Subterranean Karst Environments as a Global Sink for Atmospheric Methane

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Abstract

The air in subterranean karst cavities is often depleted in methane (CH₄) relative to the atmosphere. Karst is considered a potential sink for the atmospheric greenhouse gas CH₄ because its subsurface drainage networks and solution-enlarged fractures facilitate atmospheric exchange. Karst landscapes cover about 14% of earth’s continental surface, but observations of CH₄ concentrations in cave air are limited to localized studies in Gibraltar, Spain, Indiana (USA), Vietnam, Australia, and by incomplete isotopic data. To test if karst is acting as a global CH₄ sink, we measured the CH₄ concentrations, δ¹³C(CH₄), and δ²H(CH₄) values of cave air from 33 caves in the USA and three caves in New Zealand. We also measured CO₂ concentrations, δ¹³CCO₂, and radon (Rn) concentrations to support CH₄ data interpretation by assessing cave air residence times and mixing processes. Among these caves, 35 exhibited subatmospheric CH₄ concentrations in at least one location compared to their local atmospheric backgrounds. CH₄ concentrations, δ¹³C(CH₄), and δ²H(CH₄) values suggest that microbial methanotrophy within caves is the primary CH₄ consumption mechanism. Only 5 locations from 3 caves showed elevated CH₄ concentrations compared to the atmospheric background and could be ascribed to local CH₄ sources from sewage and outgassing swamp water. Several associated δ¹³C(CH₄) and δ²H(CH₄) values point to carbonate reduction and acetate fermentation as biochemical pathways of limited methanogenesis in karst environments and suggest that these pathways occur in the environment over large spatial scales. Our data show that karst environments function as a global CH₄ sink.
1. Introduction

Atmospheric methane (CH$_4$) is a greenhouse gas and its concentration is increasing in the atmosphere (Dlugokencky et al., 2011; Sussmann et al., 2012; Ciais et al., 2013). The present globally averaged CH$_4$ concentration is 1.87 ppmv which is 2.5 times higher than preindustrial levels (Nisbet et al., 2016). The increase in atmospheric CH$_4$ is due to an imbalance between CH$_4$ sources and sinks. Anthropogenic and natural sources combine to contribute about 680 Tg a$^{-1}$ of CH$_4$ to the atmosphere while reactions with hydroxyl (-OH) and chlorine radicals in the troposphere and stratosphere remove about 600 Tg a$^{-1}$ (Kirschke et al., 2013). Methanotrophic consumption in soils, the next largest sink, removes 30 Tg a$^{-1}$ (Kirschke et al., 2013). Despite improvements in estimating individual sources and sinks of atmospheric CH$_4$, the associated errors remain large (Kirschke et al., 2013). Recent studies suggest that caves may act as an additional CH$_4$ sink (Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016; Webster et al., 2016; Lennon et al., 2017).

Caves and associated karst landscapes may be an important overlooked sink for atmospheric CH$_4$ because they are estimated to cover as much as 10 to 20 % of the continental surface with more precise estimates suggesting about 13.8 % (Palmer, 1991; Ford and Williams, 2007). Karst landscapes are frequently associated with the chemical dissolution of limestones, but can form in any soluble rock body. The resulting caves, solution-enlarged fractures, and internal drainage networks that function to transport mass from high elevations to low elevations also allow for subsurface-surface atmospheric exchange (Kowalczyk and Froelich, 2010; Garcia-Anton et al., 2014). The total volume and surface area of karst conduits able to interact with the atmosphere is unknown, in part due to small fractures and the difficulty of imaging the subsurface with...
geophysical methods. Karst caves, due to their accessibility, provide opportunities for non-invasive, *in-situ* analyses and sampling.

Cave and karst landscapes form in two common ways, each of which influences karst’s capacity to act as a CH$_4$ sink. Epigenic karst forms through the interaction of limestone with carbonic acid derived from the dissolution of atmospheric and soil CO$_2$ into surface waters. By contrast, hypogenic caves form when corrosive water from deep sources migrates into and dissolves limestone bedrock. Epigenic caves are more widespread, and atmospheric to subatmospheric CH$_4$ concentrations of 1.8 ppmv to < 0.1 ppmv have been observed in these settings (Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016; Webster et al., 2016; Lennon et al., 2017). For comparison, in some hypogenic caves elevated CH$_4$ concentrations from 2 ppmv to 1% have been observed in association with CH$_4$-rich springs or seeps related to fluid migration from deep hydrocarbon-bearing sedimentary rocks, i.e. seepage processes that are widespread on Earth (Sarbu et al., 1996; Hutchens et al., 2004; Jones et al., 2012; Webster et al., 2017). The dominance of epigenic karst suggests these regions are functioning as a CH$_4$ sink at the global scale, but more observations are needed.

