Consumption of atmospheric methane in a limestone cave in Indiana, USA

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Abstract

Recent observations suggest that karst landscapes may be an unaccounted sink for atmospheric CH₄, but questions remain about the processes contributing to sub-atmospheric CH₄ mole fractions in caves. The CH₄ dynamics associated with karst environments were studied over 18 months at 6 locations in Buckner Cave, Southern Indiana by measuring the mole fractions and stable isotopic composition of CH₄ and carbon dioxide (CO₂). CO₂ mole fractions were used to infer seasonal changes in airflow. Samples were obtained on a monthly basis. CH₄ mole fractions ranged from 1.9 ± 0.1 ppm near the cave entrance to 0.1 ± 0.1 ppm in the more remote parts of the cave. The carbon and hydrogen stable isotopic compositions of CH₄ in the cave ranged from -58.7 to $+7$ ‰ (VPDB) and -170 to $+10$ ‰ (VSMOW), respectively. The isotopic data suggest that the CH₄ dynamics of Buckner Cave can be

described by a seasonally variable mixing system in which atmospheric CH₄ enters primarily through the main entrance and is subsequently consumed by methanotrophs. Additionally, at least two smaller CH₄ sources are evident when air has been stagnant. The results suggest that subterranean karst cavities are an important sink for atmospheric CH₄.

Keywords: Cave air, Methane, Carbon Dioxide, Cave, Methanotrophy, Greenhouse Gas

1. Introduction

The rapidly increasing abundance of atmospheric methane (CH₄), a greenhouse gas, has been linked to anthropogenic activity (Craig and Chou, 1982; U.S. EPA, 2010; Ciais et al., 2013). Accurate climate forecasting requires a detailed understanding of CH₄ sources and sinks affecting atmospheric abundances. Despite progress quantifying CH₄ sources and sinks, the uncertainties of individual sources and sinks remain large (Kirschke et al., 2013). Major CH₄ sources to the atmosphere include natural sources such as wetlands, freshwaters, and geologic emissions (seepage), as well as anthropogenic sources such as agriculture and fossil fuel use (Ciais et al., 2013). The major sink of atmospheric CH₄ is oxidation by the hydroxyl radical ($\cdot\text{OH}$) which accounts for 90 % of the total losses, whereas oxidation by CH₄ consuming (methanotrophic) microorganisms in soils is the next largest sink and accounts for about 2 % of the losses (Naik et al., 2013; Dlugokencky et al., 2011). The advective flow of air through porous rock units, including karst, causes the atmosphere to interact with the subsurface which, if oxidizing agents are present, would aid in the removal of CH₄ from the atmosphere.

Karst landforms cover 10 – 20 % of the continental surface (Palmer, 1991), and result from the interaction between soluble rock units and water. Mature karst landscapes exhibit solution-enlarged fractures, caves, and internal drainage networks which also provide conduits for atmospheric exchange (Atkinson et al., 1983; Gregorič et al., 2014). The surface area of the conduits capable of interacting with the atmosphere is unknown, in part because much of the surface area occurs in fractures that are too small for easy exploration (Curl, 1966). Despite the fact that some subsurface karst environments are inaccessible, the accessibility of caves provides a way to assess atmospheric exchange with the subsurface.

Limestone caves form as acidic water dissolves carbonate bedrock. Two common types are (1) epigenic caves, which form as meteoric water enriched in carbonic acid dissolves limestone, and (2) hypogenic caves, which form where deep-sourced waters, usually with sulfuric acid, migrate upwards. The different chemistry of the fluids entering these two types of caves also influences the paths of dissolved ions and gases, including CH₄, into the cave. Subterranean measurements of CH₄ mole fractions in karst air come from caves formed or forming from carbonic acid (epigenic speleogenesis) and those currently undergoing sulfuric acid speleogenesis (hypogenic). Epigenic caves dominate the total land area represented by karst and the CH₄ dynamics of these caves are more representative of the typical biogeochemical conditions present in karst. CH₄ mole fractions in the air of epigenic caves in Gibraltar, Spain, and Australia (Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016) are generally below that of the atmospheric background of 1.8 ppm (ppm, parts per million, is used to abbreviate $\mu\text{mol mol}^{-1}$, dry air mole fraction) (Dlugokencky et al., 2003; Adushkin and Kudryavtsev, 2010; Dlugokencky et al., 2011). The observations of sub-atmospheric CH₄ mole fractions in caves and karst have led to suggestions that they are

functioning as CH₄ sinks, however questions regarding the processes contributing to low CH₄ mole fractions in caves remain. In contrast, CH₄ mole fractions in the air of caves undergoing sulfuric acid speleogenesis have ranged from 3 ppm to 1 % by volume and have been reported from only three locations (Sarbu et al., 1996; Hutchens et al., 2004; Engel et al., 2004; Jones et al., 2012). These mole fractions are higher than the atmospheric background.

The sub-atmospheric CH₄ mole fractions observed in the systems in Gibraltar Spain, and Australia were proposed to have been caused by different mechanisms. The mechanism put forward to explain low CH₄ mole fractions in the Gibraltar cave relied on increases in $\delta^{13}\text{C}_{\text{CH}_4}$ values that coincided with decreasing CH₄ mole fractions. These data fit a Rayleigh distillation model caused by methanotrophy (*i.e.*, CH₄ being consumed by microorganisms, Matthey et al., 2013). This hypothesis is supported by the observation of methanotrophs in other epigenic cave systems (Pašić et al., 2010; Porca et al., 2012; Ortiz et al., 2013; McDonough et al., 2016). In contrast, the low CH₄ mole fractions observed in the air of Spanish caves were hypothesized to have been caused by radical reactions with CH₄ that were initiated by the radioactive decay of radon (Fernandez-Cortes et al., 2015).

