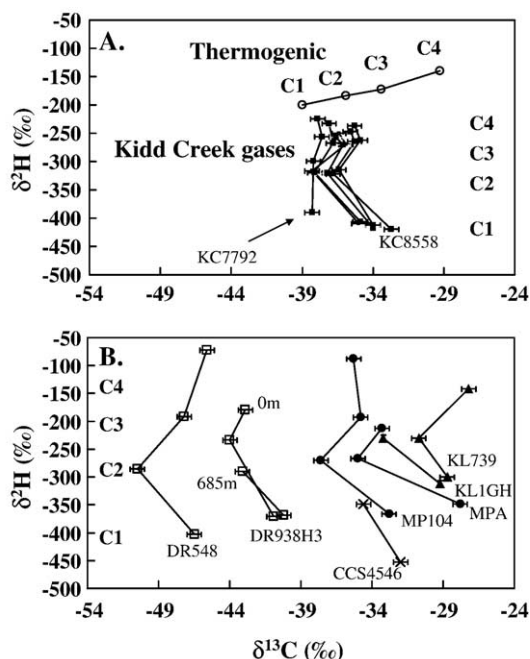| DR9IPC clone (Genbank accession number) | Percent of library | Nearest cultivated relative | Identity (%) |
|---|---|---|---|
| 16S rRNA 1 (AY604061) | 40.0 | *Methanobacterium curvum* | 93 |
| mcrA 1 (AY604057) | 23.7 | *Methanosarcina barkeri* | 99 |
| mcrA 2 (AY604058) | 73.7 | *Methanobacterium bryantii* | 86 |
| mcrA 3 (AY604059) | 2.7 | *Methanobacterium aarhusense* | 83 |

Fig. 1. Plot of $\delta^{13}C$ versus $\delta^2H$ values for $C_1$–$C_4$ for Kidd Creek (A) and for 4 new Precambrian Shield sites (B). In the lower panel, the most $^{13}$C-enriched samples at each site — Driefontein (open squares); Mponeng (closed circles), Kloof (closed triangles) and CCS (crosses) all show the characteristic $^{13}$C depletion and $^2$H enrichment between $CH_4$ and ethane similar to the proposed abiogenic gases at Kidd Creek (data from (Sherwood Lollar et al., 2002) – closed squares– upper panel). In marked contrast, the pattern for thermogenic gas reservoirs is a positive correlation of $\delta^{13}C$ and $\delta^2H$ values for $CH_4$ and ethane due to increasing isotopic enrichment in both $^{13}$C and $^2$H with increasing molecular weight for the $C_1$–$C_4$ homologues (open circles — upper panel). This pattern results from production of the hydrocarbon gases by thermal cracking of a high molecular weight organic precursor (Des Marais et al., 1981; Schoell, 1988). Error bars on $\delta^{13}C$ values are $\pm 0.5$‰. Error bars on both A and B on $\delta^2H$ values are $\pm 5$‰ and are smaller than the plotted symbols.

ethane as the proposed abiogenic gases from Kidd Creek (Fig. 1B; Table 2). Interestingly, for each site only the most $^{13}$C-enriched samples show this pattern.

### 4.2. Mixing trends

Whereas the Kidd Creek data set is remarkably uniform, one sample (KC7792) has a $\delta^{13}C_{CH4}$ value significantly more depleted in $^{13}$C and enriched in $^2$H than other samples from this site (Fig. 1A). Sherwood Lollar et al. (2002) suggested that this might be due to mixing with a more $^{13}$C-depleted and $^2$H-enriched microbial $CH_4$ component in this borehole. In the current study, the hydrocarbon gas samples analysed for 4 new Precambrian Shield sites show a much wider range of $CH_4$ isotopic signatures than at Kidd Creek (Figs. 1B