Different hypotheses have been put forward to explain the low CH$_4$ concentrations observed in epigenic cave air. The combination of subatmospheric CH$_4$ concentrations and the stable carbon isotopic ratio of CH$_4$ in the air of caves in Gibraltar led to the hypothesis that microorganisms were responsible for the removal of CH$_4$ (Mattey et al., 2013). In turn, low CH$_4$ concentrations in Spanish caves, in the presumed absence of CH$_4$-consuming (methanotrophic) bacteria, led to the hypothesis that CH$_4$ oxidation was induced by ions and ·OH generated by the radioactive decay of radon and daughter nuclides (Fernandez-Cortes et al., 2015). Since these initial observations, datasets from caves in Australia, the USA, and Vietnam have pointed towards
methanotrophic CH₄ oxidation (McDonough et al., 2016; Webster et al., 2016; Lennon et al., 2017, Waring et al., 2017).

The chemical composition of cave air results from the mixing of the atmosphere and air from the overlying soils and epikarst. These processes should also influence the CH₄ concentrations of cave air. Previous studies have shown that CH₄ concentrations have been inversely correlated with CO₂ concentrations in cave air (Mattey et al., 2013; Ferndandez-Cortes et al., 2015; McDonough et al., 2016; Webster et al., 2016). Cave air CO₂ concentrations are positively correlated with radon (Rn) concentrations and Rn is known to track cave air residence time (Cunningham and LaRock, 1991; Batiot-Guilhe et al., 2007; Kowalczk and Froelich, 2010; Mattey et al., 2010; Gregorič et al., 2011, 2014). Additionally, the stable C isotope composition of CO₂ (δ¹³CCO₂), can track the sources of CO₂ in the environment. For example, δ¹³CCO₂ values of −24 ‰ are associated with soil CO₂, while atmospheric CO₂ has δ¹³CCO₂ values ranging from −8.5 ‰ to −10 ‰ (Amundson et al., 1998; Keeling et al., 2010; Peyraube et al., 2013). Thus CO₂, Rn, and δ¹³CCO₂ in cave air can help determine the influence of cave air mixing processes on CH₄.

The stable C and H isotope compositions of CH₄ (δ¹³CCH₄ and δ²HCH₄) also provide tools for understanding the sources and sinks of CH₄ in caves because different CH₄ sources are associated with characteristic δ¹³CCH₄ and δ²HCH₄ values. For example, CH₄ produced from carbonate reduction has δ¹³CCH₄ and δ²HCH₄ values that range from −112 to −60 ‰ VPDB and from −350 to −100 ‰ VSMOW respectively (Whiticar, 1999). Atmospheric CH₄ has δ¹³CCH₄ and δ²HCH₄ values around −47.5 and −100 ‰ (Miller et al., 2002; Townsend-Small et al., 2012). The δ¹³CCH₄ and δ²HCH₄ values of CH₄ can also be altered through secondary processes such as oxidation and mixing. The oxidation pathways of CH₄ by methanotrophs or the ·OH have
fractionation factors that cause the residual CH$_4$ to show increases in $\delta^{2}$HCH$_4$ values of 8.5 ‰ for every 1 ‰ increase in $\delta^{13}$CCH$_4$ value and increases in $\delta^{2}$HCH$_4$ values of 72 ‰ for every 1 ‰ increase in $\delta^{13}$CCH$_4$ value, respectively (Feisthauer et al., 2011; Saueressig et al., 2001). Mixing between two different CH$_4$ sources creates a linear trend between the two members. Thus, measuring the $\delta^{13}$CCH$_4$ and $\delta^{2}$HCH$_4$ of cave air should allow for the determination of cave air CH$_4$ sources.

The objective of the present work is to extend the karst CH$_4$ dataset and test the hypothesis that karst systems act as a CH$_4$ sink on a global scale. To this aim, we studied CH$_4$ concentrations, $\delta^{13}$CCH$_4$, and $\delta^{2}$HCH$_4$ in cave air from 33 epigenic caves in the USA and three epigenic caves in New Zealand. CO$_2$, $\delta^{13}$CCO$_2$, and Rn were also measured to support CH$_4$ data interpretation via assessing cave air residence times and mixing processes. Data analysis is focused on determining CH$_4$ concentrations, origin, mixing processes and isotopic fractionations.