The stable isotopic ratios of ¹³C to ¹²C and of ²H to ¹H in CH₄ are known to reflect the process by which the CH₄ was generated, and may help determine the sources and sinks of CH₄ in caves. Atmospheric CH₄ has $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values near −47 ‰ and −100 ‰, respectively (Miller et al., 2002; Townsend-Small et al., 2012). Microorganisms produce CH₄ through two major pathways, carbonate reduction and acetoclastic methanogenesis; the CH₄ produced by both of these pathways is enriched in light isotopologues (Whiticar, 1999). CH₄ generated from carbonate reduction is characterized by $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values from −110 to

–60 ‰ and –250 to –100 ‰, respectively (Whiticar, 1999, Tazaz et al., 2013). In contrast, CH₄ generated from acetate fermentation has a narrow range of $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values encompassing values from –65 to –48 ‰ and –370 to –260 ‰, respectively (Whiticar, 1999).

The stable isotopic ratios of ^{13}C to ^{12}C and of ^2H to ^1H in CH₄ are also known to reflect process, such as oxidation, that affect CH₄ after it has formed. CH₄ oxidation by $\cdot\text{OH}$ causes the residual $\delta^2\text{H}_{\text{CH}_4}$ value to increase by 72 ‰ for every 1 ‰ increase in $\delta^{13}\text{C}_{\text{CH}_4}$ values (Saueressig et al., 2001). Methanotrophy, in contrast, imparts an increase in the $\delta^2\text{H}_{\text{CH}_4}$ value of 8.5 ‰ for every 1 ‰ increase in $\delta^{13}\text{C}_{\text{CH}_4}$ values (Feisthauer et al., 2011). These differences in the kinetic isotope fractionation factors associated with CH₄ oxidation can help assess the oxidation process present in $\delta^2\text{H}$ - $\delta^{13}\text{C}$ space. Thus, the $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values may help determine the sources and sinks of CH₄ in caves.

We studied the carbon and hydrogen stable isotopic compositions and mole fractions of CH₄ and carbon dioxide (CO₂) in Buckner Cave, Southern Indiana to better characterize the CH₄ dynamics of epigenic karst landscapes. We hypothesized that if CH₄ in caves has an atmospheric source and cave processes decrease CH₄ mole fractions in cave air, then CH₄ should become more depleted as the distance from the cave entrance increases. We also asked, what are the biogeochemical processes that govern the mole fractions and stable isotopic composition of CH₄ in karst cavities? Our work presents time-series data over 18 months and the first measurements of the ratio of deuterium to hydrogen ($^2\text{H}/^1\text{H}$) in CH₄ from caves. Additionally we present a high resolution temporal study of a daily change in CH₄ mole fractions in Buckner Cave. Our work shows that the stable isotopic composition of CH₄ in

Buckner Cave can be described by CH₄ oxidation by methanotrophs as well as CH₄ production from at least two different biogeochemical pathways.

2. Geological Setting

Buckner Cave is located within the Crawford Upland physiographic province of Southern Indiana (Figure 1). The rock outcrops at the surface in the Crawford Upland consist of the interbedded sandstones and limestones of the Stephensport and West Baden groups (Conner, 1987; Lakey and Krothe, 1996). These rocks are resistant to erosion and cause an increase in topographic relief. Surface erosion by streams locally exposes the underlying limestones of the Blue River Group *i.e.* the Paoli Formation., the Saint Louis Formation, and the Saint Genevieve Formation. Buckner Cave is hosted in rocks of the Blue River Group, and is primarily hosted in the Saint Genevieve Fm., but the highest sections of the cave are located in the Paoli Limestone (Conner, 1987). The total vertical relief of Buckner Cave is 36.6 m with the lowest room having an elevation of 215.5 m a.s.l.(elevation at surface 259 m) and the highest room having an elevation of 252.1 m a.s.l (elevation at surface 255 m).

Buckner Cave has been managed by the Richard Blenz Nature Conservancy since 2005. Buckner Cave is a wild cave and is visited by about 6 people per week. There is one natural entrance to Buckner Cave (Figure 2). A second entrance (Bull's Eye Pit) was blasted in 1992 to provide access for rescue purposes. This entrance has been closed, and is thought to be air tight. Air flow in Buckner Cave is also thought to be connected to the nearby Queen Blair Cave, which is lower in elevation than Buckner Cave, based on air tracer experiments (DesMarais,

1971). Additionally, fractures in the limestone may allow for exchange with the atmosphere. The average air temperature of Buckner Cave is 12.8 °C.

Buckner Cave is a branchwork cave with some anastomosing passages (Palmer, 1991). Some of the cave passages exhibit features indicative of formation by vadose processes while others appear to have formed by phreatic processes. Buckner Cave has two small drainages that eventually outflow at Big Blair Spring (DesMarais, 1981) (Figure 2).