and 2). In all cases, the proposed abiogenic signature was observed only for the samples with the most $^{13}$C-enriched $CH_4$ values at each site (DR938H3 at both 0 and 685 m, DR548; KL739 and KL1GH; CCS4546; and both samples from Mponeng — MP104 and MPA; Fig. 1B). The distinct $^{13}$C–$^2$H isotopic patterns between $CH_4$ and ethane for these samples (Fig. 1B), as well as their significantly depleted $\delta^2H$ values compared to typical thermogenic gases (Fig. 2), do not support a thermogenic origin for these $^{13}$C-enriched end-members. Furthermore, as at Kidd Creek, the majority of the data points from CCS, Driefontein and Kloof Mines fall along linear trends between the most $^{13}$C-enriched and $^2$H-depleted end-members, and samples with more $^{13}$C-depleted and $^2$H-enriched values (Fig. 2), raising the possibility of mixing between these two end-members. For CCS, Kloof and Kidd Creek, all data fall along a single trend from the $^{13}$C-enriched end-members identified in Fig. 1B, whereas for Driefontein, two possible trends can be drawn through the data, one for DR938H3 and one for DR548. At Mponeng, both samples MP104 and MPA are $^{13}$C-enriched and show the proposed abiogenic signature (Fig. 1B) and this is consistent with the lack of any mixing trend in Fig. 2.

In Fig. 2, solid lines indicate the conventionally accepted fields of $\delta^{13}C$ and $\delta^2H$ values for microbial and thermogenic $CH_4$ based on empirical studies (Schoell, 1988). The hatched trend lines indicate that $^{13}$C-depleted and $^2$H-enriched end-members at these sites could theoretically be either thermogenic or microbial in origin based on the distribution of the $\delta^{13}C$ and $\delta^2H$ fields. Other lines of evidence indicate that this end-member is microbial however, and suggest that microbial $CH_4$ mixing with abiogenic gases produces the observed trend lines. For borehole DR9IPC, 16S rRNA gene cloning and *mcrA* (methyl coenzyme M reductase) gene cloning indicate the presence of methanogens (Table 3). In addition, the $\delta^{13}C$ and $\delta^2H$ values for DR9IPC are similar to the values for microbial $CH_4$ identified in several other sites in the Witwatersrand Basin (Ward et al., 2004). In particular, fracture water from Masimong and Merriespruit, located 150 km southwest of Driefontein, have $\delta^{13}C$ and $\delta^2H$ values for $CH_4$ of $-60.7$ and $-207$‰ (MM5); and $-53.7$ and $-194$‰ (MR1), respectively (Fig. 2). The microbial origin of $CH_4$ from Merriespruit is also supported by 16S rRNA gene and by enrichment cultures which indicate the presence of methanogens (Ward et al., 2004).

In contrast, 16S rRNA gene amplifications for the Kloof samples (KL739, KL441H2, KL443HWND1, KL443HWDN) did not yield any evidence of methanogens (Ward et al., 2004; Kieft et al., 2005). While this
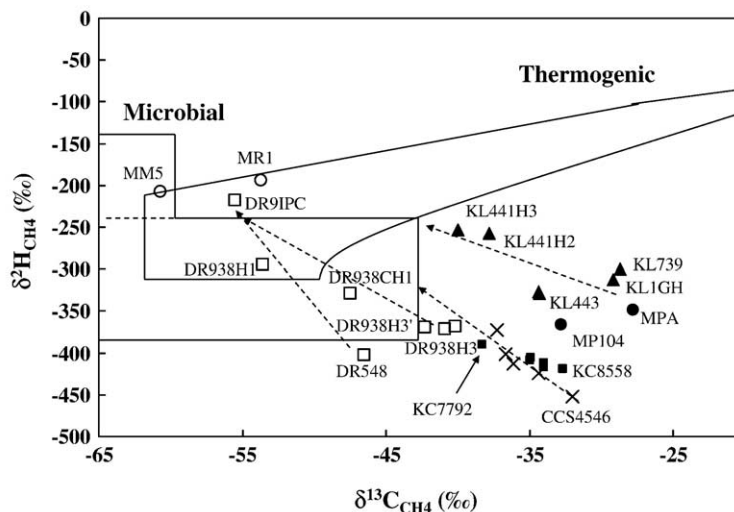
Fig. 2. Plot of $\delta^{13}C$ versus $\delta^2H$ values for $CH_4$ for sites from this study compared to the conventional fields for microbial and thermogenic $CH_4$ after Schoell (1988). Except for the two Mponeng samples (see text), samples fall along linear trends from the most $^{13}$C-enriched end-members to more $^{13}$C-depleted and $^2$H-enriched end-members. Symbols are as in Fig. 1. DR938H3 and DR938H3′ are samples taken from the same borehole 8 months apart. Unfortunately such temporal sampling was not possible for the other holes, due to the common practice of sealing them immediately after completion of drilling. Error bars are $\pm 0.5$ ‰ on $\delta^{13}C$ values and $\pm 5$ ‰ on $\delta^2H$ values and are smaller than the plotted symbols. Data from Masimong (MM) and Merriespruit (MR) are from Ward et al. (2004) for comparison. Data from Kidd Creek are from Sherwood Lollar et al. (2002).