2. Methods

2.1. Sampling and analyses

Air samples from the study caves were collected over a timespan of roughly four years (Fig. 1; Table 1). Study caves fell into three broad groups, those from the Appalachian fold and thrust belt (16); those in gently warped intracratonic basins of the USA (17); and those from the North Island of New Zealand (3). Cave air was sampled using in-situ and discrete methods. In-situ CH$_4$, CO$_2$, and Rn abundance analyses were carried out using a suite of instruments (Table 2). Discrete samples of cave air were collected in pre-evacuated 50-mL serum vials, in 1 to 3-L Tedlar® bags, or in 4-L glass bottles. CH$_4$ and CO$_2$ concentrations of discrete samples were measured via gas chromatography.
We assessed cave air mixing processes through a variety of techniques. A qualitative estimate on cave air residence time was obtained by comparing CH₄ to CO₂ concentrations at individual locations in each cave. Additionally, we measured the Rn concentrations of caves 32 through 36 to assess the relationship between cave air residence time, CH₄ concentrations, and CO₂ concentrations. δ¹³C_CO₂ data were used to assess the sources of CO₂ and thus of air entering the caves. We also assessed the distance from each sampling location to cave entrances as another tool to understand cave air mixing processes.

CH₄ and CO₂ concentrations from discrete air samples were measured at Indiana University using a Varian 450 gas chromatograph (GC) (Varian – Agilent Technologies, Palo Alto, California). The GC was fitted with a flame ionization detector (FID) for CH₄ and a thermal conductivity detector (TCD) for CO₂. Standard gas mixtures from Air Liquide America Specialty Gasses LLC (Plumsteadville, Pennsylvania) were used for 3-point calibration curves to convert signals measured on the GC to concentrations. CH₄ standards measured on the GC had errors of ± 5 to ± 14 % of the reported concentrations. CH₄ concentrations are reported with the uncertainty associated with the standard curve unless the calculated uncertainty was ≤ 0.1 ppmv. Samples with calculated uncertainties ≤ 0.1 ppmv were assigned uncertainties of 0.1 ppmv based on replicate measurements. The uncertainty associated with standard curves for CO₂ concentrations varied from < ± 1 to 5 %. CO₂ concentrations were assigned uncertainties based on their associated standard curve.

The stable carbon isotope ratios of CH₄ and CO₂ and hydrogen stable isotope ratios of CH₄ were measured on a ThermoFinnigan Delta Plus XP mass spectrometer in the Stable Isotope Research Facility at Indiana University. Carbon stable isotope ratios are expressed as conventional δ¹³C_CH₄ and δ¹³C_CO₂ values in ‰ along the scale anchored to Vienna Pee Dee Belemnite (VPDB).
Hydrogen stable isotope ratios are expressed as $\delta^2\text{HCH}_4$ values in ‰ along the scale anchored to Vienna Standard Mean Ocean Water (VSMOW). CH$_4$ samples were measured in continuous-flow mode using CH$_4$ preconcentration, cryofocusing (Miller et al., 2002), and a gas chromatography-oxidation/pyrolysis-isotope ratio mass spectrometer (GC-ox/pyr-IRMS) interface. Varying sample extraction times were used to isolate roughly 0.45 and 0.90 µmol of CH$_4$ prior to the introduction of the sample to the GC-ox/pyr-IRMS for analysis of $\delta^{13}\text{CCH}_4$ and $\delta^2\text{HCH}_4$ values, respectively. In-house CH$_4$ standards methane #3, methane #6, and methane ALM with $\delta^{13}\text{CCH}_4$ and $\delta^2\text{HCH}_4$ values of [+19.86 ± 0.05; +2.2 ± 1.2] ‰, [−39.40 ± 0.02; −153 ± 2] ‰, and [−58 ± 1; −272.2 ± 3.4] ‰ were used for 2-point normalizations. Errors associated with $\delta^{13}\text{CCH}_4$ and $\delta^2\text{HCH}_4$ values were calculated using a standard curve that accounted for the peak size of the measurement. Analytical repeatability of internal standards ranged from 0.14 to 0.6 ‰ for $\delta^{13}\text{CCH}_4$ and from to 7 to 18 ‰ for $\delta^2\text{HCH}_4$.

$\delta^{13}\text{C CO}_2$ values 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 ‰.

2.2 Data elaboration and quality control

In-situ measurements were preferentially used when statistically analyzing gas concentration data. When in-situ measurements were not available, concentrations measured on the GC were used in the statistical analyses. Samples and data were screened for quality control by comparing the samples with in-situ measurements and visual estimation of the volume of sample bags. If a sample bag had been shown to exhibit a leak for one analyte, data from that
sample were discarded. CH4 and CO2 concentrations measured by both GC-FID and FTIR showed
strong agreements (GC(CO2) = 0.92 ± 0.04 * FTIR(CO2) + 100 ± 300, r² = 0.99, p = 5*10⁻¹⁹;
GC(CH4) = 0.7 ± 0.2 * FTIR(CH4) + 0.2 ± 0.2, r² = 0.62), and the stable isotopic composition of
the samples was not related to their storage time (δ13CCH4 = 0.2 ± 0.3*day⁻⁴⁷ ± 2, r² = 0.03, p =
0.32; δ2HCH4 = 0.03 ± 0.14*day⁻⁹⁶ ± 11, r² = 0.005, p = 0.72; δ13CCO2 = −0.05 ± 0.16*day⁻¹⁹.9 ± 1.8, r² = 0.006, p = 0.57). In locations where more than one sample was taken with in-situ methods, the values of the samples were averaged. In total, 199 CH4 concentrations, 192 CO2
concentrations, 32 δ13CCH4 values, 26 δ2HCH4 values, and 60 δ13CCO2 values are reported in this
study (Supplemental Tables 1, 2). All samples are reported with 95 % confidence intervals.

