MASTERS THESIS

Gas flux and isotopic sampling of diffuse gas at a site of serpentinization for the purposes of sourcing methane

By:

Liam Morrissey (B.Eng)

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Department of Earth Sciences
Memorial University of Newfoundland

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Abstract

The flux of natural CH_4 released and CO_2 sequestered at a site of serpentinization were determined and methods for sourcing dissolved and diffuse CH_4 at the site were tested. Greenhouse gas fluxes (CO_2, CH_4, N_2O) at an ultra-basic pool associated with a site of serpentinization in the Tablelands, Gros Morne, NL were measured to determine the impact on atmospheric heating. It was calculated that the site had a small net reduction on atmospheric heating over a time horizon of 100 years and a net increase on atmospheric heating over a time horizon of 30 years. Methods for sourcing methane were also examined in this thesis. Several common collection and concentration methods were tested in the laboratory and at the Tablelands and were shown to be non-isotopically fractionating for CH_4. Additionally, a metadata analysis showed that a carbon fractionation factor of above 1.04 better described microbial CH_4 and below better described abiogenic CH_4. Results from this thesis are the first to calculate the flux of both CH_4 released and CO_2 sequestered at a site of serpentinization and highlight the need for an understanding of the natural baseline of these sites.

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Table of Contents

List of Tables

List of Figures

(Appendix 3.6). At this time it was assumed that chemical equilibrium was achieved in the headspace (operationally defined as when the CH_4 concentrations of 3 samples were within 5% of each other). The solid horizontal line represents the known $\delta^{13}C$ of the CH_4 (-41.3 ‰) that was dissolved in the water that was used in the laboratory testing of the vacuum extraction and gas stripping methods (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 0.5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

Figure 3.6. δD of CH_4 gas cryogenically trapped in the laboratory when (A) 480 mL of CH_4 was transferred to 160 mL vial, (B) 720 mL of CH_4 was transferred to 160 mL vial (Appendix 3.7). The solid line represents the known δD of the CH_4 that was used to test concentration methods in the laboratory (-167‰) (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

Figure 3.7. Carbon and hydrogen isotopic fractionation factors ($\alpha\ ^{13}C$ and α D, respectively) during the formation of microbial and abiogenic methane. Zones are provided for areas indicating microbial and abiogenic methane. Microbial methane isotopic fractionation factors are from Kohl et al. (2016) and references therein and include methane generated from both acetate fermentation (filled triangle) and CO_2 reduction (unfilled triangle). Abiotic methane isotopic fractionation factors are a review of reported data from both laboratory and field data (Kelley and Früh-Green 1999; Proskurowski et al. 2008; Sherwood et al. 1988; Taran et al. 2010; Fu et al. 2007)

List of Nomenclature and Symbols

‰ – per mil, also known as parts per thousand

CSIA – compound specific isotope analysis

FTT - Fisher-Tropsch Type

GC-IRMS- gas chromatography-combustion-isotope ratio mass spectrometer

GHG – greenhouse gas

ppbv - parts per billion by volume

RF – Radiative Forcing

V-PDB – Vienna – Pee Dee Belemnite, international isotope ratio standard for carbon

V-SMOW – Vienna -Standard Mean Ocean Water, international isotope ratio standard for hydrogen and oxygen

WHC- Winter House Canyon

1 Chapter 1 Introduction and Overview

1.1 Atmospheric Carbon Dioxide and Methane and the Global Climate

As global greenhouse gas (GHG) levels reach record highs research is now focusing on ways that these gases can be removed from the atmosphere. One proposed method involves the potential for sites of serpentinization to sequester carbon dioxide (CO_2), a major greenhouse gas, from the atmosphere and reduce our global atmospheric CO_2. Serpentinization involves the hydration of ultramafic rock to produce serpentine and hydrogen gas, that in a continental setting results in ground waters with high pH and elevated concentrations of dissolved calcium (Coleman and Keith 1971). In addition, in high serpentine environments, Ca-silicates can react and produce high concentrations of Ca^{2+}, along with an increase in pH (Frost and Beard 2007). Under these circumstances, dissolved inorganic carbon precipitates with Ca and O as solid calcium carbonate, a potential long-term storage option for atmospheric CO_2. However, the released hydrogen gas may also react (through microbial or abiogenic processes) with inorganic carbon to produce methane (CH_4), which is a more potent GHG compared to CO_2 (over a 20 year and 100 year time horizon). Therefore sites of serpentinization have the potential to act as both a source of CH_4 and a sink for CO_2. However, while the chemistry behind these reactions has been demonstrated (Coleman and Keith 1971) to our knowledge, the combination of the flux of CH_4 released and CO_2 sequestered at a given site of serpentinization has not been comprehensively measured. In addition, $CH_{4,}$ while also being a greenhouse gas, is of particular interest due to its use as a fuel source (i.e., natural gas), and a potential indicator of life on other planetary bodies and moons. However, the presence of CH_4 alone does not permit the determination of its source and more lines of evidence are needed to differentiate between the various potential sources (thermogenic, microbial, abiogenic). In this context there is a need to better understand how to collect and concentrate low concentrations of CH_4 without changing its geochemical fingerprint.

1.2 Thesis Overview and Purpose

The overall purpose of this Masters Thesis was to determine the flux of CH_4 released and CO_2 sequestered at a site of continental serpentinization and to test methods of collection and concentration of CH_4 at these sites for isotopic fractionation. To accomplish this a closed floating chamber that can contain gases (both entering and leaving the system) was built and tested in the laboratory and deployed at a site of serpentinization in the Tablelands, Gros Morne, NL, Canada. Next, methods for collecting and concentrating diffuse and dissolved CH_4 at a site of serpentinization without changing its geochemical fingerprint (i.e., stable carbon and hydrogen isotope values) were tested. Results from this project provide a better understanding of the carbon sequestering potential at sites of continental serpentinization and sourcing low concentrations ov methane.

The thesis has been written in a manuscript format with four chapters. Chapter 1 details the background and important literature relevant to the field of CH_4 sourcing and gas flux measurements. Chapter 2 focuses on the collection of GHG and the calculation of gas fluxes at a site of continental serpentinization. Chapter 2 begins with deployment of a closed floating chamber that was used to collect gas in its headspace while placed over a pool of water discharging from serpentinized rock in the Tablelands, NL, Canada. Measured gas concentrations were then used to calculate the flux of CH_4 released and

CO_2 sequestered. Finally, calculated gas fluxes were used to determine the impact the site had on atmospheric heating and to calculate the net radiative forcing of the site.

Chapter 3 evaluations methods of collection and concentration methods of dissolved CH_4 for stable carbon and hydrogen isotope measurements. These measurements are typically used for CH_4 sourcing. Specifically, gas stripping and vacuum extraction methods were tested in the laboratory and the field to determine if they changed the carbon and hydrogen isotope value of the CH_4 (i.e., isotopic fractionation). Next, methods to cryogenically concentrate low concentrations of CH_4 were tested in the laboratory for carbon and hydrogen isotopic fractionation. Chapter 3 concludes with a metadata analysis of abiogenic isotopic fractionation factors that were then compared with microbial fractionation factors measured by others to develop another line of evidence for sourcing CH_4.

Chapter 4 summarizes the findings and provides a thematic overview of the results of the study. For instance, where Chapter 2 studies ways to calculate the fluxes at sites of serpentinization, Chapter 3 looks at ways to collect and concentrate gases at these sites for the purposes of sourcing. Finally this chapter also outlines the next logical steps for future research.

1.3 Applications of Research

1.3.1 Environmental

The potential for carbon sequestration at sites of continental serpentinization can be better understood through quantifying the fluxes of CH_4 sources and CO_2 sinks at these sites. Previous research has shown that there is a potential to inject atmospheric CO_2 into these sites to enhance the carbon sequestration and bring global greenhouse gas levels to pre-industrial levels (Keleman and Matter 2008). However, this research did not consider the impact of the CH_4 released at these sites. Findings from this thesis demonstrated that when both the CO_2 sequestered and the CH_4 released are considered, over a 20 year period, the site would have an atmospheric warming effect; however, due to the short residence time of CH_4 in the atmosphere, over a 100 year period the site would have a cooling effect. On the other hand, recent research has shown that microbes sometimes found at sites of serpentinization are capable of converting CO_2 to CH_4 (Kohl et al. 2016). Therefore, injecting CO_2 into these sites may only create more CH_4 and add to atmospheric heating.

1.3.2 Oil and Gas

The results could also have an impact on the oil and gas industry for both exploration and pipeline integrity. The isotopic signature of the methane can indicate the source, providing a metric to direct exploration activities. Offshore exploration is costly and so any information that can better direct exploration operations is critical to reducing costs. This project designed a method to collect diffuse and dissolved CH_4 that does not isotopically fractionate the sample so that it can be accurately sourced. Moreover, the project verified that common collection methods did not result in isotopic fractionation of the samples, thereby verifying accuracy of previously published results. In addition to exploration applications, the results can also be used for checking the integrity of gas pipelines, but normally the sniffers only detect the CH_4 and are unable to differentiate between the various potential sources. A modified CH_4 collection system based on the designed sample collector and concentrator could be used in tandem with CH_4 sniffers to verify that the CH_4 is from the pipeline, and not for instance from a nearby microbial source.

1.3.3 Planetary Science and Astrobiology

The potential presence of CH_4 on other planets and moons has generated significant attention from both planetary scientists and the general public. For example, CH_4 has been detected on Mars (Mumma et al. 2009; Webster et al. 2015). However, because current CH_4 destruction mechanisms cannot explain the spatial and temporal changes of CH_4 on Mars, many scientists have questioned the observations of Martian CH_4 (Zahnle et al. 2011). Specifically, it has been questioned whether the recent CH_4 measurements were a result of error due to competing telluric absorption lines between the Earth's atmosphere and Mars (Zahnle et al. 2011). However, in December 2014 using a tunable laser spectrometer, Curiosity (also knowns a Mars Science Laboratory (MSL)) reported background levels of Martian CH_4 at 0.69 parts per billion by volume (ppbv) with elevated spikes of 7.2 ppbv at Gale Crater, suggesting a new source that is episodically producing CH_4 (Webster et al. 2015). While these discoveries have received publicity as potential indicators of life, the detected CH_4 must first be accurately sourced to determine if it is abiogenic, thermogenic or microbial. For example, serpentinization has been proposed to be a major reaction that took place on early Mars due to the suspected presence of water and peridotite (Zahnle et al. 2011). Since large amounts of CH_4 have been associated with serpentinization on Earth, then wide spread serpentinization may have released large amounts of CH_4 and created a CH_4 rich Martian atmosphere (Etiope et al. 2011a). Moreover, due to the low concentrations of CH_4 that have been observed, the CH_4 would first need to be concentrated before isotopic analysis for the purposes of sourcing. However, current concentration methods have not been tested to ensure that they maintain isotopic integrity of the sample.

1.4 Literature Review

1.4.1 CH_4 Sources

On Earth, there are three known mechanisms for CH_4 production: microbial, thermogenic, and abiogenic (Schoell 1988). For the purposes of identifying past or present life both microbial and thermogenic CH_4 is considered a biogenic signature since the carbon source for thermogenic was once plant life. Microbial CH_4 is formed through two primary microbial metabolic pathways: fermentation and CO_2 reduction (Whiticar et al. 1986). Fermentation derived CH_4 involves the transfer of a methyl group from a substrate (primarily acetate) and is considered to be the major pathway for microbial CH_4 production (about 70%) in freshwater environments (Whiticar et al. 1986). Alternatively, in marine environments where sulfate levels are higher, sulfate-reducing bacteria (SRBs) outcompete methanogens for acetate and therefore, CO_2 reduction pathway is dominantly used by methanogens (Whiticar et al. 1986).

Thermogenic CH_4 refers to CH_4 produced by high temperature chemical reactions that involve the degradation of sedimentary organic matter (SOM) such as the cracking of kerogen (Hunt 1996; Whiticar 1999). Approximately 80% of commercial natural gas is thermogenic in origin (Schoell 1988).

Finally, abiogenic CH_4 is produced through chemical reactions that do not involve life. The most widely invoked mechanism for the generation of abiogenic CH_4 is Fisher-Tropsch Type (FTT). FTT reactions typically occur at higher temperatures and pressures compared to the more moderate values seen in microbial and thermogenic CH_4. (Etiope et al. 2011b; Foustoukos and Seyfried 2004; McCollom and Seewald 2006). In addition to FTT reactions, the hydration of ultramafic rock, in a process known as serpentinization, can produce hydrogen gas, which may then react with CO_2 to produce abiotic CH_4.

1.4.2 Serpentinization

Continental serpentinization involves the hydration of ultramafic rock to produce serpentine, ultra-basic groundwater, and hydrogen gas (H_2) (Coleman and Keith 1971). The produced H_2 may then react with inorganic carbon to produce CH_4 (McCollom and Seewald 2006; Taran et al. 2007). However, while sites of continental serpentinization often lead to abiogenic CH_4 production, these sites can also feature thermogenic and/or microbial CH_4 (Brazelton et al. 2006; Kelley et al. 2005; Morrill et al. 2013; Szponar et al. 2013). Regardless of the source, CH_4 can migrate with the groundwater and get discharged at the surface where it volatilizes, or, in some cases bubbles out of the spring, in both cases acting as a CH_4 source to the atmosphere.

In addition to producing CH_4, the characteristically high pH of serpentinizing systems creates conditions where atmospheric CO_2 can dissolve in the spring water and react with dissolved Ca to form solid carbonates, a long-term storage option for CO_2. Research has demonstrated ways to enhance the CO_2 sequestering potential of these sites by injecting atmospheric CO_2 into serpentinizing systems to bring global CO_2 levels to pre-industrial values (Kelemen and Matter 2008). However, while this method shows the promising potential of enhanced CO_2 sequestration, it only considered the sequestered gas, and not the gases that may be released to the atmosphere (Kelemen and Matter 2008). Moreover, the fluxes of both the CH_4 released and CO_2 sequestered for a given site have not been calculated. Therefore, the natural effect these sites have on atmospheric heating remains to be quantified. Additionally, if the CH_4 at these sites is formed from CO_2 reduction, the impact of the CH_4 generation due to CO_2 injection needs to be studied. Therefore the source of the CH_4 at these sites must also be determined.

1.4.3 Sourcing CH_4

Stable isotopes can be used to differentiate sources of CH_4 based on the isotopic ratios of both the carbon ($^{13}C/^{12}C$) and hydrogen ($^{2}H/^{1}H$, often referred to as D/H). However, isotope values of CH_4 should always be interpreted within the geological and chemical context from which they were sampled. The combination of carbon and hydrogen isotope values can be plotted on a ^{13}C - D plot to create general sourcing fields (Schoell 1980; Whiticar 1999). However, these plots have only been successful in differentiating biogenic sources (microbial and thermogenic CH_4) and abiogenic sources have been show to overlap with the biogenic areas (Horita and Berndt 1999; McCollom and Seewald 2006). Moreover, these plots only consider the isotopic values of the product and do not account for formation mechanisms and reactants. However, it remains to be seen whether isotopic fractionation factors during the production of the CH_4 may provide another line of evidence.

1.4.4 Isotopic Fractionation as a means of sourcing

Isotopic fractionation is the enrichment of one isotope relative to another in a compound due to a chemical or physical process. Apparent isotopic fractionation factors consider both the products and reactants during the production of CH_4. For example, previous studies have shown that microbial formation pathways (i.e. CO_2 reduction and acetate fermentation) can be distinguished by plotting the isotopic fractionation factors of the both the hydrogen and the carbon during the production process (from reactant to product) (Whiticar et al. 1986). However, in addition to formation pathways of microbial CH_4, isotopic fractionation factors may also provide a line of evidence in differentiating sources of CH_4 (Sherwood Lollar et al. 2008). In short, more research is needed in order to determine whether isotopic fractionation factors can be used to differentiate abiogenic CH_4 from biogenic sources.

1.4.5 Isotopic Fractionation During Collection and Concentration

In order to isotopically analyze dissolved gases present at low concentrations for the purposes of sourcing, CH_4 first needs to be extracted from the water and, in some cases, concentrated before isotopic analysis. Gas collection is done using either the vacuum extraction method or the gas stripping method (Rudd et al. 1974). Typically, isotope geochemists have used the vacuum extraction method for samples that will be isotopically analyzed (Sherwood Lollar et al. 2008; Slater et al. 2008). In contrast, the gas stripping method is less commonly used by isotope geochemists as it has been assumed that the method does not quantitatively convert all dissolved gas from the liquid to the gas phase (Penny Morrill, Personal Communication, September 15 2014). In addition to collecting CH_4 dissolved in the water, CH_4 diffusing from the water can also be isotopically analyzed. Moreover, depending on the concentrations of dissolved gas in the water, the CH_4 may need to be concentrated before isotopic analysis. Concentrating a gas sample can be achieved through cryogenic trapping to reducing the temperature of the gas such that more moles of gas can occupy a fixed volume. Overall, these extraction and concentration methods, as well as diffusion, must be tested for isotopic fractionation to ensure that they maintain the isotopic integrity of the sample.