3. Methods

3.1 Air Sampling

Air sampling was carried out in two discrete sampling campaigns. A preliminary study determined seasonal patterns of CO₂ mole fractions in Buckner Cave by measuring monthly CO₂ mole fractions at six locations from August 2010 through July 2012 to (Figure 2). Sampling locations were selected based on their intersections with other passages, their distance from the main entrance, and their elevation. Distance to the main entrance was defined as the distance along the shortest passageway from a sampling location in Buckner Cave to the main entrance and was measured using the map of Buckner Cave shown in Figure 2. CO₂ mole fractions were measured *in-situ* with a portable gas monitor (Gasman – Crowcon Detection Instruments Ltd., Erlanger, Kentucky, USA; range 0 – 50,000 ppm; uncertainty, $\pm$ 100 ppm). Daily high and low temperatures and total precipitation were measured at the Monroe County Airport approximately 5 km northwest of Buckner Cave (NCEI Asheville, NC). Data from this early part of the study indicate two distinct seasons of airflow for Buckner Cave (Figure 3). We defined summer airflow to encompass periods when CO₂ mole fractions were above 2000 ppm.

Data from this study also indicated that air exchange through the sealed Bull's Eye Pit entrance was not a major influence on airflow in Buckner Cave.

In the second, more detailed part of the study from July 2013 to March 2015, samples from sites 1 through 5 were collected monthly and analyzed for the mole fractions and isotopic compositions of CH₄ and CO₂ in Buckner Cave (Figure 2). Air sampling occurred during evenings from 6 – 8 pm. Air from sampling locations was collected in Tedlar[®] bags using 60 mL or 140 mL syringes *via* three way valves. Each Tedlar[®] bag was flushed three times with 60 or 140 mL of air and was emptied before collecting the final air sample of ~ 550 to 650 mL. Samples were acquired in less than a minute.

An experiment to capture a daily change in the CH₄ mole fraction of Buckner Cave air took place September 29, 2014. The high and low atmospheric temperatures on the 29th were 27.8 and 10.0 °C, respectively. Temperatures had been oscillating above and below the average cave temperature for 16 days prior to the experiment. At three times over 4 hours, 0.6 L of pure CH₄ and 6 mL of pure sulfur hexafluoride (SF₆) were injected into the crawl way of Buckner Cave to check for differing behavior between the two gases (Figure 2). 60 mL of cave air was sampled roughly once every six minutes for five hours using pre-evacuated 50 mL serum vials. CH₄ mole fraction data were recorded once every 72 seconds using an open-path tunable diode laser system (OPL) (GasFinder 2.0, Boreal Laser Inc., Spruce Grove, Alberta, Canada, wavelength = 1.653 μm) following the methods of Webster et al. (2015). The path between the laser and the retroreflector mirror was 30 m.

3.2 Chemical and Isotopic Analyses

A Varian 450 gas chromatograph (GC) (Varian – Agilent Technologies, Palo Alto, California) was used to measure CH₄, CO₂, and SF₆ mole fractions. The chromatograph was fitted with a flame ionization detector (FID) for CH₄, a thermal conductivity detector (TCD) for CO₂, and an electron capture detector (ECD) for SF₆. CH₄ mole fraction standards of 10 and 100 ppm in a balance of N₂ from Air Liquide America Specialty Gasses LLC (Plumsteadville, Pennsylvania), and dilutions of the 10 ppm standard to 2 ppm, and a pure He blank were used to determine sample CH₄ mole fractions. The FID exhibited an analytical repeatability better than 5 %. A ± 0.10 ppm uncertainty was assigned to CH₄ mole fraction measurements based on replicates of low mole fraction samples, *i.e.* < 2.0 ppm. CO₂ standards of 100, 1000, and 10,000 ppm in balances of N₂ (Air Liquide), and a pure He blank were used to determine sample CO₂ mole fractions. CO₂ samples were assigned an uncertainty of ± 5 % based on the analytical repeatability of the CO₂ standards. CO₂ mole fraction data were also measured using a GasBench II inlet connected to a Delta Plus XP isotope-ratio mass-spectrometer (IRMS). Samples measured for mole fraction on the IRMS were given errors of 50 ppm for samples below 600 ppm (95 % confidence intervals) and were given errors of 5 % for samples above 600 ppm. SF₆ mole fractions were calculated with in-house standards created from pure SF₆ by adding known volumes of pure SF₆ to a balance of Air Zero (Air Liquide, purified air, with CO₂, H₂O, and hydrocarbons removed). SF₆ standards ranged from 0 to 700 ppb, and the repeatability of the standards were ± 1 %.

The carbon stable isotopic compositions of CH₄ and CO₂ were measured with an IRMS and are reported as conventional $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^{13}\text{C}_{\text{CO}_2}$ values in ‰ along the carbon isotopic scale anchored to Vienna Pee Dee Belemnite (VPDB). CH₄ samples were measured in continuous-flow mode using CH₄ preconcentration and cryofocusing (Miller et al., 2002) and a

gas chromatography-oxidation/pyrolysis-IRMS (GC-ox/pyr-IRMS) interface as briefly described by Cadieux et al., (2016). A variable sample extraction time was used to isolate approximately 0.4 μmol of CH_4 from each sample prior to introduction to the GC-ox/pyr-IRMS. CH_4 standards with $\delta^{13}\text{C}$ values of -38.25 ± 0.03 , $+19.89 \pm 0.05$, -39.40 ± 0.01 , and -57.75 ± 0.03 ‰ (Methane #1, #3, #6, and ALM, <http://pages.iu.edu/~aschimme/welcome.html>) were used for 2-point calibrations to normalize measured $^{13}\text{C}/^{12}\text{C}$ ratios to the VPDB scale. Ratios of $^{13}\text{C}/^{12}\text{C}$ in CO_2 from cave air were measured in continuous-flow mode using a GasBench II inlet (Tu et al., 2001). Measured $^{13}\text{C}/^{12}\text{C}$ ratios of CO_2 from cave air were converted to the VPDB scale using a single isotopically characterized in-house standard that has a value of 12.0 ± 0.2 ‰.