Three different modeling techniques were used to assess trace gas sources and sinks in the
studied caves. Keeling plots of δ13CCH4, δ2HCH4, and δ13CCO2 were used to assess the possibility of
a two end member mixing systems. The stable isotopic composition of CO2 entering the caves
(δ13C₃) was assessed through equation 1

δ13C₃ = (δ13Cm − F atm * δ13C atm) (1 − F atm)⁻¹

where δ13Cm is the δ13CCO2 of the sample, δ13C atm is the δ13CCO2 of the atmosphere, and F atm is the
fraction of atmospheric CO2 in the CO2 concentration of the sample (Peyraube et al., 2016). We
used values of −10 ‰ for δ13C atm and 400 ppmv for the concentration of atmospheric CO2.

Rayleigh distillation models were used as the theoretical basis to examine changes in the
stable isotopic composition of CH4 in cave air caused by methanotrophy or -OH oxidation. The δ-
value of an isotope system in a chemical compound of interest (e.g., CH4) in cave air can be
modeled as
\[ \delta_c = (\delta_i + 1000)f^{(-\alpha + 1)} - 1000 \] (2)

where \( \delta_c \) is the instantaneous \( \delta \)-value of a particular isotope system in cave air after partial consumption, \( \delta_i \) is the initial \( \delta \)-value of the isotope system in cave air, \( f \) is the fraction of the compound remaining, and \( \alpha \) is the kinetic isotope fractionation factor (Mattey et al., 2013). \( \alpha \) values of 1.018 and 1.1353 were used to model changes in \( \delta^{13}\text{C}_{\text{CH}_4} \) and \( \delta^2\text{H}_{\text{CH}_4} \) caused by methanotrophy (Coleman et al., 1981; Feisthauer et al., 2011). \( \alpha \) values for changes in \( \delta^{13}\text{C}_{\text{CH}_4} \) caused by methanotrophy have been observed to range from 1.003 to 1.039 (Templeton et al., 2006; Feisthauer et al., 2011); 1.018 was selected based on observations of methanotrophy in soils and its similarity to the \( \alpha \) value of 1.012 observed in St. Michael’s Cave in Gibraltar (Feisthauer et al., 2011; Mattey et al., 2013). \( \alpha \) 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). The initial stable isotopic composition of atmospheric \( \text{CH}_4 \) was modeled with a \( \delta^{13}\text{C}_{\text{CH}_4} = -47.5 \) ‰ (VPDB), and \( \delta^2\text{H}_{\text{CH}_4} = -100 \) ‰ (VSMOW) based on the work of Townsend-Small et al. (2012).

We examined the possibility of additional \( \text{CH}_4 \) sources entering the cave systems through forward modeling. We assumed that two different sources of microbially produced \( \text{CH}_4 \) contribute to cave air in addition to the atmosphere. We modeled \( \text{CH}_4 \) produced from acetate fermentation as S1 \( (\delta^{13}\text{C}_{\text{CH}_4} = -49 \) ‰ VPDB, \( \delta^2\text{H}_{\text{CH}_4} = -325 \) ‰ VSMOW) and from carbonate reduction as S2 \( (\delta^{13}\text{C}_{\text{CH}_4} = -63 \) ‰ VPDB, \( \delta^2\text{H}_{\text{CH}_4} = -125 \) ‰ VSMOW) respectively (Whiticar, 1999; Etiope and Sherwood Lollar, 2013). These sources were mixed with cave air both prior to and after partial theoretical methanotrophic oxidation. Methanotrophic oxidation was modeled with the aforementioned \( \alpha \) values of 1.018 and 1.1353.
3. Results