2 Chapter 2: Flux of diffuse methane release and carbon dioxide sequestration at Winterhouse Canyon, Gros Morne, Newfoundland, Canada; a site of continental serpentinization.

Abstract

We measured CO_2, CH_4, and N_2O gas fluxes from a pool of ultra-basic water discharging from serpentinized rock in Winterhouse Canyon, Gros Morne, Newfoundland. The flux of CH_4 released and CO_2 sequestered were calculated to be 4.6 x 10^{-7} mol/m^2min and 1.9 x 10^{-5} mol/m^2min, respectively, whereas N_2O concentrations showed little change. The net radiative forcing due to the changing concentrations of CO_2 and CH_4 during the sampling period was -0.21, suggesting that the ultra-basic pool in WHC has a net cooling effect on the atmosphere. Similarly, the net global warming potential over a time horizon of 100 years was -7, also suggesting a small cooling of the atmosphere. Overall this study was the first to consider the impact of green houses coming into and out of an ultra-basic pool above serpentinized rock and demonstrated the need for more research on the net global impacts of serpentinization.

2.1 Introduction

2.1.1 Serpentinization: a source for CH_4 and a sink for CO_2

Serpentinization involves the hydration of ultramafic rock to produce serpentine, ultra-basic groundwater, and hydrogen gas (H_2). The produced H_2 may then react with inorganic carbon to produce CH_4 (McCollom and Seewald 2006; Taran et al. 2007). However, while sites of serpentinization often lead to abiogenic CH_4 production, these sites can also feature thermogenic and/or microbial CH_4 (Brazelton et al. 2006; Kelley et al. 2005; Morrill et al. 2013; Szponar et al. 2013). For example, microbial CH_4 has been proposed for samples in the Precambrian shield (Canada) (Sherwood Lollar et al. 1993) , the Lost City Vents (Mid Atlantic Ocean) (Kelley et al. 2005) and at the Cedars (United States) (Morrill et al. 2013; Kohl et al. 2016). In addition, if sedimentary organic matter is present beneath the serpentinizing ultramafic body then thermogenic CH_4 is also a possibility. Regardless of the source, CH_4 can be transported with the groundwater and become discharged at the surface where it volatilizes, or, in some cases bubbles out of the spring, acting as a CH_4 source to the atmosphere.

In addition, in high serpentine environments, Ca-silicates can react and produce high concentrations of Ca^{2+}, along with an increase in pH (Frost and Beard 2007). Under these circumstances, dissolved inorganic carbon can precipitate with Ca and O as solid calcium carbonate (Equations 1a and 1b), a potential long-term storage option for atmospheric CO_2.

$$(1) \qquad \text{(A) } CO_{2\,(g)} \rightarrow CO_{2(aq)} \rightarrow H_2CO_{3(aq)} \rightarrow HCO_3^{-}{}_{(aq)} \rightarrow CO_3^{2-}{}_{(aq)}$$

$$\text{(B) } CO_3^{2-}{}_{(aq)} + CO_2^{2+}{}_{(aq)} + Ca^{2+}{}_{(aq)} \rightarrow Ca_2CO_{3\,(s)}$$

In addition, research has also shown the potential for utilizing these types of systems to reduce global CO_2 levels (Kelemen and Matter 2008). For example, adding 1 wt% CO_2 to the Semail ophiolite in Oman would consume approximately 25% of atmospheric CO_2 (Kelemen and Matter 2008). However, although these methods show the promising potential of enhanced CO_2 sequestration, they only consider the sequestered gas, and not the gases that may be released to the atmosphere. Therefore there still exists a knowledge gap with respect to natural fluxes of both the CO_2 sequestered and the CH_4 released for sites of serpentinization. Additionally, to our knowledge, the greenhouse, N_2O has not been measured at sites of serpentinization.

2.1.2 Gas flux

A common method used to determine the diffusive gas flux between surface waters and atmosphere assumes that the gas transfer is a function of the concentration gradient between the two phases and the gas exchange coefficient at a given temperature via the following equation (Raymond and Cole 2001):

$$flux = \alpha k(Cgasw - Csat) \tag{2}$$

where k is the gas transfer velocity (m/s); α is the coefficient of chemical enhancement (dimensionless); C_{gasw} is the aqueous concentration of the dissolved gas in the surface water (mol/L); and C_{sat} is the equilibrium aqueous gas concentration (mol/L).

Chemical enhancement occurs when a gas is reactive with the water molecules or hydroxide ions in the surface boundary, and therefore it is a function of temperature, pH and ionic strength (Wanninkhof 1992; Wanninkhof and Knox 1996). Therefore, in ultra-basic pools fed by waters discharging from serpentinizing systems like the site in question, chemical enhancement is relevant for a molecule such as CO_2 which participates in hydrolysis reactions, and less relevant for molecules that do not participate in hydrolysis such as CH_4 and N_2O. The chemical enhancement for CH_4 would be set to a value of 1 by convention (Wanninkhof and Knox 1996). Moreover, while research has been conducted on the CO_2 chemical enhancement factors in oceans and lakes, to our knowledge a CO_2 chemical enhancement factor for high pH pools of water discharging from serpentinized rock has yet to be determined.

In addition to the above method, gas flux between the aqueous fluid and the atmosphere can also be measured directly by monitoring gas concentrations in a closed floating chamber. Using this method, a chamber is placed over a water body and gases are sampled from the chamber's headspace at specific time intervals. Based on the ideal gas law, the gas flux is calculated using the initial and final gas concentrations (Equation 3). Therefore, this empirical method could be used to determine unknown parameters in the theoretical equation 2.

$$Flux = \frac{V\,(P_2 - P_1)}{RTA(t_2 - t_1)} \tag{3}$$

where V is the volume of the chamber (m^3); R is the ideal gas constant (m^3 Pa/K mol); T is the air temperature (K); A is the surface area of chamber opening (m^2); P_1 and P_2 are gas partial pressures at two different sampling times (Pa); t_1 and t_2 are times at which the samples were taken (min).

This second method assumes a linear relationship between time and gas concentrations. This assumption can be validated with intermittent gas sampling between t_1 and t_2. The possible deployment duration for the closed floating chamber depends on the time it takes for the gas in question to equilibrate between the water and the headspace in the closed chamber. Equilibration times of 20-40 minutes for CO_2, and up to 24 hours for CH_4 have been reported (Podgrajsek et al. 2014).

2.1.3 Gas flux at sites of serpentinization

Current research on gas flux at sites of serpentinization has been limited to measuring the flux of CH_4 and CO_2 released from the Chimera gas seep, a system of gas vents from the Tekirova ophiolites in Turkey (Etiope et al. 2011b). This site featured subsurface CO_2 venting to the surface and the atmosphere. Fluxes were measured using a closed-chamber system using a linear regression of gas concentration in the chamber (Etiope et al. 2011b). The study tested 27 locations with diffuse CH_4 seepage and fluxes were calculated to be on the order of $4.3x10^{-3}$ to $4.3x10^{-2}$ mol/(m^2*minute). In comparison, CO_2 fluxes ranged from $1.6x10^{-4}$ to $1.0x10^{-3}$ mol/(m^2*minute) (Etiope et al. 2011b). While this study provided an approximate value for CH_4 and CO_2 fluxes at a site of serpentinization, the gas vents studied only released CO_2 and did not show any sequestration. Therefore, these results cannot be used to estimate the rates of CO_2 sequestration at an ultra-basic pool of water discharging from serpentinized rock.

2.2 Materials and methods

2.2.1 Field site description

To determine the impact of greenhouse gas fluxes from a site of serpentinization, CH_4, CO_2, and N_2O gases were sampled above a small reservoir of water that pooled at the discharge point of an ultra-basic spring in Winterhouse Canyon of the Tablelands, a Paleozoic ophiolite complex in the Gros Morne area of western Newfoundland, Canada. The Tablelands is mainly composed of peridotite rocks from an ophiolite complex that was formed approximately 485 ma ago during the closure of the Iapetus Ocean (Elthon 1991). Previous studies at this site have shown that the serpentinization is driven by groundwater and the reactions at this site produce several active, highly reducing (~ -609 mV) and ultra-basic (pH 10-12) groundwater springs discharging at the surface (Szponar et al. 2013). These springs can be identified by the white carbonate that surrounds the rim of the pool (Szponar et al. 2013). This carbonate was likely recently deposited as atmospheric CO_2 that then dissolved into the pooled water and precipitated due to the high pH of the system (Equation 1). Therefore, these ultra-basic pools are potentially sinks of atmospheric CO_2.

This study focused on one of these springs, WHC2, situated in the valley of Winterhouse Canyon. WHC2 is a pool of ultra-basic water that is approximately 40 cm deep and 126 cm wide and is exposed to the atmosphere. Geochemical parameters of the spring water have been studied previously and the water has been shown to have characteristically high pH values along with elevated dissolved concentrations of CH_4 ranging from $2.5x10^{-6}$ mol/L to $2.4x10^{-5}$ mol/L (Szponar et al. 2013). Overall, there are two ultra-basic groundwater discharge points (WHC2a and WHC2b) at the bottom of the WHC2 pool that are characterized by a relatively higher pH and lower Eh values compared to the rest of the pool water. WHC2 is surrounded calcium carbonate deposits (see Figure 2.1) consisting of calcite (90%) and aragonite (10%), indicating potential carbon sequestration (Szponar et al. 2013).

2.2.2 Field sampling

Gas samples for flux calculations were collected using a closed floating chamber (Figure 2.1). An 18.9 L (5-gallon) container was turned upside down and submerged 14 cm into the WHC2 pool. A weighted styrofoam platform was used to support the chamber over the water during the experiment. The chamber had been previously modified to allow 0.5 m of HDPE tubing to connect through an opening in the top of the inverted bucket, which was secured using a gas-tight O-ring seal. A two way luer lock valve was fitted to the other end of the tubing for sampling purposes. During headspace sampling, the needle of a 60 mL syringe was pushed through a rubber septum on the end of the two-way luer lock valve. 60 mL of headspace gas was then slowly drawn into the syringe and, once filled, the valve was closed and the needle was removed from the septa. The 60 mL gas sample was transferred to a 45 mL evacuated bottle sealed using a blue butyl septa. This procedure was repeated such that each sample 45 ml bottle received 120 mL of gas (over pressurizing the sample).

This study focused on headspace sampling during the initial 3 hours for CO_2 and the final 5 hours for CH_4, knowing that CO_2 in the pool typically equilibrates with the atmosphere within a few hours, whereas CH_4 equilibration can take over 24 hours (Podgrajsek et al. 2014). In addition, N_2O concentrations in the headspace were measured during the duration of the experiment to determine if there was any change over the 24 hours. Headspace samples were taken from the closed floating chamber at 0 minutes, 10 minutes, 25 minutes, 1 hour, 2 hours and 11 minutes (131 minutes), 3 hours and 45 minutes (225 minutes), 13 hours and 39 minutes (819 minutes), 15 hours and 35 minutes (935 minutes), 18 hours and 5 minutes (1085 minutes) and 20 hours and 7 minutes (1207 minutes). All samples were stored in a cooler and were analyzed within 8 weeks of sampling. Concentration values for duplicate samples were within 9% (see Appendix).

The pH, conductivity, and temperature of the water were taken in-situ prior to, and directly after, the 24 hour flux experiment. Conductivity amd pH were measured using an Oakton 10 series (Eutech Instruments) handheld pH meter and temperature was measured with a hand-held alcohol thermometer respectively.

Water samples were taken for dissolved CO_2 and CH_4 concentration analyses prior to, and directly after, the 24 hour flux experiment. Dissolved CO_2 and CH_4 were extracted from the water using a modified gas stripping method (Rudd et al. 1974). In short, this method involved stripping the dissolved gases from the water by vigorously shaking a 60 mL sealed syringe containing 25 mL of He gas and 25 mL of water sample for 5 minutes. After stripping the gas, two syringes with 25 mL of the gas phase each were injected into a 25 mL Wheaton vial that was prefilled with degassed nanopure water and sealed with a conditioned blue butyl septa. Holding the Wheaton vial upside down, the gas sample was pushed into the bottle and the degassed nanopure water left the vial through an exit needle.

2.2.3 Analytical methods

2.2.3.1 CH_4 and CO_2 concentrations

CH_4 and CO_2 concentrations were measured using a SRI 8610 gas chromatograph with a flame ionization detector (GC-FID). A Carboxen 1010 fused silica capillary column with a helium carrier gas and a temperature program of 40°C hold 6 minutes, ramp 15°C/minute to 120°C, hold 5 minutes, was used to separate the specific gases. After column separation a methanizer converted the CO_2 to CH_4 so that it could be analyzed using the FID.

Daily calibration curves were created for CH_4 and CO_2. The CH_4 calibration curves were made by injecting varying volumes (3-30 µl) of a Restek 34522 standard containing 100 ppm of CH_4 using a 50-microliter gas tight locking Hamilton syringe. Similarly, the CO_2 calibration curves were made by injecting varying volumes (7 – 15 µl) of a Restek 34512-PI gas standard containing 5% CO_2 using a 25 µl gas tight locking Hamilton syringe. The lower detection limit for the GC-FID for CO_2 was a concentration of $4.7\text{x}10^{-5}$ mol/L and the lower limit for CH_4 was a concentration of $3.3\text{x}10^{-7}$ mol/L.

2.2.3.2 N_2O concentrations

N_2O concentrations in the headspace were measured using a gas chromatograph with an electron capture detector (ECD). A HayeSep D column with a helium carrier gas and a temperature program of 40°C hold 5 minutes, ramp 20°C/minute to 220°C, hold 5 minutes, was used to separate the specific gases. Daily calibration curves were created for N_2O by injecting varying volumes (0.3-1 mL) of a standard containing 2.1 ppm by volume of N_2O using a 50-microliter gas tight locking Hamilton syringe. Standard error through multiple 1 mL injections was determined to be +/- 10% and the detection limit was 2.2×10^{-8} mol/L.

2.2.4 Flux calculations

To calculate the fluxes using the closed floating chamber method Equation 3 was applied i.e., the difference between gas concentrations in the headspace at two separate time points. This method requires non-equilibrium conditions between the gas and liquid phases in the chamber. For this method to be accurate there must be a linear relationship of gas concentration with respect to time. This linearity was tested in our experiments using the concentration data collected at intermediate time points.

2.3 Results

2.3.1 Geochemical characterization

The temperature, pH, dissolved gas concentrations, and conductivity were measured at the WHC-2 spring at the beginning (Sept 1, 2015) and end of the sampling (Sept 2, 2015) period (Table 1). Air and water temperatures for the beginning and end of the experiment were within 1.5% of each other while pH and dissolved methane concentration values were within 5%. However, dissolved carbon dioxide concentrations in the pool dropped 60% from the initial measurement to the final measurement after sampling.

2.3.2 CH_4 flux

CH_4 concentrations in the flux chamber increased over time during the 24 hr experiment (Figure 2.2A). Initially CH_4 concentrations were below our detection limits (<3.3 x10^{-7} mol/L), and the CH_4 concentrations remained that way for the first 2 hours and 12 minutes (132 minutes). Once detected, CH_4 concentrations continued to increase over time to a final concentration of 2.7 x 10^{-6} (± 4.9x10^{-8}, 1σ, n=2) mol/L. Changes in CH_4 concentrations measured between 2 hours and 12 minutes (132 minutes) to 20 hours and 7 minutes (1207 minutes) were well described by a linear approximation (r^2 = 0.96). Therefore the CH_4 concentrations at these two times points were used in Equation 3 to calculate a CH_4 release of 4.6 x 10^{-7} mol/m^2min out of the WHC2 ultra-basic pool.

2.3.3 CO_2 flux

Conversely, CO_2 concentrations in the flux chamber decreased over time during the 24 hr experiment (Figure 2.2B). At the beginning of the experiment there was 8.9 x 10^{-5} mol/L (± 3.1x10^{-6}, 1σ, n=2) of CO_2 in the chamber. During the first phase of sampling (i.e., 0 minutes to 3 hours and 46 minutes (226 minutes) the CO_2 concentrations declined to a value of 5.62x10^{-5} mol/L (± 1.3x10^{-6}, 1σ, n=2) of CO_2. During the second phase of sampling (i.e., 13 hours and 40 minutes (820 minutes) to 20 hours and 7 minutes (1207 minutes) CO_2 concentrations were below our detection limits (i.e., <4.7x10-5 mol/L). Changes in CO_2 concentrations measured between 12 minutes, and 3 hours and 46 minutes (226 minutes) were well described by a linear approximation (r^2 = 0.91). The CO_2 concentrations at these time points were then used in Equation 3 to calculate a CO_2

sequestration of 1.9 x 10^{-5} mol/m^2min into the WHC2 ultra-basic pool. Therefore, CO_2 was being sequestered 41 times faster than CH_4 being released.