The hydrogen stable isotopic compositions of CH_4 are reported along the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) isotopic scale. Samples are reported in ‰ as $\delta^2\text{H}_{\text{CH}_4}$ values. $\delta^2\text{H}_{\text{CH}_4}$ values were measured with an IRMS in continuous-flow mode using CH_4 preconcentration and cryofocusing (Miller et al., 2002) and a GC-ox/pyr-IRMS interface. CH_4 standards with $\delta^2\text{H}_{\text{CH}_4}$ values of -161 ± 2 , $+2.2 \pm 1.2$, -153 ± 2 , and -273 ± 3 ‰ (Methane #1, #3, #6, and ALM, <http://pages.iu.edu/~aschimme/welcome.html>) were used for 2-point calibration along the VSMOW-SLAP scale. A variable sample extraction time was used to isolate roughly 0.8 μmol of CH_4 from each sample prior to introduction to the GC-ox/pyr-IRMS.

Errors associated with $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values were calculated using a standard curve that accounted for the peak size of the measurement. Larger errors are associated with small

peak sizes. Analytical repeatability of internal standards ranged from 0.13 to 0.4 ‰ for $\delta^{13}\text{C}_{\text{CH}_4}$ and from 3 to 20 ‰ in $\delta^2\text{H}_{\text{CH}_4}$.

3.3 Quantitative analyses

Samples and data were screened for quality control. If a sample bag had been shown to exhibit a leak for one analyte, analyses of other analytes concurrent or after that point in time from that bag were discarded. Supplemental materials 1 and 2 show all of the collected data. All samples are reported with 95 % confidence intervals.

Several different modeling techniques were used to assess the processes affecting trace gas geochemistry in Buckner Cave. Linear regression models were used to determine the effect of distance and elevation on CH_4 and CO_2 mole fractions in Buckner Cave. The sources of CO_2 entering Buckner Cave were assessed using forward modeling techniques assuming mixing between an atmospheric endmember and endmembers of varying isotopic signatures following the methods of Spötl et al. (2005). Due to noise in samples below 650 ppm CO_2 , source signatures were only calculated for samples with CO_2 mole fractions greater than or equal to 650 ppm.

Rayleigh distillation models were used as the theoretical basis to examine changes in the stable isotopic composition of atmospheric CH_4 caused by methanotrophy or $\cdot\text{OH}$ oxidation. The δ value of an isotope system in a molecule of interest in cave air can be modeled as

$$\delta_c = (\delta_i + 1000) f^{(-\alpha + 1)} - 1000 \quad (1)$$

where δ_c is the current δ -value of a particular isotope system in cave air after partial consumption, δ_i is the initial δ -value of the isotope system in cave air, f is the fraction of the molecule of remaining, and α the kinetic isotope fractionation factor (Barker and Fritz, 1981; Feisthauer et al., 2011). α values of 1.018 and 1.2413 were used to model bacterial methanotrophy (Coleman et al., 1981; Feisthauer et al., 2011), and α values of 1.0039 and 1.294 were used to model changes in $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values caused by $\cdot\text{OH}$ oxidation (Saueressig et al., 2001).

4. Results

The mole fractions and stable isotopic compositions of CH_4 and CO_2 in Buckner Cave varied both spatially and seasonally, following a pattern of thermally-driven circulation in winter and stagnation in summer (Figure 4). CH_4 mole fractions were highest in Buckner Cave during winter and lowest in Buckner Cave during summer whereas CO_2 mole fractions were lowest in winter and highest in summer (Figure 4B). CH_4 mole fractions in Buckner Cave ranged from < 0.1 to 1.9 ± 0.1 ppm, and CO_2 mole fractions ranged from 330 ± 50 to 5300 ± 500 ppm. The outside atmosphere showed little variation in CH_4 and CO_2 mole fractions with CH_4 ranging from 1.86 ± 0.13 to 2.17 ± 0.10 ppm and CO_2 ranging from 400 ± 20 to 520 ± 30 ppm. $\delta^{13}\text{C}_{\text{CH}_4}$, $\delta^2\text{H}_{\text{CH}_4}$, and $\delta^{13}\text{C}_{\text{CO}_2}$ values of the external atmosphere also exhibited narrow ranges with $\delta^{13}\text{C}_{\text{CH}_4}$ values ranging from -49 ± 3 ‰ to -42.7 ± 0.4 , $\delta^2\text{H}_{\text{CH}_4}$ values ranging from -110 ± 30 ‰ to -80 ± 30 ‰, and $\delta^{13}\text{C}_{\text{CO}_2}$ values ranging from -10.5 ± 0.5 ‰ to -9.2 ± 0.4 ‰. Winter cave air was compositionally more similar to the outside atmosphere than summer cave air and had higher CH_4 mole fractions than summer air (Independent 2-group Mann-Whitney U

Test, $W = 254.5$, $p = 0.0006$). $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values of cave air were more variable during summer than during winter and were more similar to the respective values of outside atmospheric CH_4 during winter than during summer (Figure 4E, F). $\delta^{13}\text{C}_{\text{CO}_2}$ values were more similar to the respective values of the outside atmosphere during winter than during summer (Figure 4D). Winter $\delta^{13}\text{C}_{\text{CO}_2}$ values ranged from -20.1 ± 0.2 to -13.8 ± 0.4 ‰, while, with the exception of Site 1, summer values were close to -23 ‰, ranging from -23.68 ± 0.11 to -20.96 ± 0.16 ‰.