Each of the 36 caves showed atmospheric to subatmospheric CH₄ concentrations in at least one location. Only five locations from three different caves showed elevated CH₄ concentrations relative to the atmosphere (Fig. 2, Supplemental Table 1). The CH₄ concentration in the atmosphere at study sites ranged from 1.8 ± 0.3 to 2.8 ± 0.7 ppmv. CH₄ concentrations in cave air ranged from ≤ 0.1 ± 0.1 ppmv to 5 ± 1 ppmv, and were generally observed to decrease with the distance from cave entrances (Fig. 3, Supplemental Table 1). Two thirds of the caves where three or more air measurements and distance data were recorded showed decreases in CH₄ concentration from cave entrances to interiors. For example, caves 7, 8, and 9 from Kentucky all showed progressive decreases in CH₄ concentration from about 2 ppmv at the entrance of the cave, down to zero or near zero ppmv in the more inner rooms (from 2 to 0 in caves 8 and 9 and from 1.9 to 0.3 ppmv in cave 7). Additionally, CH₄ concentrations were negatively correlated with CO₂ concentrations in cave air following an inverse power law relationship ([CH₄] = 17.5[CO₂]⁻⁰.⁴¹, r² = 0.26) (Fig. 2). In the caves where Rn concentrations were measured, the average CO₂ concentration of cave air was correlated with the average Rn concentration of cave air ([CO₂] = (1.42 ± 0.09)[Rn] + 400 ± 120, n = 4, r² = 0.99, p = 0.009).

Values of δ¹³C CO₂ in cave air ranged from −10.7 ± 0.4 to −23.81 ± 0.10 ‰. Analysis of δ¹³C CO₂ values from samples with CO₂ concentrations above 600 ppmv showed that δ¹³C ranging from −28 ‰ to −20 ‰ contributed to the composition of CO₂ in cave air. Pooled analysis of the CO₂ dataset shows that the average apparent source δ¹³C CO₂ value is −23.3 ± 0.5 ‰ (δ¹³C CO₂ = 4600 [CO₂]⁻¹ − 23.3 ± 0.5 ‰, r² = 0.83) (Fig. 4).
Values of $\delta^{13}C_{CH_4}$ and $\delta^2H_{CH_4}$ in cave air ranged from $-57.2 \pm 0.6$ to $-27.1 \pm 0.2 \%$ and $-196 \pm 10$ to $+2 \pm 18 \%$, respectively. Keeling plots of $\delta^{13}C_{CH_4}$ did not suggest that a two end member model was an adequate fit for the system ($\delta^{13}C_{CH_4}$ vs. $[CH_4]^{-1}$: $r^2 = 0.05$, $\delta^2H_{CH_4}$ vs. $[CH_4]^{-1}$: $r^2 = 0.12$). Some cave air samples plotted near the theoretical relationship between $\delta^{13}C_{CH_4}$ values and CH$_4$ concentrations caused by methanotrophy (Fig. 5). However, many points fell below and to the left of the line representing the theoretical incomplete oxidation of atmospheric CH$_4$ (Figs. 5, 6). When $\delta^2H_{CH_4}$ and $\delta^{13}C_{CH_4}$ values were plotted against each other, many samples clustered tightly near the signature of atmospheric CH$_4$ (Fig. 7). Some points, like those from caves 25 and 26, plotted near the expected trend of partial atmospheric CH$_4$ oxidation by methanotrophy. The modeled $\delta^{13}C_{CH_4}$ and $\delta^2H_{CH_4}$ values of cave air overlapped with our collected samples (Figs. 5, 6, 7, Supplemental Table 3).

4. Discussion

4.1. Subsurface-Surface Atmospheric Exchange

The concentrations and stable isotopic compositions of CH$_4$, CO$_2$, and Rn in cave air overlapped and diverged from those of the atmosphere. This suggests that atmospheric and internal cave processes influenced the composition of cave air. The majority of cave air samples were depleted in CH$_4$ and enriched in CO$_2$ relative to the local atmosphere. This points to processes like in-situ CH$_4$ oxidation and diffusion of air from the epikarst to decrease CH$_4$ and increase CO$_2$ concentrations (Fig. 2). Many study locations local atmospheric CH$_4$ concentrations were above the globally averaged atmospheric background which may have been due to their proximity to roads or pastures with local CH$_4$ sources (Gioli et al., 2012; Harper et al., 2014).
The decreases in CH$_4$ concentration in cave air were associated with increases in cave air residence time. CH$_4$ concentrations were correlated with increases in the distance from a cave entrance, CO$_2$ concentration, Rn concentration, and decreases in $\delta^{13}$C$_{CO_2}$ values (Figs. 2, 3). The relationship between the distance to a cave entrance and the residence time of air in a particular cave is multivariate and we observed departures from this trend in several caves. For example, caves 13 and 15 experienced fast airflow, cave air was flowing out of the entrance of cave 26, cave 24 has multiple entrances which likely result in multiple flow paths, in cave 20 the distance scale of the measurements may have been too small to observe a decrease in CH$_4$ concentration, and internal CH$_4$ sources, which were present in cave 9, may obscure the relationship.