may be due to the very low biomass in these samples and does not entirely rule out a small microbial $CH_4$ contribution, it is nonetheless consistent with the predominantly abiogenic origin proposed for $CH_4$ in the Kloof samples. No microbiological work was carried out at the other boreholes at Kloof, Copper Cliff South or Mponeng, but for Driefontein, 16S rRNA gene amplification and culture enrichments for methanogens were attempted for both borehole DR9IPC (results above) and for one of the proposed abiogenic end-members, borehole DR938H3. While enrichments were unsuccessful, PCR-amplified 16S rRNA gene indicated a bacterium (closely related to the sulfate-reducing *Desulfotomaculum* species) and three archaeal taxa within the family *Methanobacteriaceae*. The low biomass in this sample suggests that concentrations of $CH_4$ in the 100 uM range derived from microbial methanogenesis would be insufficient to isotopically overprint the >17 mM abiogenic $CH_4$ signature however (Moser et al., in press).

The possibility of mixing between microbial hydrocarbons (characterized by $^{13}$C-depleted and $^2$H-enriched $CH_4$ values) and abiogenic end-members can be further evaluated using the traditional scheme shown in Fig. 3, adapted from Hunt (1996). In this plot, microbial hydrocarbons fall in the upper left corner based on their $^{13}$C-depleted $CH_4$ values and $CH_4/C_2^+$ ratios >1000 (Hunt, 1996). We have modified the scheme to evaluate abiogenic-microbial mixing, using the abiogenic end-members for each site suggested by Fig. 1B. For the microbial