The CH_4 mole fraction in Buckner Cave was observed to be capable of daily fluctuations (Figure 5). CH_4 mole fractions were relatively high in the crawl way near the entrance at the start of the experiment. The wind direction was also felt blowing into the cave at the beginning of the experiment and blowing out of the cave at the end of the experiment (Webster and Mirza, field observations). Over the course of the experiment baseline CH_4 mole fractions decreased from 1.53 ± 0.03 to 0.339 ± 0.017 ppm. CH_4 and SF_6 showed the same behavior when injected into Buckner Cave. Increases in CH_4 mole fractions were registered before increases in SF_6 mole fractions, but SF_6 was measured as CH_4 mole fractions exhibited minor stability before falling back to or below CH_4 mole fractions prior to the injection of the gases.

Atmospheric CH_4 mole fractions in Buckner Cave were also related to the distance, d (m), from the Main Entrance (Figure 6). Rooms farther from the Main Entrance showed lower CH_4 mole fractions during both summer and winter based on a correlation analysis ($[\text{CH}_4]_{\text{summer}} = -0.0025 \pm 0.0008 d + 1.4 \pm 0.3$, $r^2 = 0.59$; $p = 8 \times 10^{-7}$; $[\text{CH}_4]_{\text{winter}} = -0.0024 \pm 0.0006 d + 1.9 \pm 0.2$, $r^2 = 0.62$, $p = 4 \times 10^{-8}$). CH_4 and CO_2 mole fractions were not related to the elevation of sites in Buckner Cave ($[\text{CH}_4]_{\text{summer}}$, $r^2 = 0.0$, $p = 0.9$; $[\text{CH}_4]_{\text{winter}}$, $r^2 = 0.09$, $p = 0.09$;

$[\text{CO}_2]_{\text{summer}}, r^2 = 0.17, p = 0.30; [\text{CO}_2]_{\text{winter}}, r^2 = 0.20, p = 0.23$). The CH_4 mole fraction in Buckner Cave was related to the CO_2 mole fraction by an inverse power function ($[\text{CH}_4] = 354.5[\text{CO}_2]^{-0.92}, r^2 = 0.63$).

Following the approach of Spötl et al., (2005) we inferred CO_2 sources with $\delta^{13}\text{C}_{\text{CO}_2}$ values ranging from -35 ± 2 to $-20 [-1, +0.8] \text{‰}$ are entering Buckner Cave (Figure 7). Winter sources of CO_2 have $\delta^{13}\text{C}$ values that range from -35 ± 2 to $-24 \pm 2 \text{‰}$, with an average value of $-28.7 \pm 0.4 \text{‰}$. The sources of CO_2 entering Buckner Cave during summer airflow appear to range from -26.7 ± 0.5 to $-20 [-1, +0.8] \text{‰}$ with an average value of $-24.08 \pm 0.08 \text{‰}$. Samples from winter show the input of more negative $\delta^{13}\text{C}_{\text{CO}_2}$ than summer samples (two sample t -test unequal variance, $t_{18} \text{ stat} = -3.33, p = 0.0019$).

Cave air $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values ranged from -58.7 to $+7 \text{‰}$ and from -170 to $+10 \text{‰}$ respectively (Figure 4). Several points exhibit more negative $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values than would be expected if the oxidation of atmospheric CH_4 by methanotrophy or $\cdot\text{OH}$ oxidation were the only process acting on CH_4 in Buckner Cave (Figures 8, 9). A plot of the data in $\delta^2\text{H}_{\text{CH}_4}$ vs. $\delta^{13}\text{C}_{\text{CH}_4}$ space shows that a cluster of points plots near the values for atmospheric CH_4 (Figure 10). Other points, *i.e.* points that plot to the left of the atmosphere and below the atmosphere, suggest that non-atmospheric CH_4 sources are entering Buckner Cave (Figure 10).

5. Discussion

5.1 Air Flow and Trace Gas Mole Fractions in Buckner Cave

The patterns in CH₄ mole fractions in Buckner Cave suggest that CH₄ is being removed from cave air, and that Buckner Cave is dynamically ventilated. Observations of daily and seasonal changes in CH₄ and CO₂ mole fractions in Buckner Cave suggest that external air enters Buckner Cave through the main entrance when outside temperatures are cooler than cave air temperatures (Figures 3, 4). Additionally, CH₄ mole fractions were close to atmospheric mole fractions as air flowed into the cave on Sep. 29th, but CH₄ mole fractions decreased as air flow in the cave reversed (Figure 5). Seasonality in Buckner Cave resulted in mole fractions of CH₄ and CO₂ that were more similar to the atmosphere in the winter than in the summer (Figure 4). Seasonality has been observed in other caves and results in high CO₂ mole fractions during times of low atmospheric influence, whereas low CO₂ mole fractions relate to times of high atmospheric influence (Mattey et al., 2010; Kowalczyk and Froelich, 2010; Gregorič et al., 2011; Gregorič et al., 2013; Baldini et al., 2008).