The concentration of Rn in cave air is known to track cave air residence time and has been observed to correlate with CO$_2$ concentrations. We observed correlations between cave air CO$_2$ and Rn concentrations. This matches other observations from the literature (Kowalczk and Froelich, 2010; Gregorič et al., 2011; as well as others). CO$_2$ concentrations were negatively correlated with CH$_4$ concentrations. This provides strong evidence that CH$_4$ concentrations decreased with cave air residence time. Additionally, the $\delta^{13}$C$_{CO_2}$ values exhibited a continuum between atmospheric values of $-10\%$ at low concentrations and decreased as CO$_2$ concentrations increased. The projected $\delta^{13}$C$_s$ values ranged from $-28$ to $-20\%$ with an average $\delta^{13}$C$_{CO_2}$ value of $-23.3 \pm 0.5\%$ entering the caves. This matches the $\delta^{13}$C$_{CO_2}$ of soils of $-24\%$ (Amundson et al., 1998) (Fig. 4). It is possible that the observed source values that are more negative than $-24\%$ may be due to dripwater degassing or fast airflow (Spötl et al., 2005; Garcia-Anton et al., 2014). The $\delta^{13}$C$_s$ values that are more positive than $-24\%$ may be caused by differential abundances of C3 and C4 plants above the caves (Breecker et al., 2012). Our CO$_2$ data show that cave air
residence time increased as the CH$_4$ concentrations and $\delta^{13}$C$_{CO_2}$ values of cave air decreased, and that the caves in our study are not atypical compared to other caves in the literature.

In some of the caves where we measured Rn concentrations, cave air flow was relatively fast. The average CO$_2$ and Rn concentrations from these caves were relatively low (cave 33, [CO$_2$] = 540 ± 20 ppmv, [Rn] = 20 ± 100 Bq m$^{-3}$; cave 34, [CO$_2$] = 560 ± 30 ppmv, [Rn] = 200 ± 400 Bq m$^{-3}$). Despite the similarity to atmospheric CO$_2$ concentrations in these caves, average CH$_4$ concentrations were still depleted relative to the atmosphere (cave 33, [CH$_4$] = 1.07 ± 0.06 ppmv; cave 34, [CH$_4$] = 1.34 ± 0.06 ppmv). These observations show that an in-situ process is removing CH$_4$ from the cave air because subatmospheric CH$_4$ concentrations in cave air are still observed in the absence of large increases in CO$_2$ concentrations as would be expected if CH$_4$ were only diluted by the arrival of CH$_4$-free air into caves from soils. These observations agree with other observations of fast CH$_4$ oxidation in caves (Fernandez-Cortes et al., 2015; Lennon et al., 2017; Warring et al., 2017). Landscape scale CH$_4$ flux data from karst areas are needed to estimate the size of the karst sink.

### 4.2. Sources and Stable Isotopic Composition of Methane

Our CH$_4$ concentration, $\delta^{13}$C$_{CH_4}$, and $\delta^2$H$_{CH_4}$ data from caves show that caves have sources of non-atmospheric CH$_4$. Additional sources of CH$_4$ entering caves were detected by CH$_4$ concentrations above the atmospheric background in three caves, namely (i) cave 3, Indiana, (ii) cave 22, Tennessee, and (iii) cave 32, New Zealand. Caves with CH$_4$ concentrations above the atmospheric background appear to be uncommon, and understanding if there is a systematic change in karst landscapes from lower order to higher order drainages awaits future work.
The departures of $\delta^{13}\text{CCH}_4$ and $\delta^{2}\text{HCH}_4$ values from the theoretical oxidations lines of atmospheric CH$_4$ show that caves have non-atmospheric CH$_4$ sources. Our data point to the methanogenic sources of acetate fermentation and carbonate reduction (Fig. 7). Other studies have also shown microbially produced CH$_4$ entering caves (Mattey et al., 2013; Webster et al., 2016). Mixing between residual atmospheric CH$_4$ from partial methanotrophic oxidation and CH$_4$ from acetate fermentation in the soil-epikarst-cave system will generally cause a decrease in the $\delta^{13}\text{CCH}_4$ and $\delta^{2}\text{HCH}_4$ values of cave air compared to the oxidation of atmospheric CH$_4$, and this is supported by our modeling (Figs. 5, 6). For example, caves 23, 24, and 26 all appear to be influenced by acetoclastic methanogenesis.

CH$_4$ produced from carbonate reduction is inferred to enter the caves based on samples that had $\delta^{2}\text{HCH}_4$ values that were roughly equal to, or more positive than atmospheric values (Fig. 7). The stable isotopic compositions of CH$_4$ from caves 5, 15, 25, and 27 can be explained by partial methanotrophic consumption of CH$_4$ generated from carbonate reduction with the strongest source signal in cave 5 (Fig. 7). CO$_2$ reduction is typically observed in lake sediments, but has been observed in oxidizing environments such as biological soil crusts in deserts after rain events (Angel et al., 2011). We hypothesize that karst environments, which are less oxidizing, exhibit similar behavior. Our data show that carbonate reduction and acetate fermentation can occur in similar environments over large spatial scales and are not limited to aquatic and arctic environments.