end-member, while microbial $CH_4$ has been identified at Kidd Creek and Copper Cliff South mines based on microbial cultures (Doig, 1994), no information on the isotopic composition of that $CH_4$ exists to date. Whereas a microbial end-member has been identified at Driefontein (DR9IPC) based on both isotopic evidence and gene cloning (Table 3), we do not have the same information for Kloof. The same dolomitic Transvaal Supergroup overlies Kloof mine however and as at Driefontein, fractures intersecting both the Ventersdorp and overlying Transvaal provide a conduit for mixing of fluids and gases between these two formations. For all samples then, as an initial test of the model, we used a $\delta^{13}C$ value of −55‰ for the microbial end-member based on the carbon isotope value of DR9IPC (Fig. 3). By this approach, data from Kloof are consistent with mixing of the most $^{13}$C-enriched end-member (KL739) with up to 43% of a microbial hydrocarbon end-member with a $\delta^{13}C_{CH_4}$ value of −55‰ (Fig. 3). All estimates are insensitive to whether the microbial end-member has $CH_4/C_2^+$ ratios of $10^3$–$10^4$ or even higher. For Driefontein, DR938H1 is consistent with mixing with up to 84% microbial $CH_4$ (based on mixing with DR548), while mixing between DR9IPC and DR938H3 defines a separate trend line, as in Fig. 2. Given that the gases occur in hydrogeologically isolated fractured rock it is not surprising that some sites exhibit a range of end-members and different possible mixing lines, as fracture controlled systems are rarely homogeneous. In fact, the mixing
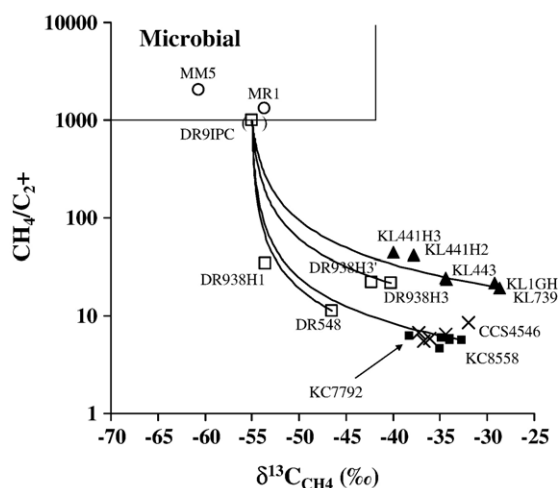
Fig. 3. Plot of $\delta^{13}C$ values for CH$_4$ versus CH$_4$/C$_2^+$ ratios adapted from Hunt (1996). Data fall along mixing lines between the most $^{13}C$-enriched CH$_4$ abiogenic end-member at each site as suggested by Fig. 1B, and a $^{13}C$-depleted CH$_4$-rich microbial end-member, with a value of $-55$ ‰ (see text). Mixing lines are calculated based on the equations:

$$\delta^{13}C = x(\delta^{13}C_{microbial}) + (1 - x)(\delta^{13}C_{abiogenic});$$

where $x$ is the percent of the microbial end-member; and,

$$CH_4 / C_2+ = (x(I_{microbial}) + (1 - x)(I_{abiogenic}))^{-1};$$

where $I = C_2^+/CH_4$, the inverse of CH$_4$/C$_2^+$. The estimated percent microbial component for each sample (see text) is calculated by projecting its $\delta^{13}C$ value onto the mixing lines on Fig. 3. As noted in the text, the estimates of % microbial gas are relatively insensitive to CH$_4$/C$_2^+$ ratios but are dependent on the selected $\delta^{13}C$ values for the two end-members, and hence provide only a conservative estimate of the relative fraction of microbial gas. In particular, the mixing lines as depicted assume that the most $^{13}C$-enriched end-member at each site is entirely free of a microbial contribution. If the most $^{13}C$-enriched samples are already themselves the product of some mixing with microbial CH$_4$ with an even more $^{13}C$-enriched end-member, then the estimates of the fraction of microbial contribution would be even larger. Symbols are as in Fig. 1. MM5 and MR1 (circles) are microbial CH$_4$ end-members previously identified for the Witwatersrand Basin based on CH$_4$ isotopic values, 16S rRNA gene and enrichment cultures (Ward et al., 2004). Unfortunately air contamination of sample DR9IPC means that C$_2^+$ values are diluted to below detection limit, so a CH$_4$/C$_2^+$ ratio cannot be calculated for this sample. Based on the similarity in isotopic composition of this sample to MR1 and MM5, it has been plotted (in brackets) close to those samples for comparison purposes. Error bars are ± 0.5‰ on $\delta^{13}C$ values and ± 7‰ on CH$_4$/C$_2^+$ ratios and are smaller than the plotted symbols.

lines could be recalculated for a microbial end-member with a $\delta^{13}C_{CH4}$ value close to MM5 ($-60$‰) (Fig. 4) rather than close to DR9IPC ($-55$‰), and the data are still consistent with this simple two-component mixing between microbial and abiogenic gas, although the estimates of the fraction of microbial mixing vary some-