2.3.4 N_2O flux

N_2O concentrations in the gas phase in the chamber remained within our analytical error during the 24 hr experiment (Figure 2.2C). The average concentration of N_2O was of 3.2 x 10^{-8}(± 2.6x10^{-9}, 1σ, n=10) mol/L. Therefore, the flux of N_2O in the chamber over the 24 hr experiment was negligible for the WHC2 ultra-basic pool.

2.4 Discussion

2.4.1 CO_2 coefficient of chemical enhancement

In this study we calculated CO_2, CH_4 and N_2O gas fluxes into and out of ultra-basic serpentinization-associated groundwater discharging and pooling in Winterhouse Canyon of Gros Morne National Park, NL, Canada. These gas fluxes were calculated using Equation 3 and gas concentrations determined from samples collected over a 24 hour period. However, as mentioned previously, this is not the only method used for calculating flux. A less labor-intensive method for estimating flux could have been used if the site-specific parameters such as the gas transfer velocity (k), the coefficient of chemical enhancement (α), and initial gas concentration differences between the measured value and the theoretical concentration at equilibrium with the overlying atmosphere were known. While the α for CH_4 is, by convention, set to 1 because it does not participate in hydrolysis reactions (Wanninkhof and Knox 1996), the α for CO_2 for serpentinizing systems is unknown. However, we can use the information gained in this study to determine the unknown parameters in equation 2, potentially allowing for future studies of this site and other similar sites of serpentinization to avoid the labor intensive methods described above.

The k_{CH4} can be calculated for our study system using Equation 2 by substituting the measured CH_4 flux (4.6 x 10^{-7} mol/ m^{-2} min) and setting α_{CH4} to 1. Once the k value is known within a system for a specific gas and temperature it can be calculated for any other gas based on the ratios of the Schmidt numbers via the following equation (Jähne et al. 1987):

(4) $$k_{CO2} = k_{CH4}(\frac{Sc_{CO2}}{Sc_{CH4}})^n$$

where Sc_{CO2} and Sc_{CH4} are the Schmidt numbers 783 and 798 for CO_2 and CH_4, respectively. The variable n ranges from –2/3 for a smooth water surface and 1/2 for a turbulent surface and was set to -2/3 because the surface was smooth (Jähne et al. 1987).

C_{sat} values for CH_4 and CO_2 (1.3 x 10 $^{-5}$ mol/m^3 and 1.0x 10 $^{-1}$ mol/m^3 respectively) were calculated using Henry's law (applicable for ideal gas mixtures and dilute solutions) (MacIntyre et al. 1995; Raymond and Cole 2001):

(6) $$C_{sat} = C_g R T K_h$$

Where C_g is the initial concentration of gas in the atmosphere (taken as the initial concentration in the headspace of the closed chamber) (3.3 x 10 $^{-7}$ mol/L and 8.9x 10 $^{-5}$

mol/L for CH_4 and CO_2 respectively); R is the universal gas constant (m^3 Pa/k mol); T is temperature (285 K and 291 K during the beginning and end of the sampling for CO_2 and CH_4 respectively); and K_h is a temperature-dependent Henry's constant ($1.6x\ 10^{-5}$ mol/m^3 Pa and .0005 mol/m^3 Pa for CH_4 and CO_2 respectively at 283K) (Sander 2015). To solve for α in Equation 2, the measured dissolved gas concentrations from the beginning of the experiment was used for C_{gasw} (Table 2.1).

We calculated an α for CO_2 of 22.7 by substituting our CO_2 flux (-1.9×10^{-5}mol*m^{-2}*min^{-1}), k_{CO2} (7.9×10^{-6} m/min), CO_{2gasw} (3.2×10^{-8} mol/L), CO_{2sat} (1.0×10^{-4} mol/L) values into Equation 2. To our knowledge, there are no other studies that report CO_2 enhancement factors in waters associated with sites of serpentinization. In the absence of site-specific α values, high pH lakes may be considered the closest analogues to our system. For example, CO_2 enhancement factors at a high pH lake were between 3.5 to 7.5 for a pH range of 9.45 to 9.75 (Bade and Cole 2006). Similarly, CO_2 chemical enhancement factors at both the Mono Lake (pH 9.8) and Big Soda Lake (pH 9.5) were 4.9 and 27.5 respectively for CO_2 invasion into water (Wanninkhof and Knox 1996). Therefore, our calculated α_{CO2} falls within the range of high pH lakes. If pH were the only factor affecting the chemical enhancement of CO_2, then we would have expected the chemical enhancement calculated from data collected at WHC2, a water body with a pH of >12, to be higher than the chemical enhancement of CO_2 from the lower pH lakes mentioned above. Factors such as temperature and ionic strength also clearly affect the chemical enhancement at WHC2.

2.4.2 Natural global warming potential

In this study we observed that CO_2 is sequestered from the atmosphere 41 times faster than CH_4 gas is emitted to the atmosphere at the WHC2 ultra-basic pool in the Tablelands. However, the direct climate-change effect of this exchange cannot be determined using fluxes alone since CH_4 is a more powerful greenhouse gas than CO_2 and the gases have different atmospheric residence times. The Intergovernmental Panel on Climate Change (IPCC) uses radiative forcing to calculate the effect of a change is gas concentration on the overall energy balance (in W/m^2) between incoming solar radiation and the energy re-radiated back into space (IPCC 2013), such that the radiative forcing caused by CO_2 sequestration can be directly compared to the radiative forcing of CH_4 being emitted at the surface of the WHC2 ultra-basic pool. To determine the current effect this has on the atmosphere the radiative forcing can be used to calculate a gas' affect on the overall energy balance. Using Equations 6a and 6b for CO_2 and CH_4 respectively the net radiative forcing (RF) of this site was calculated. The gas concentrations at 2 hours and 12 minutes (132 minutes), and 3 hours and 46 minutes (226 minutes) were used in Equations 6a and 6b because they are the only times in the linear range where neither CO_2 nor CH_4 were below detection. In addition, the measured N_2O concentration in the headspace was used in the following equation:

$$\text{(6a)} \qquad RF_{CO2} = \alpha \ln (C/C_0)$$

$$\text{(6b)} \qquad RF_{CH4} = \alpha (\sqrt{M} - \sqrt{M_0}) - (f(M, N_0) - f(M_0, N_0))$$

Where RF_{CO2} and RF_{CH4} are radiative forcing values for CO_2 and CH_4 respectively, α is 5.35 and 0.036 for CO_2 and CH_4 respectively, M is CH_4 in ppb (taken at 3 hours and 46 minutes), N is N_2O in ppb (taken at 3 hours and 46 minutes), and $f(M,N) = 0.47 \ln[1+ 2.01x10^{-5} (MN)*0.75 + 5.31x10^{-15} M(MN)^{1.52}]$, and the subscript 0 refers to unperturbed molar fraction of the species (taken at 2 hours and 12 minutes) (IPCC 2013).

The RF associated with the CO_2 sequestered between 2 hours and 12 minutes (132 minutes) to 3 hours and 46 minutes (226 minutes) for CO_2 was -0.22. This negative value indicated an overall atmospheric cooling effect from the CO_2 removal from the atmosphere. In contrast the RF for the CH_4 releases at the same time was +0.01. This positive value indicated a warming effect from the CH_4 addition to the atmosphere alone. However, the net RF due to the changing concentrations of CO_2 and CH_4 was -0.21. Therefore, the negative net RF value calculated at this site of serpentinization suggests that the ultra-basic pool in WHC has a net cooling effect on the atmosphere.

While radiative forcing provides a prediction of the immediate impact of changing GHG concentration it does not consider the different residence times of the gases in the atmosphere. In addition to radiative forcing, the IPCC also uses the global warming potential (GWP) to make future predictions about the impacts of different greenhouse gases. GWP is a relative measure of the heat that a greenhouse gas traps in the atmosphere over a specific time horizon as compared to the amount of heat trapped by an equivalent mass of CO_2. The GWP is calculated as a ratio of the time-integrated radiative forcing from the instantaneous release of 1 kg of a trace substance relative to the release of a 1 kg reference gas where CO_2 has a GWP of 1 (IPCC 2001). This relative value is a function of the residence time of the gas in question, such that GWP changes with time. For example, CH_4 has a GWP value of 86 over a time horizon of 20 years and a decreased GWP value of 34 over a time horizon of 100 years (IPCC 2013).

To determine the overall long-term effect of this site the GWPs of the gases must be converted to a CO_2 equivalent for various time horizons. The conversion of GWP to CO_2 equivalence is simply a 1:1 ratio because a gas's GWP is relative to that of CO_2. Over a 20-year time horizon the GWP of methane is 86 (CO_2 equivalence of 86) but the site is removing 41 times more CO_2 than methane (CO_2 equivalence of -41). Therefore, the CO_2 equivalence can be added to get a net CO_2 equivalence of 45 over 20 years, referring to a net global warming potential of 46 and a heating of the atmosphere. In contrast, over a 100 year time horizon the GWP of methane is 34 (CO_2 equivalence of 34) but the site is removing 41 times more CO_2 than methane (CO_2 equivalence of -41). Therefore, over 100 years the site has a net CO_2 equivalence of -7, referring to a global warming potential of -7, and a cooling of the atmosphere, congruent with the RF prediction. However, this calculation would only be relevant once the groundwater spring became inactive. While inactive springs have been observed based on carbonate deposits at sites of serpentinization, determining the average time for this process to occur was outside the scope of this study.

While these results show the immediate and long-term impact of this specific ultra-basic pool, this is a small site that likely makes a relatively insignificant environmental impact.

However, within the Tablelands alone there are several other ultra-basic pools where carbonates have been observed and that are potentially taking in CO_2 and releasing CH_4. In addition, carbonate has been found near sites of runoff without pooling, indicating potential sequestration in the absence of ultra-basic pools. Moreover, sites of serpentinization like the Tablelands can be found all over the world. However, fluxes for each site must be considered on a case-by-case basis. For example, the Cedars, a site of serpentinization in Sanoma, California, features bubbling gases from ultra-basic springs, likely indicating higher CH_4 concentrations and flux than measured at Winterhouse Canyon (Morrill et al. 2013). With this in mind, some of these sites, specifically the Oman ophiolite, have been proposed for enhanced carbon capture storage that would involve injecting CO_2 to enhance carbon sequestration (Kelemen and Matter 2008). However, a recent study has also shown that under certain conditions CO_2 can be converted to CH_4 microbially at the Cedars (Kohl et al. 2016). Therefore, before we focus on ways to modify these systems to enhance CO_2 sequestration we must first gain a better understanding of the natural baseline of the sites and their impacts on the environment as a whole. This study is the first to consider the impact of green house gases (CO_2, CH_4, N_2O) coming into and out of an ultra-basic pool above serpentinized rock and highlighted the need for more research on the net impact of serpentinization globally.

Tables

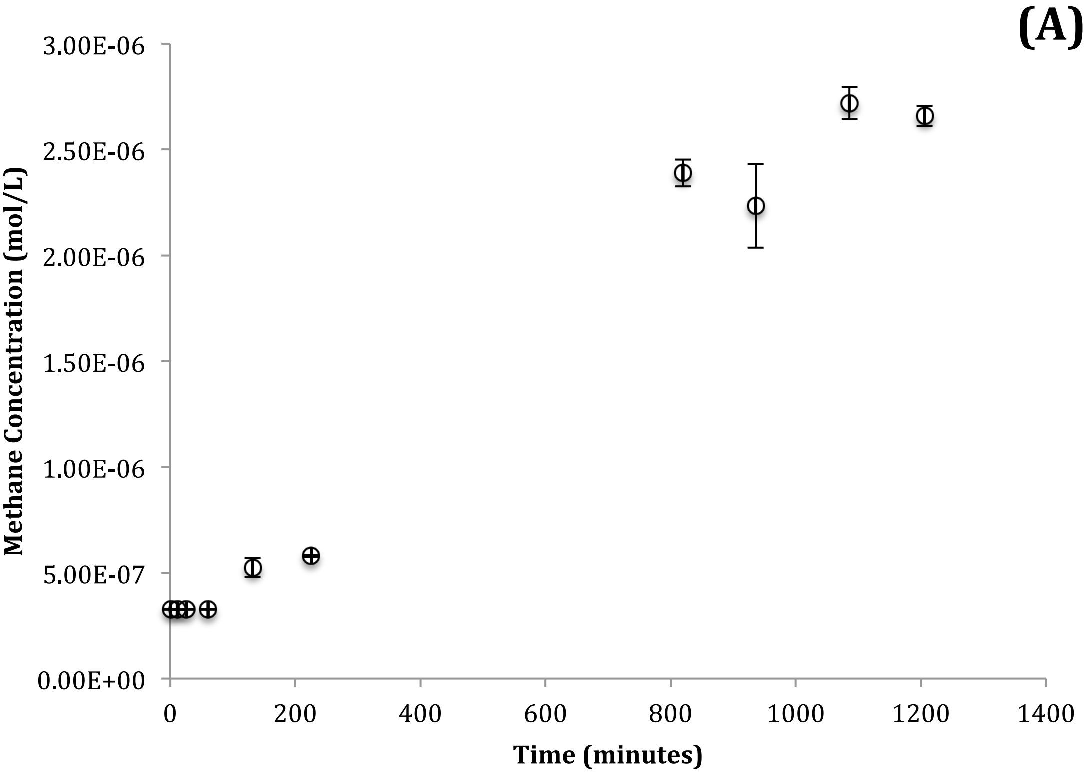
Table 2.1. Sampling conditions for Winterhouse Canyon Spring (WHC-2b)

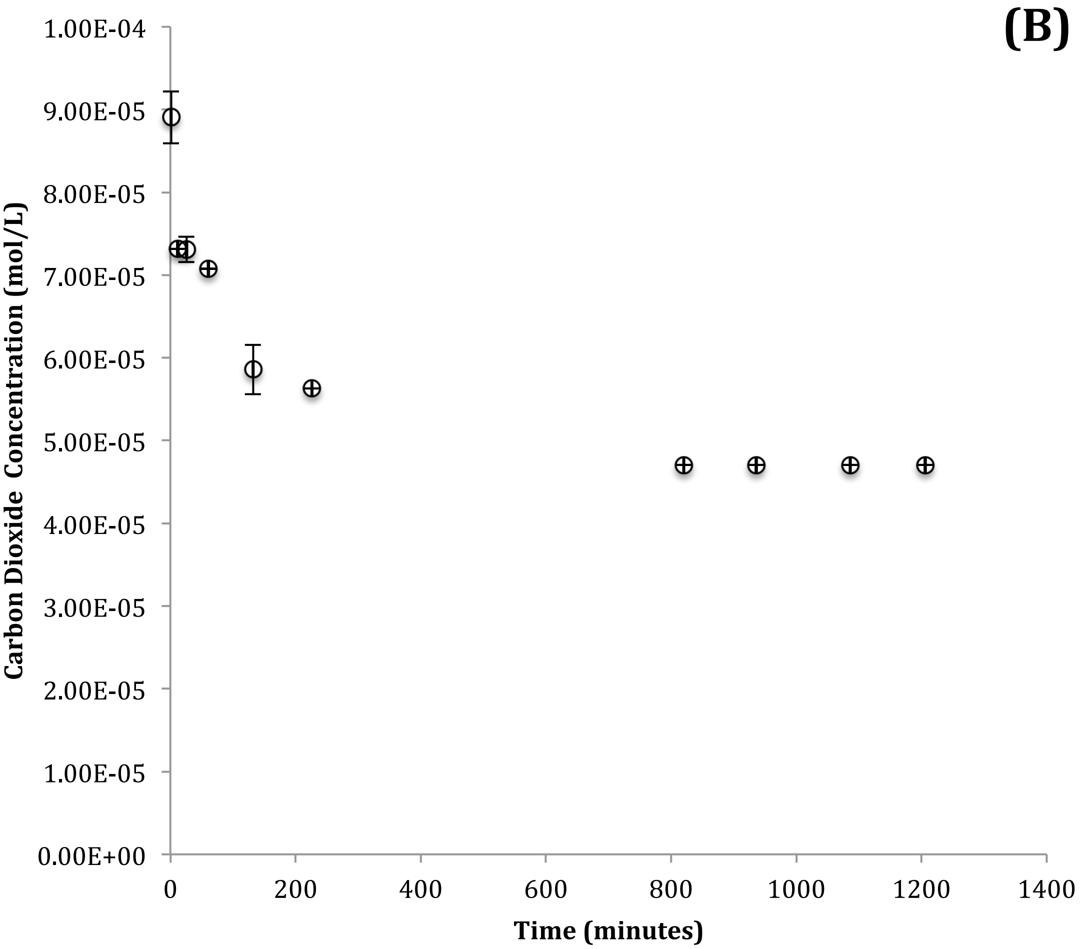
	Sep 1 2015 13:30	Sep 2 2015 16:30
Field Air Temperature (K)	285	291
Field Water Temperature (K)	288	285
Lab Air Temperature	293	
pH	12.72	12.1
Conductivity(ms)	4.37	3.32
CO_2 in Water Conc.(mol/L)	3.2E-08	1.9E-08
CH_4 in Water Conc. (mol/L)	5.9E-05	5.5E-05
Average wind *Speed (km/hr)	6.36	

* Taken as an average of the hourly reported data from "TheWeatherNetwork" for the 24- hour period at the Rocky Harbor Weather Station (http://climate.weather.gc.ca/climate_data/hourly_data_e.html?StationID=6938&timeframe=1&Year=2015&Month=9&cmdB1=Go&Day=2)

Figures

Figure 2.1:
Closed floating chamber deployed in Winter House Canyon Spring 2 (WHC2). The chamber was supported with styrofoam. Gases collect in the headspace of the chamber and were sampled over a 24 hour period from the top of the chamber. The white rock surrounding the pool is calcium carbonate.