Sites of methanogenesis in or near the studied cave systems may include waterlogged soils above caves, cave soils themselves, and the epikarst. It is possible that after rain events anoxic micro niches occur in soil, or the epikarst and the generated CH$_4$ is dissolved and later introduced into caves with drip waters. We confirmed that dissolved CH$_4$ outgasses in drip water of cave 32, which was situated underneath a wetland, by placing our CH$_4$ detecting probe near the water and...
measuring increased CH4 concentrations in its vicinity. We confirmed that in-situ CH4 production can take place in locally anoxic environments within caves by measuring CH4 concentrations close to a bat guano deposit in cave 25, Tennessee (site 4h; average CH4 concentration = 0.3 ± 0.5 ppmv). Time series measurements near the large bat guano deposit showed that CH4 concentrations oscillated between 0.5 ppmv and 0.1 ppmv over the course of seconds, presumably in response to episodic migration of CH4 bubbles through the moist guano (similar oscillations in ammonia, NH3, were also observed). Additionally, we observed circumstantial evidence for local in-situ CH4 production in cave 3 because measured CH4 concentrations upstream of a restroom in the cave were low, while measured CH4 concentrations downstream of the restroom were enhanced. Our data show that caves are capable of expressing elevated CH4 concentrations due to in-situ CH4 production when accumulations of organic matter, such as guano or plant material, foster methanogenesis or when dissolved CH4 from waters outgasses into cave air.

We observed minor amounts of thermogenic CH4 entering in at least one cave. Locally elevated CH4 concentrations in cave 9 (Mammoth Cave, Kentucky) were associated with a known hydrocarbon seep that is also transporting sulfide (Olson, 2013). Elevated CH4 concentrations, thought to derive from thermogenic CH4, have also been observed at sulfidic springs in Cueva de Villa Luz (Webster et al., 2017). Some 13CH4 and 2HCH4 values in cave air, i.e. two of the samples from cave 24, are compatible with thermogenic CH4 isotopic signatures (e.g., Schoell, 1988; Etioppe et al., 2009; Fig. 7). It cannot be excluded that small fluxes of CH4 from shales or hydrocarbon deposits underlying the limestone are entering caves through natural fractures, but our present isotope data cannot confirm this source for cave 24. The Antes and Utica shales, which contain hydrocarbon gases, are stratigraphically below cave 24 (Coleman et al., 2014), and geologic faults and joints, which are often aligned with caves, may serve as conduits for the flow.
of hydrocarbons (Powell, 1969). A confirmation of hydrocarbons entering from deep sources may be obtained through measurements of 'radiocarbon-dead' CO₂, Rn, and ethane.

4.3. Methane Oxidation Mechanisms

The combination of δ¹³CCH₄ and δ²HCH₄ values allow for inferences to be made about the CH₄ oxidizing reactions in karst environments. We distinguish between two scenarios with distinct sets of assumptions. Many isotopic compositions of CH₄ in this study cannot be accounted for in a first scenario in which we assume that (i) CH₄ enters the caves from the atmosphere, through acetate fermentation, and through carbonate reduction, and that (ii) CH₄ is removed from cave air through reactions involving the ·OH. Conversely, in a second scenario, if it is assumed that (i) sources of CH₄ in cave air include the atmosphere, acetate fermentation, and carbonate reduction, and that (ii) CH₄ is removed from cave air by methanotrophy, all of the points fall within the plausibility envelope of the model, suggesting that methanotrophy is the mechanism responsible for removing CH₄ from cave air (Fig. 7). Consideration of an additional source of thermogenic CH₄ (natural gas) from deep geologic sources enlarges the plausibility fields of both prior scenarios to encompass all of the data. Furthermore, the modeled isotopic composition of cave air, which has the same assumptions as the second scenario, overlays comparably with our data set.

Our δ¹³CCH₄ and δ²HCH₄ data also agree with observations of δ¹³CCH₄ and δ²HCH₄ from a cave in Indiana where it appeared that CH₄ from both acetate fermentation and carbonate reduction influenced cave air geochemistry (Webster et al., 2016). Additionally, our data resemble an arctic system characterized by acetoclastic and hydrogenotrophic CH₄ sources and methanotrophy (McCalley et al., 2014). Our isotopic evidence for in-situ microbial CH₄ oxidation in caves is
corroborated by recent results from in-situ mesocosm experiments in Vietnam where cave rocks with live microorganisms were shown to consume CH₄ even in cases where surface soils were very thin to non-existent (Lennon et al., 2017; Nguyễn-Thuỳ et al., 2017).