what. The key point of Figs. 3 and 4 is not necessarily to constrain a specific microbial $\delta^{13}C$ value for each site, but to demonstrate that the isotopic and compositional variation in samples observed supports a model of mixing between the $^{13}C$-enriched end-members in Fig. 1B, and microbial end-members with a range of $\delta^{13}C$ values typical of CH$_4$ produced by methanogens. If a similar approach is taken for the Canadian Shield sites, KC7792 can be explained by a mixture of the most $^{13}C$-enriched end-member at Kidd Creek (KC8558), with up to 17.5% microbial CH$_4$ (Fig. 3). The CCS data fit somewhat less well. CCS4546 is the only sample with abiogenic characteristics at this site (Fig. 1B) and is the most $^{13}C$-enriched and $^2H$-depleted value at the site (Fig. 2). Hence, whereas the CH$_4$/C$_2^+$ ratio for CCS4546 in Fig. 3 is slightly elevated (8.6 versus approximately 6 for the other samples), a mixing line through the remainder of the data to a $^{13}C$-enriched end-member with the $\delta^{13}C_{CH4}$ value of CCS4546 and a CH$_4$/C$_2^+$ ratio of 6 indicates that a mixture of up to 23% microbial CH$_4$ can account for the rest of the data at that site.

The most $^{13}C$-enriched end-members at each site are too C$_2^+$-rich to be microbial in origin (Fig. 3), and likewise too $^2H$-depleted in CH$_4$ to be consistent with a thermogenic origin (Fig. 2). Analogous to the Kidd Creek gases, the carbon and hydrogen isotopic patterns of these gases (Fig. 1B) suggest an abiogenic origin. The fact that the data from these sites in both Canada and South Africa fall on mixing lines in both Figs. 2 and 3 provides excellent support for interpretation of these data as two-component mixing between microbial
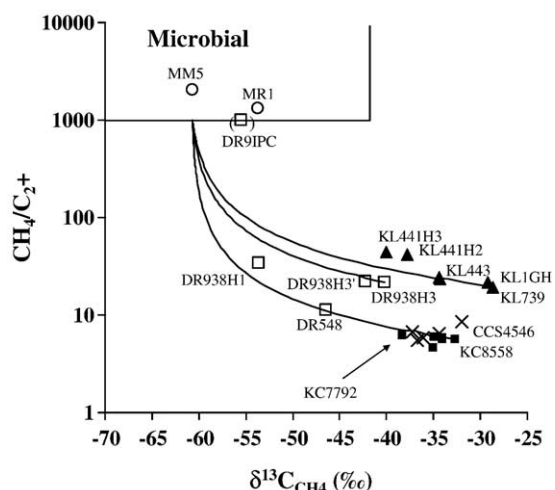


Fig. 4. Plot of $\delta^{13}C$ values for CH$_4$ versus CH$_4$/C$_2^+$ ratios adapted from Hunt (1996). In this plot, mixing lines are calculated as in Fig. 3 but for mixing between the most $^{13}C$-enriched CH$_4$ end-member at each site, and a $^{13}C$-depleted CH$_4$ rich microbial end-member with a value of $-60$‰ (see text).

and abiogenic end-members. Alternatively the presence of a thermogenic component at the sites in this study cannot be completely ruled out. Indeed, Ward et al. (2004) showed that mixing between microbial and thermogenic gases might account for gases in boreholes at Evander mine located in the quartzite, shale and minor volcanics of the Witwatersrand Supergroup sediments. Samples from South Africa in the present study however are predominantly from boreholes in a different geologic unit, the basaltic lavas of the Ventersdorp Supergroup. Furthermore, all the samples at Evander fall close to the conventional microbial and thermogenic isotope fields in Fig. 2 (Ward et al., 2004), unlike the $^{13}$C-enriched and $^2$H-depleted end-members at the sites in the present study. Hence we feel the best explanation for the data in the present study is indeed two-component mixing between $^{13}$C-enriched abiogenic and $^{13}$C-depleted microbial end-members. The fact that data from this study fit a simple two-component mixing model between these microbial and abiogenic end-members suggests that if a thermogenic component is present, it is small and does not control the isotopic and compositional variations in a major way.