The mole fraction of CH₄ in the air of Buckner Cave is related to the amount of atmospheric influence in Buckner Cave. CH₄ mole fractions were more similar to atmospheric values during winter airflow when cave air CO₂ also exhibited mole fractions and stable isotopic compositions that were more similar to atmospheric values (Figure 4). CH₄ and CO₂ mole fractions in Buckner Cave are generally inversely correlated, although smaller scale deviations from this pattern are observed (Figure 4). The small scale deviations from the pattern occur in May when both CH₄ and CO₂ mole fractions are relatively low, and in October when both CH₄ and CO₂ mole fractions are relatively high, and may be due to changing flow regimes in the cave. The trend of inversely correlated CH₄ and CO₂ mole fractions in Buckner cave matches observations of cave air from caves in Gibraltar, Spain, and Australia (Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016).

The $\delta^{13}\text{C}_{\text{CO}_2}$ values of air in Buckner Cave also suggest that cave air flow was faster in winter than in summer. Winter CO_2 sources are estimated to have an average $\delta^{13}\text{C}_{\text{CO}_2}$ value of $-28.7 \pm 0.4 \text{ ‰}$ which is more negative than the estimated average $\delta^{13}\text{C}_{\text{CO}_2}$ value of summer sources, $-24.08 \pm 0.08 \text{ ‰}$. The ultimate source of this ^{13}C -depleted CO_2 entering Buckner Cave in winter is likely soil CO_2 which has $\delta^{13}\text{C}_{\text{CO}_2}$ values near -24 ‰ (Amundson et al., 1998; Breecker et al., 2012). However, the diffusivity of light isotopologues through porous materials can be preferentially enhanced through pressure pumping, which occurs in porous materials as air flows past them (Bowling and Massman, 2011). As air flows through Buckner Cave in the winter, pressure pumping in the karst and epikarst may draw more ^{13}C -depleted CO_2 into cave air. Source $\delta^{13}\text{C}_{\text{CO}_2}$ values as low as -32 ‰ have been observed in snowpack and other caves during times of greater atmospheric influence when the source has been considered to derive from soil CO_2 (Bowling and Massman, 2011; Garcia-Anton et al., 2014). Slower airflow in summer is suggested by increased CO_2 mole fractions and stable isotopic compositions of CO_2 with $\delta^{13}\text{C}_{\text{CO}_2}$ values that correspond to values observed for CO_2 in the soils and epikarst, *i.e.* near -24 ‰ (Cernusak et al., 2009; Matthey et al., 2010; Kowalczyk and Froelich, 2010; Breecker et al., 2012; Peyraube et al., 2013; Garcia-Anton et al., 2014; Fernandez-Cortes et al., 2015).

Secondary ventilation driven by changes in external wind speed and wind direction is likely present in Buckner Cave (Kowalczyk and Froelich, 2010; Breitenbach et al., 2015). Cave air close to the main entrance may be prone to enhanced exchange by wind-forced mixing, and may explain higher CH_4 mole fractions at Site 1 (the entry room) during summer when the interior of Buckner Cave is relatively less ventilated. The summer relationship between CH_4 mole fractions and distance from the cave entrance may follow an inverse power function

(Figure 6). This relationship may suggest that two fluids with distinct composition are physically close but experience a barrier to mixing. If this is applicable to Buckner Cave, it may suggest that airflow in the entry room is fundamentally different from flow in the rest of the cave during summer.

5.2 CH₄ Dynamics of Buckner Cave

The CH₄ mole fractions, $\delta^{13}\text{C}_{\text{CH}_4}$ values, and $\delta^2\text{H}_{\text{CH}_4}$ values offer additional insight into the sources and sinks of CH₄ in Buckner Cave (Figures 8, 9, 10). While the general pattern of increasing $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values as CH₄ mole fraction decreases indicates that CH₄ oxidation is taking place in Buckner Cave, data that plot below and to the left of the theoretical oxidation trends show that non-atmospheric sources of CH₄ with low $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values are entering parts of the cave (Figures 8,9). When $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values are plotted against each other, CH₄ generated from other biogeochemical pathways appears to be entering the cave (Figure 10).

CH₄ produced from carbonate reduction appears to be entering Buckner Cave. Several samples from Buckner Cave plot near known CH₄ produced from carbonate reduction (Figure 10). Oxidation of this CH₄ source in Buckner Cave appears to result in a positive shift in the $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values that could be caused by methanotrophy or $\cdot\text{OH}$ oxidation. CO₂ reduction has been observed in the anoxic interior of biological soil crusts in desert soils (Angel et al., 2011), and it is possible that CO₂ reduction occurs in locally anoxic soil microenvironments above karst, or locally anoxic epikarst environments. This CH₄ appears to

be partially oxidized resulting in slightly more positive values (*i.e.* $\delta^{13}\text{C}_{\text{CH}_4}$ value nears -62‰ and $\delta^2\text{H}_{\text{CH}_4}$ values near -100‰) than are typically observed from carbonate reduction.