(A)
3.00E-06
2.50E-06
2.00E-06
1.50E-06
1.00E-06
5.00E-07
0.00E+00
Methane Concentration (mol/L)
0
200
400
600
800
1000
1200
1400
Time (minutes)


(B)
1.00E-04
9.00E-05
8.00E-05
7.00E-05
6.00E-05
5.00E-05
4.00E-05
3.00E-05
2.00E-05
1.00E-05
0.00E+00
Carbon Dioxide Concentration (mol/L)
0
200
400
600
800
1000
1200
1400
Time (minutes)

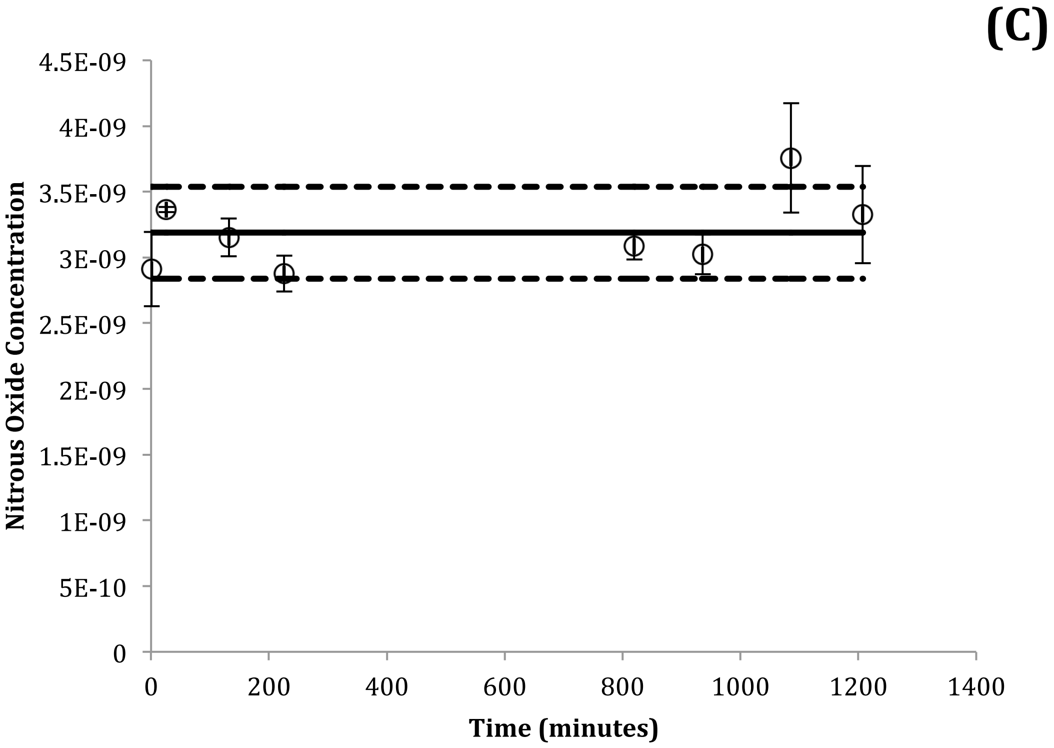


Figure 2.2. Time series data for gas concentrations sampled from the closed chamber floating over an ultra-basic pool created by a groundwater springs associated with serpentinization in the Tablelands, Gros Morne, NL: (A) methane, (B) carbon dioxide, and (C) N_2O (See Appendix 2.2). For (C) the dashed lines indicate the analytical error associated with the GC-ECD analysis. Error bars on all plots indicate standard deviation of the mean plotted point based on sample duplicates.

3 Chapter 3: Sourcing Dissolved and Diffuse Methane: Do common collection and concentration methods isotopically fractionate?

Abstract

Typical CH_4 sourcing methods involve using stable isotope analysis to genetically discriminate the sources (Schoell 1980). However, if common collection and concentration methods result in isotopic fractionating of either C or H in the sample it would change these isotopic signatures and make sourcing difficult. In this paper several common collection and concentration methods for isotopic analysis of dissolved and diffuse CH_4 were tested for isotopic fractionation in the laboratory and the field. The vacuum extraction and gas stripping methods were both shown to be non-isotopically fractionating for carbon and hydrogen isotopes (within the +/- 0.5‰ and +/- 5‰ error for carbon and hydrogen respectively) and are therefore suitable for gas collection for the purposes of sourcing. After testing gas collection methods the paper then tested cryogenic concentration methods for isotopic fractionation. Cryogenic concentration is typically used when gas concentrations are too low for isotopic analysis. Similarly, no carbon or hydrogen isotopic fractionation was observed for 5 of 6 cryogenically trapped samples (within the +/- 0.5‰ error and +/- 5‰ error for carbon and hydrogen respectively). Cryogenic concentration was also used to test for isotopic fractionation by diffusion across the liquid phase boundary, and again no carbon isotopic fractionation was observed (within +/- 0.5‰ error). Finally, this paper compared isotopic fractionation factors during the formation of abiogenic CH_4 (from reactants to products) to those of

microbial CH_4 to demonstrate another line of evidence for differentiating between abiogenic and microbial samples.

3.1 Introduction

3.1.1 Sourcing CH_4

Methane (CH_4) detected at the Earth's surface can typically be found either dissolved in surface waters or diffusing into the atmosphere (Schoell 1988). However, this CH_4 is rarely generated at the surface and typically originates from subsurface systems such as rock-water reactions, thermogenic production, microbial production, or even leaking pipelines (Schoell 1988). In addition to CH_4 on Earth, CH_4 has been detected on Mars by the Curiosity rover, leading to speculation whether these findings are the first example of life on other planetary bodies and moons (Webster et al. 2015; Zahnle et al. 2011). However, detecting CH_4 alone cannot determine its source and more lines of evidence are needed to differentiate between the various potential sources. CH_4 sourcing typically occurs at sites that feature high concentrations of bubbling gases and uses the $\delta^{13}C$ and δD isotopic values of the gas to determine the source. However, CH_4 concentrations discovered in the Martian atmosphere are significantly lower than those required for standard isotopic measurements, meaning gases must be concentrated prior to analysis. Moreover, there exists a knowledge gap on whether gas collection and concentration methods at sites of low concentrations of dissolved CH_4 maintain the isotopic integrity of the CH_4. Isotopic fractionation during either collection or concentration of the gas could alter the isotope ratios and make accurate sourcing more difficult. Therefore, this isotopic fractionation would either need to be removed by using another method or measured and accounted for during isotopic analysis.

On Earth, there are three known mechanisms for CH_4 production: microbial, thermogenic, and abiogenic, each yielding a characteristic carbon and hydrogen isotopic signature(Schoell 1988).

Sourcing CH_4 can be difficult and multiple lines of indirect evidence are often needed. Traditional sourcing methods involve using stable isotope analysis to genetically discriminate the different sources of CH_4 based on the isotopic ratios of both the carbon ($^{13}C/^{12}C$) and hydrogen ($^{2}H/^{1}H$, often referred to as D/H,). The combination of carbon and hydrogen isotope values are then plotted on a ^{13}C - D plot to create general regions for different sources.

Stable isotope ratios are reported using standard δ-notation ($\delta^{13}C$, δD) using the Equation 1 below (Coplen 2011):

$$(1) \qquad \delta = [(R_{sample}/R_{standard}) - 1]$$

Where R is the ratio of ($^{13}C/^{12}C$) relative to the Pee Dee Belemnite (PDB) and D/H relative to the standard mean ocean water (SMOW) (reported in parts per thousand (‰)) for carbon and hydrogen respectively.

While these plots, along with hydrogeological context and microbiological evidence, have mostly been successful in differentiating between biogenic sources (microbial or thermogenic), there are a wide range of abiogenic carbon and hydrogen isotope values that overlap with areas that were once considered to be biogenic (Horita and Berndt 1999; McCollom and Seewald 2006).

Overall, plots considering only the $\delta^{13}C$ and δD fail to account for the formation mechanisms and the role reactants and catalysts play in the production process. However, if the carbon and hydrogen isotopic fractionation factors between reactants and products (between CO_2 and CH_4, and H_2 and CH_4 respectively) are plotted, the effect of the δ values of the source materials is accounted for and the plot may provide another line of evidence.

Isotopic fractionation is the enrichment of one isotope relative to another in a compound due to a chemical or physical process. To quantify this enrichment an isotopic fractionation factor is used to compare the relative presence of an isotope before and after a process. For example, Equation 2 shows the carbon fractionation factor from products to reactants ($\alpha_{p\text{-}r}$) in the production of CH_4 from CO_2:

$$(2) \qquad \alpha_{p\text{-}r} = \frac{({}^{13}C_{CH4}/{}^{12}C_{CH4})_p}{({}^{13}C_{CO2}/{}^{12}C_{CO2})_r}$$

Isotopic fractionation factors during the formation of the CH_4 can potentially provide another line of evidence for sourcing. For example, previous studies suggest that microbial formation pathways can be distinguished by plotting the isotopic fractionation factors of both the hydrogen and the carbon during the production process (from reactant to product) (Whiticar et al. 1986). Microbial CH_4 is formed through two primary microbial metabolic pathways: fermentation and CO_2 reduction (Whiticar et al. 1986). Fermentation-derived CH_4 involves the transfer of a methyl group from a substrate (primarily acetate) and is considered to be the major pathway for microbial CH_4 production (approximately 70%) in freshwater environments (Whiticar et al. 1986). While Whiticar et al. (1986) provided ranges of isotopic fractionation factors for differentiating microbial CH_4 produced via fermentation and CO_2 reduction, a comprehensive review of carbon and hydrogen isotope data for microbial and abiogenic formation mechanisms covering is needed.

In addition to differentiating pathways of microbial $CH_{4,}$ creating a plot of isotopic fractionation factors for carbon and hydrogen during the production process of CH_4 may provide an additional line of evidence in the sourcing of $CH_{4.}$ Sherwood Lollar et al. (2008) plotted fractionation factors for a Kidd Creek field sample of potential abiogenic origin with previous data for microbial CH_4. The abiogenic samples fell outside of the microbial zones (Sherwood Lollar et al. 2008). More research is needed in order to determine whether isotopic fractionation factors can be used to differentiate abiogenic CH_4 from microbial sources. To accomplish this a meta-data analysis of microbial and abiogenic fractionation factors is calculated and plotted on a fractionation-fractionation plot.

3.1.2 Isotopic fractionation during collection and concentration

In order to isotopically analyze dissolved gases at low concentrations for the purposes of sourcing, CH_4 first needs to be extracted from the water, and if its concentration is low then it must be concentrated before isotopic analysis. However, extraction and concentration methods must be tested to ensure that they do not change the isotope values of the CH_4 sample.

Collection of dissolved CH_4 samples from water for isotopic analysis is typically done using the vacuum extraction method as it has been assumed to quantitavely convert all dissolved gas to the gas phase (Sherwood Lollar et al. 2008; Slater et al. 2008). However, in addition to this method, the gas stripping method is sometimes used to extract dissolved CH_4 from water for isotopic analysis (Etiope et al. 2016). However, in contrast to the vacuum extraction method, this method has long been assumed by isotope geochemists to be isotopically fractionating as it potentially does not quantitatively convert all dissolved gas from the liquid to the gas phase (Penny Morrill, Personal Communication, September 15 2014). To the best of our knowledge there is currently no comprehensive study whether these processes maintain isotopic integrity of the CH_4.

In addition to CH_4 being extracted from the water, CH_4 diffusing from the water into the air may also be collected for isotopic analysis. However, depending on the initial concentration of CH_4 in the water, samples of dissolved and diffuse CH_4 may need to be concentrated before isotopic analysis. Concentrating a gas sample can be achieved using cryogenic trapping to reducing the temperature of the gas such that more moles of gas can occupy a fixed volume. Based on the ideal gas law (Equation 3), if the pressure (P) and volume (V) are held constant then the number of moles (n) collected in the sample is inversely proportional to the temperature of the bottle (T) multiplied by the gas constant R.

(3)

$$n = \frac{PV}{RT}$$

Cryogenic trapping has been tested previously for carbon isotope fractionation by using a single step extraction system to concentrate a sample of dissolved chlorinated ethenes with known carbon isotope values and then injecting them into a gas chromatograph isotope ratio mass spectrometer (Morrill et al. 2004). The results demonstrated that the cryogenic trap method was non-isotopically fractionating for carbon isotopes in chlorinated ethanes (Morrill et al. 2004). However, to the best of our knowledge, there are currently no studies that test for isotopic fractionation during the cryogenic concentration of CH_4.

In addition to isotopic fractionation effects from collection and concentration, if the gas sample in the bottle is under-pressurized before isotopic analysis then isotopic fractionation is possible. In a series of tests on under-pressurized samples, sample bottles were filled with CH_4 with known $\delta^{13}C$ and δD values such that the final pressure was less than 1 atm. Following bottle preparation gas, was extracted from the sealed bottles using a gas-tight locking syringe and analyzed for its carbon and hydrogen stable isotope values. Of the four injections performed, all were depleted in ^{13}C and two fell outside the standard analytical error for $\delta^{13}C$ of ± 0.5 ‰ (Ward 2002). This Study assumed hydrogen isotopic fractionation would also occur in underpressurized bottles given the observed given carbon isotopic fractionation (Ward 2002). Therefore, in order to maintain isotopic integrity of the CH_4, all sample bottles were over-pressurized prior to isotopic analysis.

3.1.3 Isotopic fractionation during diffusion

When analyzing isotopic values of CH_4 diffusing from pools of water into air it is important to also consider the potential for isotopic fractionation across the water/air phase boundary. If isotopic fractionation by diffusion is not accounted for it could alter the isotopic values and potentially lead to inaccurate sourcing.

Isotopic fractionation by diffusion is caused by the different motilities of isotopic molecules as they pass through various media. Diffusion across a phase boundary is limited by the slower diffusion rate between the two media (in this case diffusion into water) (Knox et al. 1992). However, when the system is in equilibrium (i.e., gases are both coming into and out of the water at equal rates) isotopic fractionation can be determined by the fractionation during gas dissolution (Equation 4).

$$\alpha_{eq} = \frac{(H/L)_d}{(H/L)_g} \quad (4)$$

Where H is the equilibrium concentration of the heavier isotopic species, L is the equilibrium concentration of the lighter isotopic species, and d and g are the dissolved and gas phases.

The first objective of this chapter of my thesis is to test methods of gas collection and cryogenic concentration of CH_4 for carbon and hydrogen isotopic fractionation. The second objective was to perform a metadata analysis of all previous studies that report carbon and hydrogen isotopic data for the reactants and products during the formation of microbial and abiogenic CH_4 and create a detailed plot of αD and $\alpha^{13}C$ for microbial and abiogenic CH_4 to determine if a fractionation-fractionation plot can differentiate these two CH_4 sources.