### 4.3. $H_2$-based autotrophy linked to methanogenesis

This approach to resolving mixing between abiogenic and microbial $CH_4$ in the crust has important implications for evolving research into the Earth's deep biosphere, specifically the identification of potential substrates supporting microbial communities in the deep subsurface. $H_2$-based subsurface microbial communities associated with autotrophic methanogenesis have been proposed in the Columbia River basalt aquifer (Anderson et al., 1998; Stevens and McKinley, 1995), Lidy Hot springs (Chapelle et al., 2002); and in carbonate-rich serpentinized peridotites at an off-axis hydrothermal vent field near the mid-Atlantic ridge (Kelley et al., 2001; 2005). Interest in such systems is high since they may be modern terrestrial analogues for subsurface microbial ecosystems under reducing conditions on the early Earth (Shock and Schulte, 1998) or on other planets or Jovian satellites (Chapelle et al., 2002; McCollom, 1999). Several of the boreholes sampled in this study have high concentrations of $H_2$ gas as well as the $CH_4$ and higher hydrocarbons already discussed. $H_2$ concentrations in samples from Mponeng, Driefontein and Kloof are as high as 11.5% (Table 1). $H_2$ levels in samples from CCS, Kidd Creek and other sites in the Precambrian Shield rocks of Canada have been reported in concentrations as high as 1–58% (Table 1; (Sherwood Lollar et al., 1993b)). Over several

years, re-sampling of boreholes in Canada has shown that the high $H_2$ levels are not simply an artefact of drilling (Bjerg et al., 1997) but a persistent natural phenomenon in this geologic environment (Sherwood et al., 1988; Sherwood Lollar et al., 1993b, 2002). Like the hydrocarbon gases, the high quantities of $H_2$ are likely the product of abiogenic water–rock interactions in these geologically isolated, high rock to water ratio environments. Specific $H_2$-generating reactions may vary from one geological environment to another. At sites such as Kidd Creek where significant sections of ultramafic rock occur, $H_2$ may be the product of serpentinization over geologically long time scales analogous to systems described elsewhere (Abrajano et al., 1988; Charlou and Donval, 1993; Kelley et al., 2001). In the absence of significant ultramafics, such as at the Witwatersrand Basin sites, radiolytic decomposition of water in the uranium-rich Witwatersrand Formation has been shown to produce $H_2$ at a sufficient rate to account for the elevated $H_2$ concentrations observed in these isolated fracture fluids (Lin et al., 2005). $H_2$ as an energy source for autotrophic microbial ecosystems on both the Earth (Anderson et al., 1998; Stevens and McKinley, 1995) and Mars (Boston et al., 1992) has been a topic of considerable debate in the literature. Nonetheless, the number of studies that have been able to convincingly demonstrate coupled oxidation of $H_2$ and reduction of $CO_2$ to produce $CH_4$ via the equation:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (1)$$
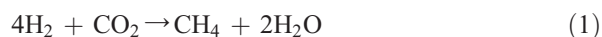
as the basis for a microbial food chain in the deep terrestrial subsurface has been limited (Chapelle et al., 2002).