A cluster of points plots below and to the top right of an atmospheric CH_4 source. Five samples in this cluster plot within the region of thermogenic CH_4 , *i.e.* CH_4 from deep geologic sources, such as shale gas (Figure 10) (Whiticar, 1999; Etiope and Sherwood Lollar, 2013). Thermogenic CH_4 could be a minor source of CH_4 in Buckner Cave. However the migration of geological CH_4 into Buckner Cave would likely occur through a geologic fault injecting CH_4 at a point source. During periods of slower air flow, specifically during summer, seepage from a geological source would likely create an elevated, quasi-constant CH_4 mole fraction in a specific part of the cave near the seep as long as the rate of influx and airflow patterns are relatively constant. The CH_4 in this room of the cave would also have a temporally similar isotopic composition due to the stable seepage source. Such a pattern was not observed which suggests that other CH_4 sources are likely contributing to the mole fractions of CH_4 , $\delta^{13}\text{C}_{\text{CH}_4}$, and $\delta^2\text{H}_{\text{CH}_4}$ values observed in Buckner Cave.

Since the migration of thermogenic CH_4 in Buckner Cave is unlikely, the data suggest that CH_4 generated by acetate fermentation is entering Buckner Cave. Acetoclastic methanogenesis may occur in the soil above the cave, the epikarst, or in the cave itself. The samples from Buckner Cave plot along a methanotrophic oxidation trend (Figure 10). Only one observation plots off of this trend. Oxidation of acetoclastically produced CH_4 may occur *in-situ* or prior to entering the cave. At Site 2, a slightly increased CH_4 mole fraction in June 2014 is compatible with the oxidation of CH_4 that had been produced by acetoclastic methanogenesis (Figures 4, 10). Additionally, the CH_4 mole fraction, $\delta^{13}\text{C}_{\text{CH}_4}$, and $\delta^2\text{H}_{\text{CH}_4}$ values associated with

these points can be modeled assuming that a localized pulsed CH₄ source enters the cave, raises the CH₄ mole fraction to about 0.5 ppm, and is subsequently oxidized by methanotrophy.

It has recently been suggested that ions and radicals associated with radon decay may be oxidizing CH₄ in caves (Fernandez-Cortes et al., 2015). Many isotopic compositions of CH₄ in Buckner Cave cannot be accounted for if it is assumed that (i) CH₄ enters Buckner Cave from the atmosphere, through carbonate reduction, and through acetate fermentation and (ii) that CH₄ is removed from the cave atmosphere through reactions involving the ·OH radical. Conversely, if it is assumed that (i) CH₄ sources in Buckner Cave include the atmosphere, carbonate reduction, and acetate fermentation and (ii) that CH₄ is removed from the cave atmosphere by methanotrophy, only one point plots outside of the plausibility envelope of the model (Figure 10). However, if a thermogenic source is also assumed to be entering Buckner Cave and the ·OH radical is a possible mechanism for CH₄ removal in Buckner Cave, then three points plot outside of the plausibility ranges of the model.

5.3 Future Directions

Quantitative measurements of the oxidative removal of atmospheric CH₄ in subterranean karst environments are needed for future incorporation of CH₄ consumption by karst into global models of greenhouse gas sources and sinks. For example, CH₄ fluxes in caves could be calculated *via* combined measurements of CH₄ mole fraction in caves and simultaneous wind speed measurements. Eddy covariance above karst landscapes may be a suitable method to determine the amount of atmospheric CH₄ being consumed by karst because this method is an effective means of measuring CH₄ flux in arctic landscapes (Sachs et al., 2008; Tagesson et al.,

2012). A third appropriate method may be quantification of CH₄ oxidation in karst by *in-situ* chamber flux experiments, followed by global extrapolation across all karst landscapes. Caveats would be represented by (i) uncertain estimates of the total subterranean pore space that is able to exchange air with the global atmosphere, and (ii) likely contrasting methanotrophic kinetics across different climates and altitudes. The simultaneous utilization of multiple approaches will yield valuable insight on subterranean CH₄ consumption in all large subterranean cavities and fractures at varying spatial and climatic scales.

6. Summary and Conclusions

Buckner Cave is a net CH₄ sink because cave air is exchanging with the atmosphere and the CH₄ mole fractions in cave air were generally below those of the outside atmosphere. Mounting evidence points to subterranean cave environments serving as a global sink for atmospheric CH₄. Different air flow regimes in Buckner Cave throughout the year resulted in distinct seasonal variations of CH₄ and CO₂ mole fractions. The pattern of stable isotopic compositions of CH₄ in Buckner Cave can be best explained by an atmospheric CH₄ source, oxidation of CH₄ by methanotrophic bacteria, and at least two isotopically different non-atmospheric sources of CH₄ (Figure 10). Our observations of non-atmospheric CH₄ sources entering the air of Buckner Cave agree with observations from St. Michael's Cave in Gibraltar where the $\delta^{13}\text{C}_{\text{CH}_4}$ values of cave air suggested methanogenesis occurred within the karst system (Mattey et al., 2013). Our data also agree with observations from Garden and Wellington caves in Australia where methanotrophy was determined to be the reason for the sub-atmospheric CH₄

mole fractions in those caves (McDonough et al., 2016). Further studies are needed to quantitatively constrain the oxidative loss of CH₄ in subterranean karst environments.

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Figure 1. Location of Buckner Cave in Southern Indiana (A) and the USA (B). Satellite image from Google Maps.

Figure 2. Simplified map of Buckner Cave showing the Main Entrance and sampling locations labeled by numbers. This map has been redrawn from the map of Buckner Cave published by the Richard Blenz Nature Conservancy in 2012. Small blue arrows indicate streams and their direction of flow.