3.2 Methods

3.2.1 Laboratory testing of collection and concentration methods

The $\delta^{13}C$ and $\delta^{2}H$ values of the laboratory CH_4 were determined before testing sampling and concentrating methods for isotopic fractionation. Two tanks of 99 % pure CH_4 were isotopically characterized. CH_4 from the first tank (17L at 40 psig of 99% CH_4 supplied by Air Liquide) was diluted in He gas by removing 5 mL of tank gas and injecting it into a 35 mL bottle prefilled with helium (He). This sample of CH_4 was then analyzed for its $\delta^{13}C$ and δD values. CH_4 from this tank was subsequently dissolved into water. This water containing dissolved CH_4 was used to test various collection and concentration methods for isotopic fractionation in the laboratory. When the first tank was close to empty, a second tank (7200L of 99% CH_4 at 1700 psi supplied by Air Liquide) was purchased and isotopically analyzed. Similar to the first tank, CH_4 was diluted by injecting 5 mL of gas into 3 different 35 mL bottles that were prefilled with He. These samples were then analyzed for $\delta^{13}C$ and δD and CH_4 from this tank was used to test for isotopic fractionation during cryogenic concentration.

To dissolve CH_4 in water a 2 L Kimble bottle was completely filled with deionized water and sealed using a black butyl septum conditioned in a NaOH solution. Next, 20 mL of 99% pure CH_4 at 1 atm with known isotopic composition was injected through the septa and allowed to completely dissolve into the water. After 72 hours at 24 °C the gas bubbles were gone, ensuring quantitative conversion of gas phase CH_4 to dissolved phase CH_4. CH_4 was then extracted from this solution to test for isotopic fractionation in collection methods.

A gas collection method, known as gas stripping, was tested to determine if it isotopically fractionated the carbon and hydrogen isotope values of dissolved CH_4. The gas stripping method involves transferring dissolved gases from the liquid phase to a gas phase. It is frequently used for concentration measurements, but is not commonly used for isotopic measurements, because of the potential for non-quantitative conversion. Twenty-five mL of He gas was taken up into a 60 mL syringe and an equal volume of water containing dissolved CH_4 was taken up in the same syringe (Rudd et al. 1974). The two phases were shaken together for 5 minutes whereby the He gas stripped the CH_4 from the water. Next, the gaseous headspace containing the CH_4 was transferred into an inverted 35 mL bottle completely filled with degassed water and sealed using a blue butyl septum. The injected headspace displaced an equal volume of water via an exit needle. The exit needle was removed when the water level approached the top of it, ensuring no gas escaped from the bottle. The whole process was then repeated without an exit needle such that 50 mL of headspace was ultimately injected into a 35 mL bottle, over pressurizing the sample by 15 mL.

Another gas collection method, known as the vacuum extraction method, was tested to determine if it isotopically fractionated the carbon and hydrogen isotope values of dissolved CH_4. The vacuum extraction method transfers gases from the liquid to gas phase via the pressure difference created by the vacuum. To test the vacuum extraction method in the laboratory 80 mL of water containing dissolved CH_4 was added to a 160 mL evacuated bottle such that an equal ratio of sample to bottle volume was achieved. This procedure was repeated with 125 mL of water and a 250 mL evacuated bottle. The large depressurization from the vacuum then transferred gases from the liquid to the gas phase in the headspace of the bottle. The larger 250 mL bottle was used for hydrogen isotope analysis due to the greater number of injected moles of H required for δD measurements.

After testing gas collection methods for isotopic fractionation, cryogenic trapping methods for gas concentration were then tested in the laboratory. To test for isotopic fractionation during cryogenic trapping various volumes of gaseous CH_4 with known isotopic values were transferred cryogenically into a cooled 160 mL bottle.

First, a 160 mL bottle was evacuated to 50 mm Hg and then sealed using a blue butyl septa conditioned in NaOH. This evacuated bottle was then connected using 0.2 m of ¼ in diameter high density polyethylene (HDPE) tubing and two-way valves to two other 160 mL bottles prefilled with 240 mL of the isotopically characterized CH_4. The evacuated bottle was then placed into a liquid nitrogen cryogenic trap that reduced the temperature in the bottle to 77 K (-196°C). The valve connecting the evacuated 77 K bottle to the CH_4-filled room temperature bottles was then opened for 5 minutes. Gas was transferred from the CH_4 filled bottles, due to the pressure and temperature differences, to the cryogenically cooled bottle. This experiment was then repeated with three (as opposed to two) 160mL bottles prefilled with 240 mL of CH_4 being concentrated into an evacuated 160 mL bottle. The purpose of this experiment was to determine if changing the amount of CH_4 being trapped into the evacuated bottle induced isotopic fractionation.

Finally, isotopic fractionation of dissolved CH_4 mass transfer from liquid to gas, and subsequent cryogenic trapping simulating field-sampling conditions (see Chapter 2), was tested. Similar to the previous experiment, a closed floating chamber was inverted and placed 4 cm into water containing dissolved CH_4 such that all diffusing CH_4 was contained in the headspace of the chamber. CH_4 concentrations in the headspace were measured intermittently until equilibrium was achieved; so as to best simulate equilibrium field conditions. Equilibrium was operationally defined as the time when CH_4 concentrations in the headspace at three consecutive time points were within 5 % of each other. After equilibrium was achieved the tubing from the top of the container was then connected using a two-way valve to an evacuated 160mL bottle that was sealed with a blue butyl septa and placed in a liquid nitrogen bath. The valve was then opened for 5 minutes allow gas from the container to transfer into the 160 mL bottle due to the pressure and temperature differences.

3.2.2 Field testing gas collection

3.2.2.1 Field site description

In addition to the laboratory experiments, dissolved gas collection methods (i.e. gas stripping and vacuum extraction methods) were also used to collect dissolved CH_4 from a groundwater spring in the Tablelands in Gros Morne from August 31st –September 1st 2015. In the Tablelands, subsurface groundwater reacting with ultramafic rock in a process known as serpentinization produces active and ultra-basic (pH 10-12) springs that can be found discharging at the surface in pools (Szponar et al. 2013). This study focused on the WHC2 groundwater discharge point, found in the valley of Winterhouse Canyon, as this spring has been previously shown to have concentrations of dissolved CH_4 (0.04 mg/L to 0.38 mg/L) (Szponar et al. 2013). Moreover, these springs produce a unique sourcing challenge, as all three types of CH_4 can be present (Szponar et al. 2013). In order for the dissolved CH_4 to be sourced by stable isotope analysis, it must be first extracted from the water by a non-fractionating means. If the CH_4 extraction method is isotopically fractionating it can change the isotopic signature of the sample and make accurate sourcing using isotopes difficult. Therefore, testing CH_4 collection methods for isotopic fractionation at these sites is critical to the sourcing process.

3.2.2.2 Field sample gas collection methods

Dissolved gas from the WHC2 pool was first collected for carbon isotope analysis using the gas stripping method described in section 3.2.1. In short, two 60 mL syringes were filled with 25mL of He and 25mL of spring water. The two phases were shaken for 5 minutes to transfer the dissolved gases from the water into the headspace. The 50 mL headspace from the two syringes was then injected into a 35 mL bottle previously filled with degassed water. The process was then repeated for hydrogen isotope analysis but with two 60mL syringes filled with 25ml of sample and 25mL of He.

Next, gas samples were extracted using the vacuum extraction method (section 3.2.1) with 80 mL and 240 mL of pool water injected into a previously evacuated 160mL and 500mL bottles for carbon and hydrogen isotope analysis, respectively.

3.2.3 Analytical methods

3.2.3.1 Stable carbon and hydrogen isotopic measurements

Gas chromatography combustion isotope ratio mass spectrometry and gas chromatography pyrolysis isotope ratio mass spectrometry were used to analyze carbon and hydrogen stable isotope values, respectively, of CH_4 ($\delta^{13}C$ and δD) using an 6890N gas chromatograph (Agilent) connected to a Delta V Plus isotope ratio mass spectrometer (IRMS) via either a GC combustion unit (GC/C III; Thermo Scientific) or through a high temperature micro pyrolysis furnace (GC/TC; Thermo Scientific). A Carboxen 1010 capillary column (30 m x 0.32 mm x 15 mm) with a 10:1 split ratio and a temperature program of 40 °C for 6 min, to 110 °C at 25 °C/min, hold 8 min was used to separate H_2,

CH_4, CO, and CO_2 for $\delta^{13}C$ of CH_4. Similarly, the same column with a 5:1 split ratio and a temperature program of 110 °C for 5.5 min, to 180 °C at 35 °C/min, hold 2 min was used to separate H_2, CH_4, CO, and CO_2 for δD of CH_4. Through testing, standard error for compound specific isotope analysis on the GC was ± 0.5‰ and ± 5‰ for $\delta^{13}C$ and δD measurements, respectively (Ward 2002). These errors were determined through tests that varied sample bottles, split settings, injection sizes, syringes and gas concentrations.

CH_4 concentrations were measured using a SRI 8610 gas chromatograph with a flame ionization detector (GC-FID). A Carboxen 1010 fused silica capillary column with a helium carrier gas and a temperature program of 40°C hold 6 minutes, ramp 15°C/minute to 120°C, hold 5 minutes, was used to separate the specific gases. After column separation a methanizer converted the CO_2 to CH_4 so that it could be analyzed using the FID. Daily calibration curves for CH_4 were made by injecting varying volumes (30-500 µl) of a Restek 34522 standard containing 100 ppm of CH_4 using a 500-microliter gas tight locking Hamilton syringe. The lower detection limit for the GC-FID for CH_4 was a concentration of 3.3×10^{-7} mol/L.

3.3 Results

3.3.1 Isotopic characterization of laboratory CH_4 gas

Before testing the collection and concentration methods in the laboratory CH_4 was first isotopically characterized for its $\delta^{13}C$ and its δD values. The 1st gas tank of CH_4 had an average $\delta^{13}C$ value of -40.1 ± 0.1 ‰ (n=5) and an average δD -167 ± 1 ‰ (n=2). The 2nd gas tank of CH_4 had an average $\delta^{13}C$ value of -41.3 ± 0.1 ‰ (n=3), and an average δD -205 ± 4‰ (n=3).

3.3.2 Testing CH_4 gas extraction methods for isotopic fractionation

3.3.2.1 Carbon isotopes

The average $\delta^{13}C$ value of the laboratory CH_4 extracted using the vacuum extraction method was -40.8 ± 0.1‰ (n=3) compared to the known $\delta^{13}C$ value for the dissolved CH_4 of -40.1 ± 0.5 (Figure 3.1 A). Therefore, while all $\delta^{13}C$ values were more negative compared the known $\delta^{13}C$ of the dissolved CH_4, they were within the total analytical error for $\delta^{13}C$ for CH_4 by CSIA (±0.5‰), and no isotopic fractionation was observed using the vacuum extraction method when the evacuated bottle is filled half way with solution.

The average $\delta^{13}C$ value of the laboratory CH_4 extracted using the gas stripping method was -40.2 ± 0.1 ‰ (n=3) compared to the known $\delta^{13}C$ for the dissolved CH_4 of -40.1 ‰ ± 0.5 (Figure 3.1 B). Therefore, there was no isotopic fractionation observed outside of the standard analytical error for $\delta^{13}C$ of CH_4 by CSIA (± 0.5 ‰) using the gas stripping method where the volume of stripping gas (in this case He) is the same as the volume of liquid that the gas is being stripped from (i.e. a 1:1 ratio)

The dissolved CH_4 sample extracted from the WHC2 spring in the Tablelands had an average $\delta^{13}C$ value of -27.5 ± 0.1‰ (n=5) when the gas was removed using the gas stripping method, compared to an average $\delta^{13}C$ value of -27.7 ± 0.3‰ (n=6) for the vacuum extraction method (Figure 3.2). Therefore, similar to the laboratory data, there was no observable difference between the $\delta^{13}C$ values for the gas stripping method and the vacuum extraction method.

3.3.2.2 Hydrogen isotopes

The average δD value of CH_4 extracted using the vacuum extraction method (125 mL of dissolved CH_4 sample in a 250 mL bottle) laboratory was -162 ± 4‰ (n=3) compared to the known δD value for the dissolved CH_4 of -167 ± 5 ‰ (Figure 3.3 A). Therefore, given a total analytical error for δD for CH_4 by CSIA of 5 ‰, there was no observable hydrogen isotopic fractionation using the vacuum extraction method when the evacuated bottle is filled half way with solution.

The average δD value of the laboratory CH_4 extracted using the gas stripping method was -163 ± 2‰ (n=3) compared to the known δD for the dissolved CH_4 of -167 ± 5‰ (Figure 3.3 B). Therefore, there was no isotopic fractionation observed outside of the standard analytical error for δD of CH_4 by CSIA (± 5 ‰) using the gas stripping method where the volume of stripping gas (in this case He) is the same as the volume of liquid that the gas is being stripped from (i.e., a 1:1 ratio).

Samples for hydrogen isotopes in the field were taken from the Tablelands but were incorrectly handled and could not be isotopically analyzed for δD.

3.3.3 Testing CH_4 gas cryogenic concentration for isotopic fractionation

3.3.3.1 Carbon isotopes

Cryogenic concentration of 480 mL of laboratory CH_4 gas into a previously evacuated 160mL bottle resulted in an average $\delta^{13}C$ value of -42.1 ± 0.1‰ (n=3) compared to the known $\delta^{13}C$ value for the CH_4 tank of -41.3 ± 0.5‰ (Figure 3.4 A). Therefore, while all $\delta^{13}C$ values were more negative compared the known $\delta^{13}C$ of the CH_4 tank, only one was outside the analytical error for $\delta^{13}C$ for CH_4 by CSIA of ± 0.5 ‰.

Cryogenic concentration of 720 mL of laboratory CH_4 gas into a previously evacuated 160 mL bottle resulted in an average $\delta^{13}C$ value of -42.1 ± 0.1‰ (n=3) compared to the known $\delta^{13}C$ value for CH_4 tank of -41.3 ± 0.5‰ (Figure 3.4B). Therefore, while all $\delta^{13}C$ values were more negative compared the known $\delta^{13}C$ of the CH_4 tank, they were within the total analytical error for $\delta^{13}C$ for CH_4 by CSIA (± 0.5 ‰) and no isotopic fractionation was observed for the cryogenic concentration of 720 mL into a 160 mL bottle.

Finally, cryogenically concentrating the laboratory CH_4 diffusing from water into the 18 L container (after reaching equilibrium) resulted in a $\delta^{13}C$ value of -40.5 ‰ compared to the known $\delta^{13}C$ value for the dissolved laboratory CH_4 of -40.1 ±0.5‰ (Figure 3.5). Therefore, given a total analytical error for $\delta^{13}C$ for CH_4 by CSIA of ± 0.5‰, there was no observable carbon isotopic fractionation for the cryogenic concentration of an 18L headspace placed over water containing dissolved CH_4 at equilibrium.

3.3.3.2 Hydrogen isotopes

Cryogenic concentration of 480 mL of laboratory CH_4 gas into a previously evacuated 160mL bottle resulted in an average δD value of -205 ± 3‰ (n=3) compared to a known δD value for the CH_4 tank of -205 ± 5‰ (Figure 3.6 A). Therefore, given a total analytical error for δD for CH_4 by CSIA of 5 ‰, there was no observable hydrogen isotopic fractionation for the cryogenic concentration of 480 mL into a 160 mL bottle.

Cryogenic concentration of 720 mL of laboratory CH_4 gas into a previously evacuated 160 mL bottle resulted in an average δD value of -199 ± 3‰ (n=3) compared to the known δD value for the CH_4 tank of -205 ± 5‰ (Figure 3.6 B). Therefore, while all δD values were more positive compared the known δD of the CH_4 tank, they were within the total analytical error for δD for CH_4 by CSIA (5 ‰) and no isotopic fractionation was observed for the cryogenic concentration of 720 mL into a 160 mL bottle.

3.4 Discussion

3.4.1 Isotopic fractionation in gas collection methods

Current CH_4 sourcing methods typically rely on measuring the carbon and hydrogen isotope values of CH_4, and then plotting these values on a $\delta^{13}C$ and δD plot. Therefore, maintaining isotopic integrity during gas collection is crucial to ensuring accurate sourcing. If isotopic fractionation were to occur during the extraction of dissolved CH_4 it would alter the isotopic values of the gas and potentially lead to inaccurate sourcing.

Of the gas extraction methods tested in the laboratory (gas stripping and vacuum extraction) there was no observable carbon or hydrogen isotopic fractionation outside of the total analytical error for $\delta^{13}C$ and δD for CH_4 by CSIA of ± 0.5 ‰ and 5‰, respectively. Similarly, it was observed that there was no observable isotopic difference between the two methods performed on field samples in the field. Therefore, because these methods maintain the isotopic integrity of the sample, they can be used when extracting dissolved CH_4 from water for the purposes of sourcing.