The approach for identifying abiogenic and microbial $CH_4$ end-members presented in this paper, and the large database on deep subsurface gases developed by our research in Canada and South Africa provides an opportunity to test this proposed reaction on a large scale. Chemolithoautotrophs growing on $H_2$ can potentially reduce a variety of different oxidants including any available oxygen, sulfur and nitrogen oxidants, metals and metalloids, as well as $CO_2$. If $H_2$ autotrophy linked to $CO_2$ reduction and methanogenesis is an important process, there may be a correlation between $H_2$ utilization and production of an isotopically $^{13}$C-depleted microbial $CH_4$. Fig. 5 is a compilation of all samples for Canada and South Africa measured by our group to date ($n = 65$), incorporating data from almost two dozen sites. A relationship exists between $H_2$ concentrations and the isotopically distinct microbial and abiogenic hydrocarbon gas end-members identified in this study. With few excep-
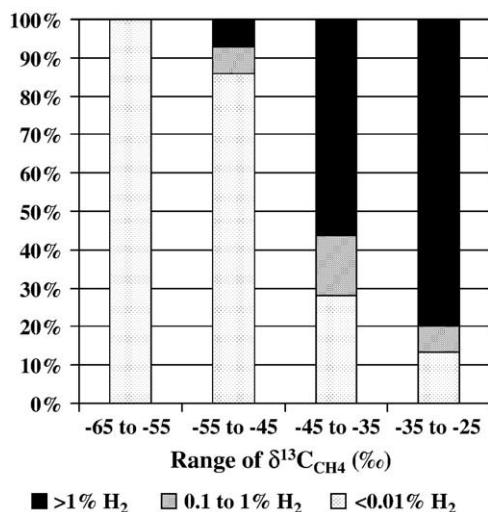
Fig. 5. Histogram of $H_2$ concentrations versus $\delta^{13}C_{CH_4}$ values for samples ($n=65$) from this study as well as for previously reported data from Precambrian Shield sites in South Africa (Ward et al., 2004) and Canada (Montgomery, 1994; Sherwood Lollar et al., 1993a,b, 2002). With few exceptions, high $H_2$ concentrations ($>1\%$) ($n=31$) are associated with the most $^{13}C$-enriched $CH_4$ end-members ($\delta^{13}C_{CH_4}$ values between $-45‰$ and $-25‰$). In contrast, samples with $^{13}C$-depleted $CH_4$ values (indicative of a significant methanogenic component) have $H_2$ values between 0.1–1% ($n=7$), to below detection limit ($<0.01\%$) ($n=27$).

tions, high $H_2$ concentrations ($>1\%$) are associated with only the most $^{13}C$-enriched $CH_4$ samples ($\delta^{13}C$ values between $-45$ and $-25‰$). In contrast, samples with $^{13}C$-depleted $CH_4$ values (indicative of a significant methanogenic component) correlate with $H_2$ values in the range of 0.1% to 1%, to below detection limit ($<0.01\%$). The observed pattern of $H_2$ concentrations and $CH_4$ isotopic signatures may be the geochemical fingerprint of $H_2$ autotrophy linked to methanogenesis via Eq. (1). This evidence supports the interpretation of the hydrocarbon isotopic data as controlled by two-component mixing between $^{13}C$-enriched $CH_4$ end-members and $^{13}C$-depleted microbial end-members and may further suggest that methanogenesis is supported by $H_2$ utilization. A scenario could be envisaged where $H_2$–$CH_4$ rich abiogenic gases are mobilized when fractures open due to tectonic activity or due to more recent disturbances due to mining activity and support a redox gradient-driven ecosystem in the Precambrian Shield deep subsurface where rapid utilization of $H_2$ by microbial communities quickly depletes $H_2$ and produces a $^{13}C$-depleted $CH_4$ signature characteristic of methanogenesis that mixes with and partially overprints the pre-existing $^{13}C$-enriched abiogenic $CH_4$ signature.

## 5. Conclusions

This study suggests that the abiogenic hydrocarbon gases first identified at Kidd Creek in fact exist at a variety of Precambrian Shield sites worldwide. Using the mixing models outlined here, the relative contribution of abiogenic versus microbial end-members can be estimated. Verification of an abiogenic component first requires identification of the end-member based on the $\delta^{13}C$ and $\delta^2H$ model illustrated in Fig. 1, followed by evaluation of the extent of mixing with other more conventional hydrocarbon sources via mixing schemes such as in Figs. 2 and 3. This combined approach has important implications for evaluation of potential abiogenic hydrocarbon reservoirs in a wide range of geologic settings, including the longstanding controversy concerning the possible contribution of abiogenic gases to economic petroleum hydrocarbon reservoirs (Gold, 1979; Kenney et al., 2002). The association of high concentrations of $H_2$ with $^{13}C$-enriched $CH_4$ end-members, and the $H_2$ depletion in $^{13}C$-depleted methanogenic end-members further suggests the possibility that abiogenic gases may support $H_2$ autotrophy linked to methanogenesis in the deep subsurface.

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