Figure 3. Results from the first part of the study identifying broad scale airflow patterns in Buckner Cave. Date is represented as mm/yy on the horizontal axis. Notice that temperatures elevated air temperatures relative to the average temperature of Buckner Cave (A) corresponded to periods of high CO₂ mole fractions in Buckner Cave (B). Summer airflow in Buckner Cave (vertical gray bars) was defined to encompass periods when the CO₂ mole fraction was above 2000 ppm. Summer airflow coincided with daily low temperatures that were generally higher than the average cave temperature (12.8 °C) and lasted until the daily high temperature was generally colder than the average cave temperature. Air temperatures in (A) were measured at

Monroe County Airport and were from the National Center for Environmental Information (NCEI). Errors bars in (B) are smaller than the data points.

Figure 4. Time series data collected during the 2013 – 2015 field campaign. Date is on the horizontal axis as mm/yy. Vertical gray bars represent times of summer airflow and vertical white bars correspond to times of winter airflow. Elevated air temperatures relative to the average temperature of Buckner Cave (A) corresponded to high and low CO₂ mole fractions (B), respectively. More negative $\delta^{13}\text{C}_{\text{CO}_2}$ values were observed during summer airflow relative to winter airflow (C). CH₄ mole fractions in Buckner Cave (D) were negatively correlated to the outside air temperature. More variable $\delta^{13}\text{C}_{\text{CH}_4}$ (E) and $\delta^2\text{H}_{\text{CH}_4}$ (F) values were observed during summer airflow compared to winter airflow. A lack of error bars indicates that the analytical uncertainty was smaller than the plotted data symbol. Air Temperatures in (A) were measured at Monroe County Airport and were retrieved from the National Center for Environmental Information (NCEI).

Figure 5: Results from the CH₄ and SF₆ addition experiment to capture a change in cave air CH₄ mole fraction in Buckner Cave. Baseline CH₄ mole fractions decrease after a reversal in airflow at 19:45. CH₄ (A) and SF₆ spikes (B) at 18:27, 19:20, and 20:10 show that CH₄ and SF₆ exhibited the same behavior in Buckner Cave. CH₄ mole fractions returned to previous base line mole fractions as SF₆ mole fractions decreased to 4 ppb. If error bars are not visible they are smaller than the data points.

Figure 6: The CH₄ mole fraction in the air of Buckner Cave decreases as the distance from the main entrance increases. The statistics show that the CH₄ mole fraction in interior rooms is lower than the CH₄ mole fraction in exterior rooms. The uncertainty associated with all points is ± 0.1 ppm.

Figure 7: The $\delta^{13}\text{C}_{\text{CO}_2}$ vs. CO₂ mole fraction in Buckner Cave air. The data suggest that atmospheric CO₂ is mixing with isotopic endmembers between -35 and -20 ‰. Summer air shows the influence of a more positive end member and winter air shows the influence a more negative end member. Soil CO₂ is modeled with a $\delta^{13}\text{C}_{\text{CO}_2}$ value of -24 ‰. The color scheme follows that of Figure 4. Site 1 = light blue, Site 2 = dark red, Site 3 = black, Site 4 = red, Site 5 = dark blue. If error bars are not visible, they are smaller than the data points.

Figure 8: CH₄ mole fraction vs. $\delta^{13}\text{C}_{\text{CH}_4}$ observed in Buckner Cave air. The expected isotopic trend (dotted line) along methanotrophic depletion of atmospheric CH₄ was calculated assuming a starting CH₄ mole fraction of 1.8 ppm, a $\delta^{13}\text{C}_{\text{CH}_4}$ value of -47 ‰, and a fractionation factor of 1.018 (Barker and Fritz, 1981; Coleman et al., 1981; Feisthauer et al., 2013). ·OH oxidation is modeled with a fractionation factor of 1.0039 (Saueressig et al., 2001).

Figure 9: CH₄ mole fraction vs $\delta^2\text{H}_{\text{CH}_4}$ values observed in Buckner Cave. The light gray dotted line represents the expected methanotrophic fractionation of an atmospheric methane (1.8 ppm, –100 ‰) using a fractionation factor of 1.2413 (Coleman et al., 1981). $\cdot\text{OH}$ oxidation is modeled with a fractionation factor of 1.294 (Saueressig, et al., 2001). The discrepancy between the observed $\delta^2\text{H}_{\text{CH}_4}$ values and the predicted $\delta^2\text{H}_{\text{CH}_4}$ values of partially degraded atmospheric methane shows the existence of a source of ²H-depleted methane.

Figure 10: Stable isotopic composition of CH₄ in Buckner Cave plotted in $\delta^2\text{H}_{\text{CH}_4}$ versus $\delta^{13}\text{C}_{\text{CH}_4}$ space. The dashed outlines in the figure represent most of the isotopic ranges of CH₄ generate by thermogenic processes and acetate fermentation (Etiope and Sherwood Lollar, 2013). Methanotrophic oxidation is modeled with a slope of 8.5 and $\cdot\text{OH}$ oxidation is modeled with a slope 75 (Saueressig et al., 2001; Feisthauer et al., 2011).

Supplemental 1: CH₄ and CO₂ mole fractions, collection dates, and analysis date for each site.

Supplemental 2: The stable isotopic compositions of CH₄ and CO₂, collection dates, and analysis date for each site.

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