While the vacuum extraction method is more commonly used for isotopic analysis, recent work on the isotopic composition of dissolved CH_4 has used the gas stripping method, often referred to as a headspace equilibration method, to extract the gas from water. For example, the gas stripping method was used to strip dissolved gases from water with 112 mL of solution and 10 mL of argon being shaken together for 5 minutes (Capasso and Inguaggiato 1998; Etiope et al. 2016). Therefore, the ratio of solution to gas was approximately 11:1 whereas in this study a 1:1 ratio as per the method by Rudd et al. (1974) was used. Generally, the gas stripping method refers to the shaking of a dissolved gas solution and another gas to strip dissolved gases into the headspace. However, the shaking times and ratio of solution to headspace vary. As a result, the conclusion from this paper that the gas stripping method is non-isotopically fractionating only applies to a

1:1 headspace to solution ratio. Further research is required to determine whether varying the headspace to solution ratio or shaking times induces isotopic fractionation.

3.4.2 Isotopic fractionation in gas concentration methods

While the gas extraction methods tested did not isotopically fractionate samples, when dissolved CH_4 concentrations are low, extracted samples typically must also be concentrated before isotopic analysis. Therefore, along with extraction methods, gas concentration methods were also tested to determine if they are isotopically fractionating for CH_4 and potentially causing inaccurate sourcing. In this study there was no observable isotopic fractionation of carbon in 5 of 6 samples and no isotopic fractionation of hydrogen in any of the 6 samples when cryogenically trapping 480 mL and 720 mL of CH_4 into an evacuated 160 mL bottle.

3.4.3 Isotopic fractionation from diffusion

After testing isotopic fractionation during cryogenic trapping, the next step was to verify whether isotopic fractionation was occurring when dissolved CH_4 diffused into the air. Since concentrations of diffuse CH_4 were too low for isotopic analysis, the previous cryogenic concentration methods were used. After 24 hours chemical equilibrium was achieved between the dissolved CH_4 solution and headspace gas (Figure 3.5). The gas was then cryogenically concentrated into a 160 mL bottle and tested for isotopic fractionation. Overall, there was no observable carbon isotopic fractionation between the standard and the sample in the headspace (Figure 3.5). However, despite cryogenic concentration, CH_4 concentration in the sample was too low to complete hydrogen isotopic analysis (given a detection limit of 0.2%). This experiment concludes that diffusion of dissolved CH_4 across the liquid air boundary does not cause observable carbon isotopic fractionation. Further study is required in order to determine if the same can be said of hydrogen in the diffuse CH_4.

Previously, a model was developed to experimentally determine the equilibrium CH_4 isotopic fractionation across the air-water boundary (Fuex 1980). Results from this paper concluded that the equilibrium isotopic fractionation for carbon in CH_4 across the air water boundary was 1.00033 +/- 0.00002. While Fuex's experiment is similar to the one completed for this study, it did not achieve quantitative conversion because not all CH_4 was dissolved into the water. The experiment dissolved 160.6 mL of CH_4 into 19.8 L, but had 9.4 mL of CH_4 not dissolved. In addition, after reaching equilibrium, the gas in the experiment did not need to be concentrated prior to isotopic analysis.

To determine the impact an isotopic fractionation factor of 1.00033 would have on the $\delta^{13}C$ value the fractionation factor can be converted to an enrichment factor ($\boldsymbol{\varepsilon}$) via Equation 5:

$$\boldsymbol{\varepsilon} = 1000(\alpha-1) \quad (5)$$

An α of 1.00033 is therefore equivalent to an enrichment factor of 0.33 ‰. Because this value is within the analytical error for CH_4 by CSIA of ± 0.5 ‰ it supports the conclusion that carbon isotopic fractionation by diffusion across the liquid-water phase boundary is not observable.

3.4.4 Isotopic fractionation to source abiogenic CH_4

Current CH_4 sourcing methods typically rely on the plotting of $\delta^{13}C$ versus δD as one line of evidence to distinguish CH_4 sources. However, while this method has been used in differentiating between microbial and thermogenic CH_4, abiotic CH_4 can also fall within these isotopic ranges and further complicates sourcing (Horita and Berndt 1999; Taran et al. 2007). Considering only the $\delta^{13}C$ and δD of the CH_4 fails to account for the formation mechanisms and the role reactants and catalysts play in the production of CH_4. However, if the carbon and hydrogen isotopic fractionation factors between reactants and products (between CO_2 and CH_4, and H_2 and CH_4 respectively) are plotted, the affects of the δ values of the source materials and isotopic fractionation associated with the reaction(s) that produce CH_4 are accounted for.

Previous research has shown that plotting carbon and hydrogen isotopic fractionation factors can be used to differentiate formation pathways for microbial CH_4 (Kohl et al. 2016; Whiticar et al. 1986). Building on these findings, it was postulated that isotopic fractionation factors could also be used to differentiate abiogenic CH_4 from microbial CH_4 (Sherwood Lollar et al. 2008). Therefore, a comprehensive literature review of studies that reported isotopic data for abiotic CH_4 in both the field and the laboratory was undertaken. Using Equation 6, the data were then converted into apparent carbon and hydrogen isotopic fractionation factors for the production of CH_4 ($\alpha\ ^{13}C_{CO2\text{-}CH4}$ and $\alpha\ D_{H2O\text{-}CH4}$).

(6) $$\alpha^{n}X_{a-b} = \frac{\delta^{n}X_{a} + 1000}{\delta^{n}X_{b} + 1000}$$

Where X is the element (C or H) and n is the heavy isotope (13 or 2), and a is the product and b is the reactant.

Apparent fractionation factors are used to compare empirical differences between the stable isotope values of various co-existing species and do not take into account the potential conversion of the substrate into CH_4. For example, carbonate can be both a product of acetate fermentation and a substrate for methanogens. These factors were then plotted and compared to the summary plot of microbial fractionation factors, which included many lab experiments (Kohl et al. 2016).

Figure 3.7 shows a large overlap for hydrogen fractionation factors for microbial and abiogenic CH_4 with the majority of the factors clustered between α= 1.1 to 1.3. However, there is a clearer divide for carbon isotopic fractionation factors between the two sources. Microbial carbon fractionation factors ranges from approximately α= 1.04 to 1.1 with the majority of the data clustered between α= 1.5 to 1.8. In contrast, abiogenic carbon fractionation factors range from α= 0.99 to 1.07 with the majority of the data clustered between α= 1.004 to 1.04. Therefore, a carbon isotopic fractionation of above 1.04 and above better describes microbial CH_4 and a carbon isotopic fractionation of below 1.04 better describes abiogenic CH_4. While there is still overlap, the sources of CH_4 were better differentiated by carbon isotope fractionation compared to hydrogen isotope fractionation. However, more research is needed on ways to separate the intermediate samples that plot close to the proposed line of differentiation.

However, it is important to note that the abiogenic fractionation factors consider both laboratory and field data for several different catalysts and various formation temperatures. Therefore, further research is needed as to the role formation temperatures and catalysts play on isotopic fractionation before this trend can confidently be used as a line of evidence in sourcing.

3.5 Figures

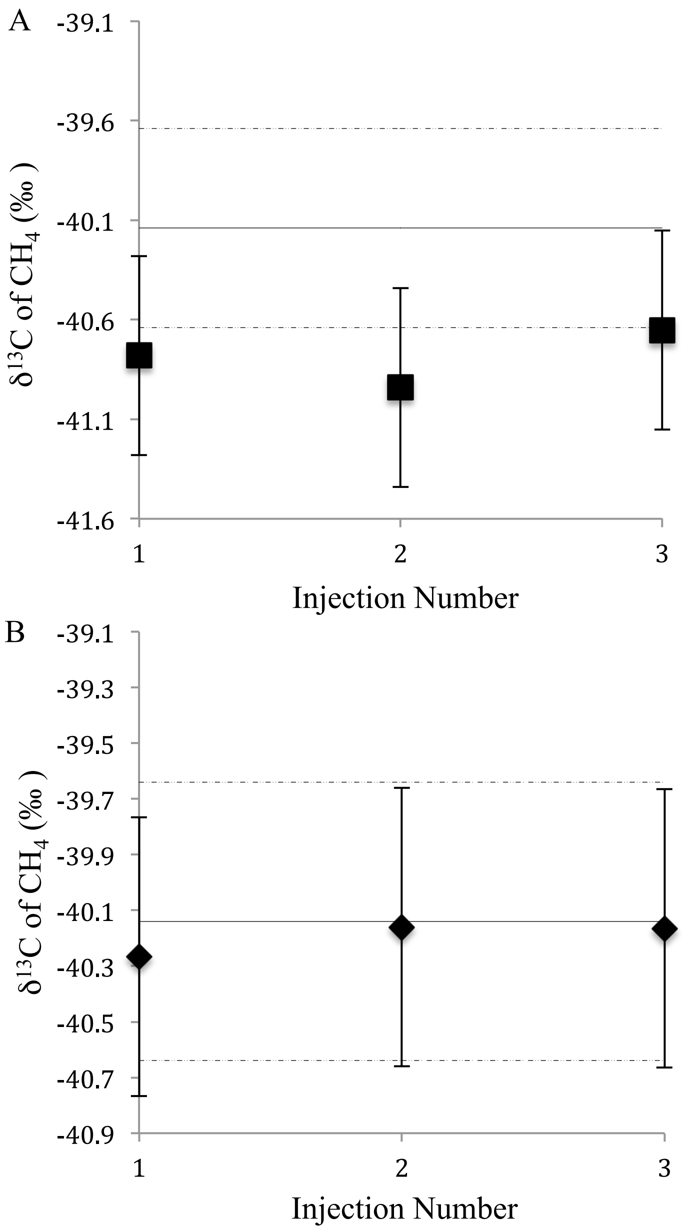


Figure 3.1. $\delta^{13}C$ of CH_4 gas samples collected from water in the laboratory using (A) the vacuum extraction method and (B) the gas stripping method (Appendix 3.3). The solid line represents the known $\delta^{13}C$ of the CH_4 (-40.1‰) that was dissolved in the water that was used in the laboratory testing of the vacuum extraction and gas stripping methods (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 0.5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

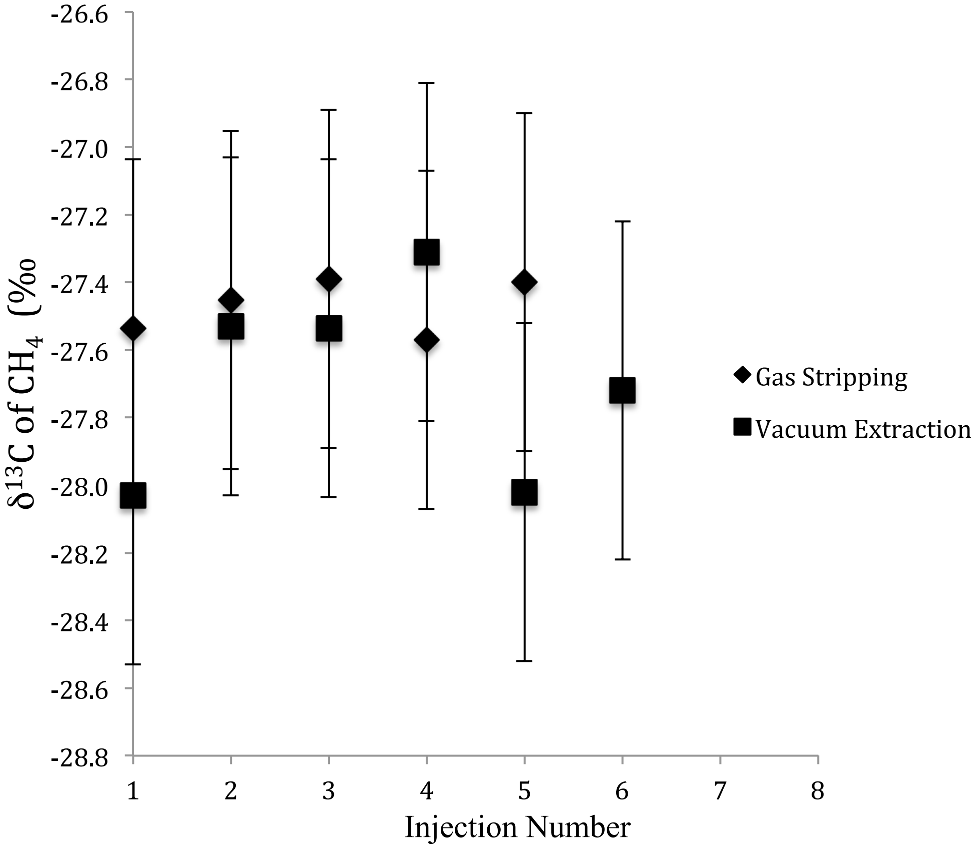


Figure 3.2. $\delta^{13}C$ of CH_4 gas samples collected from the WHC2 pool in the Tablelands, Gros Morne National Park, NL, Canada using the gas stripping method and the vacuum extraction method (Appendix 3.5). Error bars represent the +/- 0.5‰ analytical error associated with the compound specific isotope analysis of CH_4 (Ward 2002).

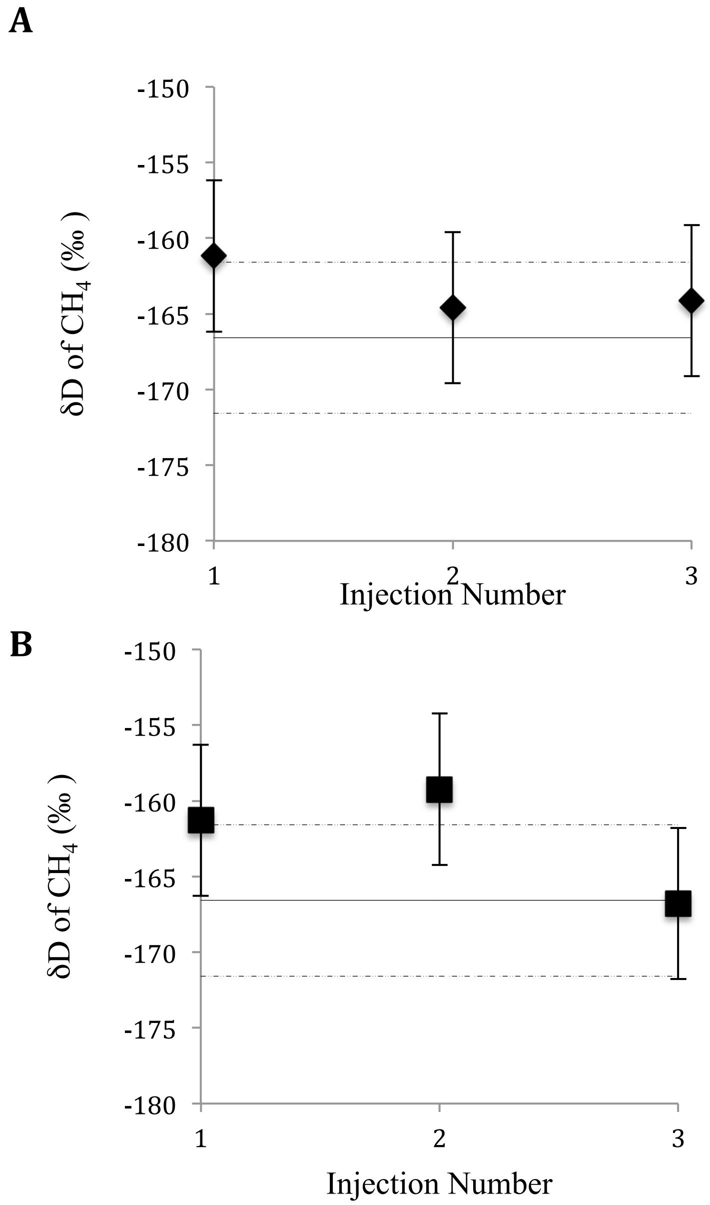


Figure 3.3. δD of CH_4 gas samples collected from water in the laboratory using (A) the vacuum extraction method and (B) the gas stripping method (Appendix 3.4). The solid line represents the known δD of the CH_4 (-167 ‰) that was dissolved in the water that was used in the laboratory testing of the vacuum extraction and gas stripping methods (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

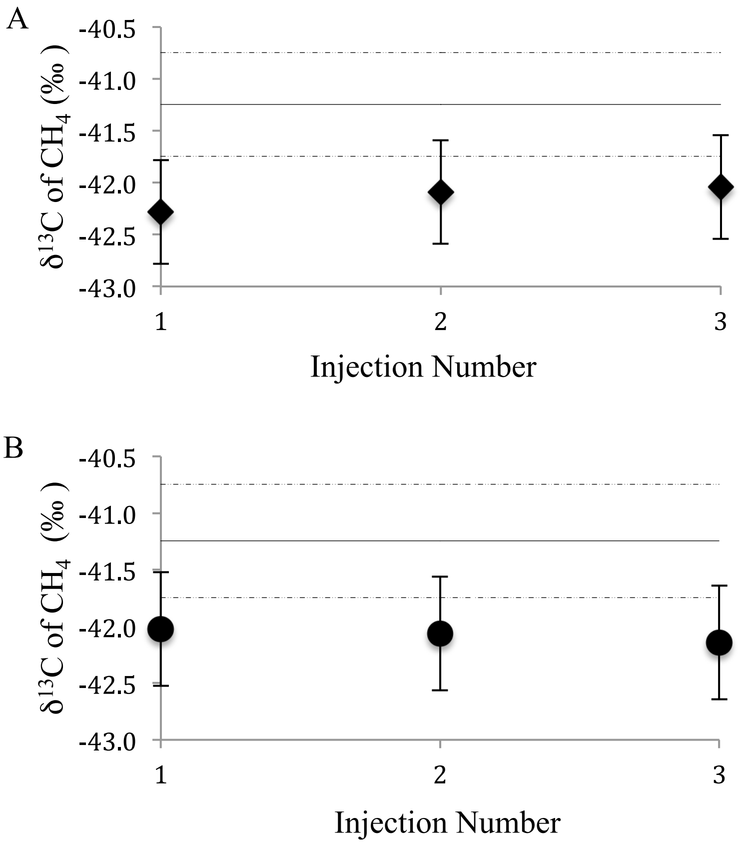


Figure 3.4. $\delta^{13}C$ of CH_4 gas cryogenically trapped in the laboratory when (A) 480 mL of CH_4 was transferred to 160 mL vial and (B) 720 mL of CH_4 was transferred to 160 mL vial (Appendix 3.6). The solid line represents the known $\delta^{13}C$ of the CH_4 that was used to test concentration methods in the laboratory (-41.3‰) (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 0.5 ‰) associated with compound specific isotope analysis of CH_4 (Ward, 2002).

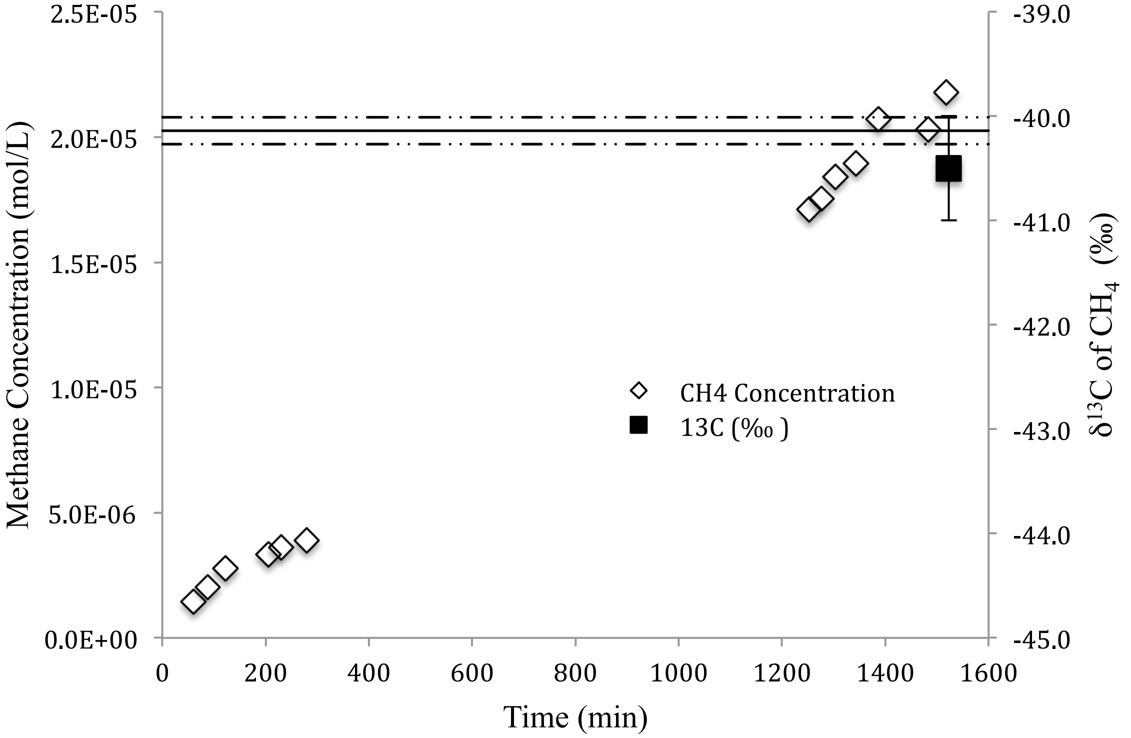


Figure 3.5. δ^{13}C value and concentrations of CH_4 gas samples diffusing from water into the headspace of a closed floating chamber over time. Headspace was cryogenically trapped for isotopic analysis 25 hours and 23 minutes after the start of the experiment (Appendix 3.6). At this time it was assumed that chemical equilibrium was achieved in the headspace (operationally defined as when the CH_4 concentrations of 3 samples were within 5% of each other). The solid horizontal line represents the known δ^{13}C of the CH_4 (-41.3 ‰) that was dissolved in the water that was used in the laboratory testing of the vacuum extraction and gas stripping methods (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 0.5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

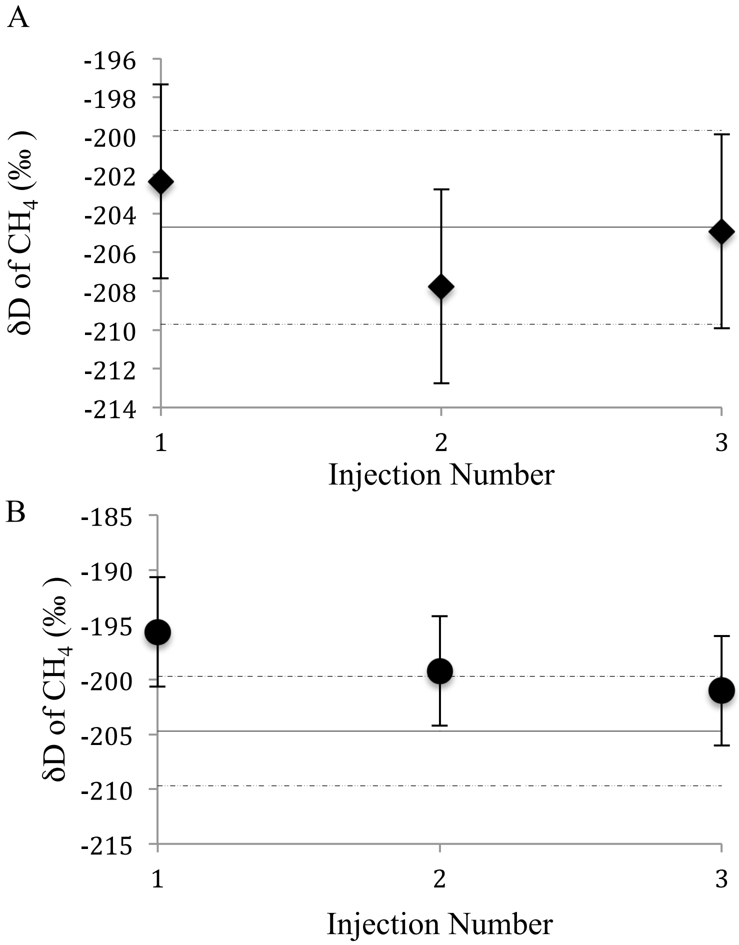


Figure 3.6. δD of CH_4 gas cryogenically trapped in the laboratory when (A) 480 mL of CH_4 was transferred to 160 mL vial, (B) 720 mL of CH_4 was transferred to 160 mL vial (Appendix 3.7). The solid line represents the known δD of the CH_4 that was used to test concentration methods in the laboratory (-167‰) (Appendix 3.2). Dotted lines represent the standard analytical error (+/- 5 ‰) associated with compound specific isotope analysis of CH_4 (Ward 2002).

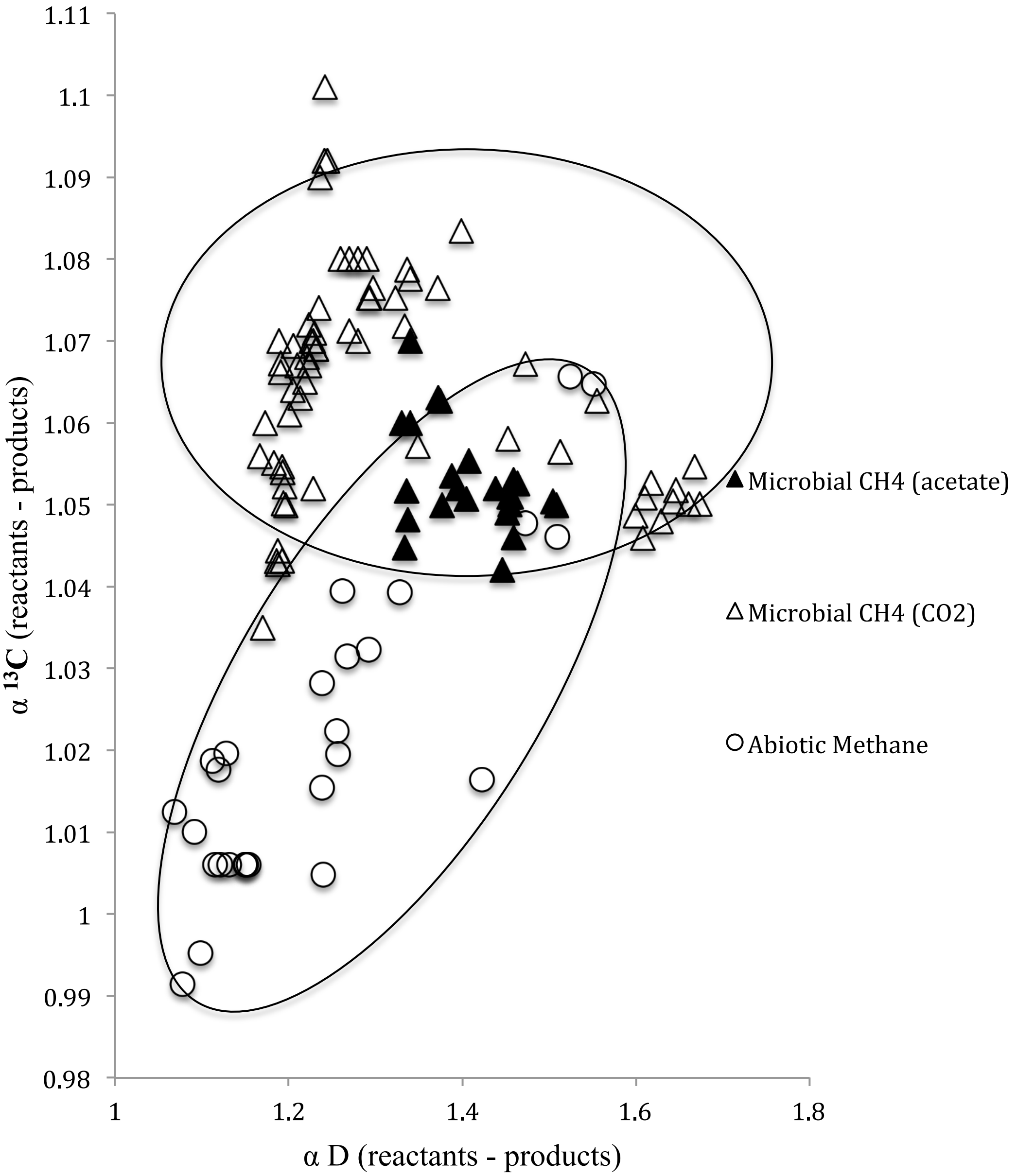


Figure 3.7. Carbon and hydrogen isotopic fractionation factors (α ^{13}C and α D, respectively) during the formation of microbial and abiogenic methane. Zones are provided for areas indicating microbial and abiogenic methane. Microbial methane isotopic fractionation factors are from Kohl et al. (2016) and references therein and include methane generated from both acetate fermentation (filled triangle) and CO_2 reduction (unfilled triangle). Abiotic methane isotopic fractionation factors are a review of reported data from both laboratory and field data (Kelley and Früh-Green 1999; Proskurowski et al. 2008; Sherwood et al. 1988; Taran et al. 2010; Fu et al. 2007)

4 Chapter 4

4.1 Summary of Findings

The overall purpose of this research project was to determine the flux of natural CH_4 release and CO_2 sequestration at sites of serpentinization and to develop methods to sample the CH_4 at such sites for the purposes of sourcing. First, in Chapter 2 I measured gas fluxes at sites of serpentinization and then used the data to determine the impact this site has on atmospheric heating. Next, in Chapter 3 I considered the CH_4 dissolved into and diffusing from the pools at these sites and investigated ways to collect and concentrate the gas sample without isotopic fractionation for the purposes of sourcing.

While sites of serpentinization have been shown to sequester atmospheric CO_2, to the best of our knowledge no paper has considered the impact of potential CH_4 release, and as a result, there exists a knowledge gap on the net impact these sites have on atmospheric heating (Keleman and Matter 2008). Chapter 2 addressed this question by building a floating closed chamber to collect gases coming into and out of a pool of high pH water discharging from serpentinized rock in the Tablelands. $CH_{4,}$ CO_2 and N_2O concentrations from a closed headspace over the WHC2 pool were measured over a 24-hour period by intermittent sampling. These concentrations were then used in a linear approximation to calculate the flux of CH_4 released and CO_2 taken in. Over the 24-hour period the pool sequestered 41 times more CO_2 than CH_4 released. While this method successfully calculated gas fluxes from changing concentrations, fluxes can also be calculated if the chemical enhancement factor is known. However, there is a lack of research on chemical enhancement at sites of serpentinization. Therefore, this study used the calculated fluxes from the linear approximation to calculate a chemical enhancement factor of 22.7, the first for a pool of ultra-basic water above serpentinized rock. This value is similar to reported values for high pH lakes and further research is needed on factors that influence chemical enhancement. Next, to answer the question of environmental impact, the global warming potential and radiative forcing values of the two gas fluxes were compared. In summary, these sites, which have been studied as a potential way to reduce global CO_2, are also releasing CH_4, another harmful green house, and have a net radiative forcing number of -.21 and a net global warming potential of -7 (100 year time horizon); both indicating a removal of heat from the atmosphere. However, if only the CO_2 sequestered was considered the site would appear to have a much larger impact on reducing atmospheric heating. Therefore, before we look for ways to harness the CO_2 sequestering potential of sites of serpentinization, more research is needed on the natural global baseline of these sites. Next research steps would include studying others sites at the Tablelands to produce an estimate for the net radiative forcing. This value could then be used to predict net radiative forcing for other large sites and then a global estimate on the impact sites of serpentinization have on atmospheric heating.

After considering the impact of these sites on atmospheric heating, Chapter 3 then addressed another pressing question at sites of serpentinization; how can we extract the dissolved gases from these pools and then concentrate the sample without isotopic fractionation for the purposes of sourcing? Sites of serpentinization create a unique sourcing challenge, as all three types of CH_4 can be present (microbial, thermogenic, abiogenic) (Szponar et al. 2013). The question of sourcing CH_4 has applications to a wide range of fields including identifying potentially harvestable natural gas to even determining whether Martian CH_4 is the first example of active life on other worlds. Typical CH_4 sourcing methods involve using stable isotope analysis to genetically zonate the sources (Schoell 1980). However, if common collection and concentration methods were isotopically fractionating the CH_4 sample it would change these isotopic signatures and make sourcing difficult. Therefore, before we can analyze the isotopic signature we need a way to extract the dissolved CH_4 and then concentrate the sample, without changing this isotopic signature.

Chapter 3 addressed this question by testing common collection and concentration methods for dissolved and diffuse CH_4 for isotopic fractionation used in the laboratory and the field. The vacuum extraction method and gas stripping methods were first tested using water samples saturated with CH_4 from an isotopically characterized tank. Extracted samples were then isotopically analyzed and results showed that there was no observable carbon or hydrogen isotopic fractionation for either method. After demonstrating that these methods were non-isotopically fractionating the study then tested for isotopic fractionation in cryogenic concentration methods. No carbon or hydrogen isotopic fractionation was observed for 5 of 6 cryogenically trapped samples. The cryogenic concentration method was then used to measure isotopic fractionation by diffusion across the liquid air phase boundary. An inverted plastic container was placed over water saturated with CH_4 such that diffusing gases were collected in the headspace.

Once equilibrium was achieved in the headspace gases were then cryogenically trapped into an evacuated 160 mL vial. The sample was then isotopically analyzed and results showed that there was no observable carbon isotopic fractionation for diffusion across a phase boundary. These results agree with previous studies that have shown $\delta^{13}C$ enrichment as high as 0.33 ‰ (within the +/- 0.5‰ error from compound specific isotope analysis). Finally, the study then considered how isotopic fractionation factors during the formation of abiogenic CH_4 (from reactants to products) may provide another line of evidence for differentiating between abiogenic and microbial samples.

Overall, this thesis considers both ways to source the low concentrations of CH_4 at sites of serpentinization sites and the relative impact the gases at these sites have on atmospheric heating. Together, Chapters 2 and 3 develop the base of knowledge about gases at sites of serpentinization and its findings can be applied a range of areas of active research including environmental impact studies, natural gas exploration, and even the search for life on other worlds.

4.2 Proposed next research steps

The next steps to continue to develop the findings from this Master's Thesis would be to first begin studying others sites at the Tablelands to produce an estimate for the net impact. The radiative forcing and global warming potential values calculated by this study were only for one small pool over a 24-hour and were insignificant on global atmospheric heating. However, the methods used to calculate flux at this site could be applied to several other pools at the Tablelands. This value could then be used to predict net radiative forcing for other large sites globally and then develop a global estimate on the impact sites of serpentinization have on atmospheric heating. In addition, more research will need to be conducted on whether the microbes that can convert CO_2 to CH_4 are common at sites of serpentinization. If these microbes are common than injecting CO_2 could have a significant detrimental effect on atmospheric heating.

In addition to a net impact of sites of serpentinization, the gas collection and concentration methods tested in the laboratory should be used at the Tablelands to obtain carbon and hydrogen isotopic values for the CH_4 diffusing from the pool. Gas samples that were collected from the chamber headspace in Chapter 2 were too low for isotopic analysis and would need to be cryogenically concentrated first (a method that was shown to be non-isotopically fractionating in Chapter 3). In addition, concentrations of CH_4 stripped from the pool using the gas stripping and vacuum extraction methods were handled incorrectly and should be done again to obtain hydrogen isotopic analysis. Finally, the methods tested in Chapter 3 should be applied at the Tablelands to help source the CH_4 at this site.

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Appendix

Compound	Injection Size (L)	Moles of Standard Injected	Peak Area
CH_4	0.00003	1.24E-10	6.86
	0.00005	2.07-10	13.88
	0.0002	8.28E-10	65.46
	0.0003	1.24E-09	87.87
CO_2	0.000007	1.45E-08	8.5518
	0.0000085	1.76E-08	29.62
	0.00001	2.07E-08	56.84
	0.000015	3.11E-08	117.6

Day	Injection size (L)	Peak area	Moles of N_2O Injected
1	0.001	136	8.70E-11
	0.0005	45.6	4.35E-11
	0.0003	15.31	2.61E-11
2	0.001	131.6	8.79E-11
	0.0005	46.3	4.35E-11
	0.0003	20.6	2.61E-11
3	0.001	128.6	8.70E-11
	0.0005	48.6	4.35E-11
	0.0003	13.23	2.61E-11

Appendix 2.1 GC-FID Calibration values for (A) CH_4 and CO_2 Calibration using Restek 34522 Standard and (B) N_2O Calibration using a 2.1 ppm standard.

Sample Number	Time	CH_4 Peak Area	CH_4 conc. (mol/L)	Average CH_4 conc. (mol/L)	CO_2 Peak Area	CO_2 Conc. (mol/L)	Average CO_2 Conc. (mol/L)	N_2O Peak Area	N_2O Conc. (mol/L)	Average N_2O Conc. (mol/L)
1.1	0	6*	3.26E-07	3.26E-07	86.73	8.68E-05	8.91E-05	15.4	2.71E-08	2.91E-08
1.2	2	6*	3.26E-07		95.53	9.13E-05		23.4	3.11E-08	
2.2	12	6*	3.26E-07	3.26E-07	59.59	7.31E-05	7.31E-05	23.66	3.11E-08	3.11E-08
3.1	25	6*	3.26E-07	3.26E-07	57.41	7.20E-05	7.31E-05	28.8	3.38E-08	3.36E-08
3.2	27	6*	3.26E-07		61.74	7.42E-05		28.2	3.35E-08	
4.1	60	6*	3.26E-07	3.26E-07	49.8	6.82E-05	7.07E-05	27.45	3.31E-08	3.17E-08
4.2	62	6*	3.26E-07		59.91	7.33E-05		22.38	3.04E-08	
5.1	131	11.147	5.56E-07	5.24E-07	34.98	6.07E-05	5.86E-05	22.2	3.05E-08	3.15E-08
5.2	133	9.72	4.92E-07		26.64	5.65E-05		26.3	3.25E-08	
6.1	225	11.59	5.76E-07	5.79E-07	28.03	5.72E-05	5.63E-05	20.9	2.78E-08	2.88E-08
6.2	227	11.75	5.83E-07		24.43	5.54E-05		24.5	2.97E-08	
7.1	819	53.2	2.43E-06	2.39E-06	8*	4.71E-05	4.71E-05	21.46	3.01E-08	3.09E-08
7.2	821	51.2	2.34E-06		8*	4.71E-05		24.4	3.16E-08	
8.1	935	51.88	2.37E-06	2.23E-06	8*	4.71E-05	4.71E-05	19.53	2.92E-08	3.02E-08
8.2	937	45.6	2.09E-06		8*	4.71E-05		23.8	3.13E-08	
9.1	1085	60.8	2.77E-06	2.72E-06	8*	4.71E-05	4.71E-05	33.6	3.46E-08	3.76E-08
9.2	1087	58.37	2.66E-06		8*	4.71E-05		44.5	4.05E-08	
10.1	1207	59.02	2.69E-06	2.66E-06	8*	4.71E-05	4.71E-05	22.5	3.06E-08	3.33E-08
10.2	1208	57.48	2.62E-06		8*	4.71E-05		33	3.59E-08	

Appendix 2.2 Time series data of gas concentrations (methane, carbon dioxide, and N_2O) sampled from the closed chamber floating over an ultra-basic pool created by a groundwater springs associated with serpentinization in the Tablelands, Gros Morne, NL

* - data is at detection limit of device

(A)	**B ISO 1** **CH_4 std** **exp.: -54.5 ± 0.2 ‰**		**T ISO 2** **CH_4 std** **exp.: -38.3 ± 0.2 ‰**	
Date	**ampl. 44**	**$\delta^{13}C$ (‰)**	**ampl. 44**	**$\delta^{13}C$ (‰)**
Apr (2016)	3676	-54.61	5413	-38.40
	3567	-54.95	5724	-38.41
	3213	-54.74	5577	-38.43
Apr (2016)	2043	-54.99	3824	-38.54
	2739	-54.87	3460	-38.64
Sep (2015)	4691	-54.56		
	2716	-54.60		
	2679	-54.57		
	2770	-54.39		
Sep (2015)	2706	-54.54		
	2709	-54.61		
	1902	-54.54		
	811	-54.44		
	507	-54.16		
Sep (2014)	2706	-54.54		
	1348	-54.04		
	2320	-54.85		
	2394	-54.86		
	1382	-54.17		
	2393	-54.88		
	2431	-54.26		
	1202	-54.55		
	457	-54.24		
	1161	-54.16		
	533	-54.30		
	686	-53.92		
	782	-54.22		
	380	-54.48		
	738	-54.83		

(B)

Date	**δ^2H B ISO 1 CH$_4$ std Cert. Value: -266 ‰**		**δ^2H T ISO 2 CH$_4$ std Cert. value: -157 ‰**	
	ampl. 2	**δ (‰)**	**ampl. 2**	**δ (‰)**
May (2016)	2596	-275.6	4240	-157.1
	2557	-271.4	4704	-160.1

Appendix 3.1 GC-IRMS Standard Calibration Data for (A) δ^{13}C of CH$_4$ and (B) δD of CH$_4$

(A)

Tank	δ ^{13}C (‰)	Tank Avg. (‰)	Tank Std. Dev.
Tank 2		-41.25	0.08
(Apr 2016)	-41.25		
	-41.17		
	-41.32		
Tank 1	-40.02	-40.14	0.13
(Sept 2015)	-39.98		
	-40.26		
	-40.25		
	-40.19		

(B)

Method	δD (‰)	Tank Avg. (‰)	Tank Std. Dev.
Gas Tank 2	-203.11	-204.71	4.24
(May 2016)	-209.51		
	-201.50		
Gas Tank 1	-167.34	-166.5857129	1.07
(May 2016)	-165.83		

Appendix 3.2 (A) δ ^{13}C and (B) δD of CH_4 from the two tanks that were used to test gas collection and gas concentration methods

Method	δ ^{13}C (‰)	Method Average (‰)	Method Std. Dev.
Gas Stripping	-40.27	-40.20	0.06
	-40.16		
	-40.17		
Vacuum	-40.78	-40.79	0.14
	-40.94		
	-40.65		

Appendix 3.3 δ^{13}C of CH_4 gas samples collected from water in the laboratory using the vacuum extraction method and the gas stripping method (September 2015)

Method	δ D (‰)	Method Average (‰)	Method Std. Dev.
Gas Stripping	-161.18 -164.59 -164.12	-163.30	1.85
Vacuum	-161.27 -159.23 -166.78	-162.43	3.90

Appendix 3.4 δD of CH_4 gas samples collected from water in the laboratory using the vacuum extraction method and the gas stripping method (May 2016).

Method	δ ^{13}C (‰)	Sample Average (‰)	Method Average (‰)
Gas Stripping	-27 -27.47	-27.54	-27.47
	-27.57 -27.49 -27.30	-27.45	
	-27.24 -27.54	-27.39	
	-27.60 -27.54	-27.57*	
	-27.59 -27.21	-27.40*	
Vacuum	-28.75 -28.32 -27.53 -27.52	-28.03	-27.69
	-27.52 -27.54	-27.53	
	-27.78 -27.29	-27.54	
	-27.30 -27.32	-27.31	
	-27.76 -28.28	-28.02*	
	-27.72 -27.72	-27.72*	

Appendix 3.5 δ^{13}C of CH_4 gas samples collected from the WHC2 pool in the Tablelands, Gros Morne National Park, NL, Canada using the gas stripping method and the vacuum extraction method (September 2014, Septmeber 2015). *Data from September 2014

Method	$\delta^{13}C$ (‰)	Method Average (‰)	Method Std. Dev.
(A) Cryo Trap 480mL	-42.28	-42.14	0.13
	-42.09		
	-42.04		
(B) Cryo Trap 720mL	-42.02	-42.08	0.06
	-42.06		
	-42.14		
(C)Cryo Trap Diffusing Methane from 18L HS	-40.50	-40.50	

Appendix 3.6 $\delta^{13}C$ of CH_4 gas cryogenically trapped in the laboratory when (A) 480 mL of CH_4 was transferred to 160 mL vial and (B) 720 mL of CH_4 was transferred to 160 mL vial and (C) an 18L headspace containing CH_4 was transferred to a 160 mL vial (April 2016).

Method	δD (‰)	Method Average (‰)	Method Std. Dev.
(A) Cryo Trap 2x160	-202.35	-205.01	2.70
	-207.76		
	-204.91		
(B) Cryo Trap 3x160	-195.66	-198.64	2.73
	-199.22		
	-201.02		

Appendix 3.7 δD of CH_4 gas cryogenically trapped in the laboratory when (A) 480 mL of CH_4 was transferred to 160 mL vial and (B) 720 mL of CH_4 was transferred to 160 mL vial (May 2016).

(A) **Sample**	**CH_4**		**Corrected δ VPDB (‰)**
	ampl. 44 (mV)	**δ VPDB (‰)**	
Methane tank 2 characterization 1	11394	-41.45	-41.24
	7031	-41.47	-41.26
Methane tank 2 characterization 2	16299	-41.67	-41.46
	9666	-41.08	-40.87
Methane tank 2 characterization 3	9974	-41.43	-41.22
	8980	-41.63	-41.42
Cryogenic Concentration 480 mL sample 1 (diluted ~40x) (laboratory)	4973	-42.42	-42.20
	4186	-42.58	-42.36
Cryogenic Concentration 480 mL sample 2 (diluted ~40x) (laboratory)	4832	-42.29	-42.07
	4730	-42.32	-42.10
Cryogenic Concentration 480 mL sample 3 (diluted ~40x) (laboratory)	4512	-42.19	-41.97
	4329	-42.33	-42.11
Cryogenic Concentration 720 mL sample 1 (diluted ~40x) (laboratory)	6838	-42.26	-42.04
	6836	-42.22	-42.00
Cryogenic Concentration 720 mL sample 2 (diluted ~40x) (laboratory)	5160	-42.01	-41.80
	3362	-42.55	-42.33
Cryogenic Concentration 720 mL sample 3 (diluted ~40x) (laborator)y	8332	-42.37	-42.15
	6529	-42.35	-42.13
Cryogenic Concentration of headspace with diffusing methane	698	-40.88	-40.68
(laboratory)	691	-40.53	-40.33

(B) Sample	CH$_4$	
	ampl. 44 (mV)	δ VPDB (‰)
Gas stripping method sample 1 (laboratory) (2015)	1413	-40.36
	2417	-40.28
	2547	-40.16
Gas stripping method sample 2 (laboratory) (2015)	3158	-40.14
	1585	-40.18
Gas stripping method sample 3 (laboratory) (2015)	1527	-40.12
	2459	-40.21
Vacuum extraction method sample 1 (laboratory) (2015)	1746	-40.87
	2679	-40.65
	2233	-40.82
Vacuum extraction method sample 2 (laboratory) (2015)	1491	-41.02
	1545	-41.07
	2113	-40.72
Vacuum extraction method sample 3 (laboratory) (2015)	789	-41.13
	6605	-40.51
	3065	-40.49
	2036	-40.48
Gas stripping method sample 1 (field) (2015)	593	-27.60
	576	-27.47
Gas stripping method sample 2 (field) (2015)	630	-27.57
	692	-27.49
	740	-27.30
Gas stripping method sample 3 (field) (2015)	561	-27.24
	536	-27.54
Gas stripping method sample 4 (field) (2014)	1310	-27.60
	1345	-27.54
Gas stripping method sample 4 (field) (2014)	881	-27.59
	838	-27.21
Vacuum extraction method sample 1	303	-28.75

(field) (2015)	301	-28.32
	667	-27.53
	632	-27.52
Vacuum extraction method sample 2 (field) (2015)	489	-27.52
	488	-27.54
Vacuum extraction method sample 3 (field) (2015)	981	-27.78
	959	-27.29
Vacuum extraction method sample 4 (field) (2015)	1087	-27.30
	1074	-27.32
Vacuum extraction method sample 5 (field) (2014)	4321	-27.76
	3714	-28.28
Vacuum extraction method sample 6 (field) (2014)	2550	-27.72
	3351	-27.72
Methane Tank 1 Characterization (2015)	2578	-40.02
	2560	-39.98
	2707	-40.26
	2098	-40.25
	2475	-40.19

Appendix 3.8 GC-IRMS data of δ^{13}C and amplitudes for all CH_4 samples for (A) April 2016 and (B) September 2015 and September 2016

Sample	**CH_4 δ^2H**		**Corrected CH_4 δ^2H**
	ampl. 2 (mV)	**δ ^2H VSMOW (‰)**	**δ ^2H VSMOW (‰)**
Methane tank 2 characterization 1	4960	-204.0	-200.1
	4486	-210.4	-206.1
Methane tank 2 characterization 2	3341	-214.5	-210.0
	4615	-213.4	-209.0
Methane tank 2 characterization 3	3963	-205.5	-201.5
Cryogenic Concentration 480 mL sample 1 (diluted ~40x) (laboratory)	2293	-206.4	-202.4
Cryogenic Concentration 480 mL sample 2 (diluted ~40x) (laboratory)	3387	-212.1	-207.8
Cryogenic Concentration 480 mL sample 3 (diluted ~40x) (laboratory)	3473	-203.7	-199.8
	3378	-214.5	-210.0
Cryogenic Concentration 720 mL sample 1 (diluted ~40x) (laboratory)	5834	-200.0	-196.3
	2412	-198.7	-195.0
Cryogenic Concentration 720 mL sample 2 (diluted ~40x) (laboratory)	2244	-193.2	-189.8
	3144	-213.0	-208.6
Cryogenic Concentration 720 mL sample 3 (diluted ~40x) (laboratory)	5979	-200.0	-196.3
	3667	-210.0	-205.8
Gas stripping method sample 1 (laboratory)	5123	-163.0	-161.2
Gas stripping method sample 2 (laboratory)	4892	-166.6	-164.6
Gas stripping method sample 3 (laboratory)	4951	-166.1	-164.1
Vacuum extraction method sample 1 (laboratory)	4967	-163.1	-161.3
Vacuum extraction method sample 2 (laboratory)	2015	-154.3	-152.9
	5052	-167.6	-165.5
Vacuum extraction method sample 3 (laboratory)	3271	-168.9	-166.8
Methane tank 1 characterization	4537	-169.5	-167.3
	3424	-167.9	-165.8

Appendix 3.9 GC-IRMS data of δD and amplitudes for all CH_4 samples for May 2016