5. Conclusions

1) CH₄ consumption is the dominant process in karst landscapes. Subterranean karst air generally shows subatmospheric CH₄ concentrations. CH₄ and CO₂ concentrations were negatively correlated in cave air showing that as the residence time of cave air increases the CH₄ concentration of cave air decreases. CH₄ concentrations decreased faster than increases in CO₂ concentration in cave air demonstrating that an in-situ process is responsible for the removal of CH₄ from cave air.

2) The stable isotopic composition of CH₄ in studied caves suggests that CH₄ is being oxidized by microbial methanotrophy. This evidence adds to reports that methanotrophy is the mechanism by which CH₄ is rapidly removed in cave air (Mattey et al., 2013; McDonough et al., 2016; Webster et al., 2016; Lennon et al., 2017, Waring et al., 2017). The observations of sub-atmospheric CH₄ concentrations in cave air from this and other studies show that karst is behaving as a global CH₄ sink.

3) The stable isotopic composition of CH₄ in the studied caves suggests that, in addition to atmospheric CH₄, at least two additional CH₄ sources are present in some caves. We suggest that the sources include CH₄ produced from acetate fermentation and from CO₂ reduction. These data corroborate recent findings of partially oxidized CH₄ entering cave air from acetate fermentation and CO₂ reduction in Indiana (Webster et al., 2016). These
observations of CH₄ production by acetate fermentation and carbonate reduction suggest that both processes happen over a wide scale in the environment.

Acknowledgements

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Figure 1. (A) Locations of the U.S. study caves in their regional context and (B) within the contiguous USA. (C) The location of the New Zealand caves. Karst land cover data were obtained from Weary and Doctor (2014) and Ford and Williams (2007).
Figure 2. CH₄ concentrations versus CO₂ concentrations in studied caves. The majority of samples appear to follow an inverse power law relationship with CH₄ concentrations being inversely related to CO₂ concentrations (with points from caves 3 and 22 removed from the overall trend). Numbers represent individual caves. For clarity, some points are shown as black dots with a line pointing to the cave number of the sample. Some error bars were omitted from the figure for clarity. Data associated with error bars are also representative for typical errors of data where no bars are shown.
Figure 3. The spatial variation of CH$_4$ mole fraction in the air of cave 8, Kentucky sampled on May 6, 2012. (A) CH$_4$ concentration dropped sharply down to 0.4 ppmv along a narrow path after the first large room, a few tens of meter from the entrance. CH$_4$ gradually decreased over roughly 100
m down to 0 ppmv in the Cathedral room, (B) while CO₂ exhibited relatively constant concentrations in cave air.

Figure 4. A Keeling plot of $\delta^{13}C_{CO_2}$ versus inverse CO₂ concentration in cave air samples. The data show that atmospheric CO₂ is mixing with apparent isotopic endmembers between −33 and −20.4 ‰. A regression analysis of the entire data set shows that the average $\delta^{13}C_{CO_2}$ value entering
the caves is $-23.3 \pm 0.5 \%$. Numbers represent individual caves. For clarity, some points are shown as black dots with a line pointing to the cave number of the sample, nor are error bars included.

Figure 5. Relationship between CH$_4$ concentration and $\delta^{13}$C$_{CH_4}$ in cave air. Some samples plot along the expected relationship between CH$_4$ concentration and $\delta^{13}$C$_{CH_4}$ caused either by methanotrophy modeled with an $\alpha$ value of 1.018, or by oxidation with $\cdot$OH modeled with an $\alpha$. 
value of 1.0039. Other samples plot below and to the left of the theoretical shifts of the oxidation trends. Numbers represent individual caves. Note that modeled cave air, represented by letters, also plots left of theoretical methanotrophic oxidation. For clarity, some points are shown as black dots with a line pointing to the cave number of the sample. If error bars are not visible, they are smaller than the data points.
Figure 6. Relationship between methane concentration and $\delta^{2}H_{CH_{4}}$ in cave air. Some samples plot along the expected relationship between CH$_{4}$ concentration and $\delta^{2}H_{CH_{4}}$ caused by methanotrophy modeled with an $\alpha$ value of 1.1353, or by oxidation with $\cdot$OH modeled with an $\alpha$ value 1.294. Other samples plot below and to the left of the shift caused by methanotrophy. Numbers represent individual caves. Note that modeled cave air, represented by letters, generally plots left of the theoretical oxidation lines. For clarity, some points are shown as black dots with a line pointing to the number of the cave the sample is from. If error bars are not visible, they are smaller than the data points.
Figure 7. Stable isotopic composition of CH₄ in cave air samples plotted in $\delta^{2}H_{CH_{4}}$ versus $\delta^{13}C_{CH_{4}}$ space. CH₄ generated by carbonate reduction, acetate fermentation, and thermogenesis are plotted within labeled fields (Whiticar, 1999). We model inputs from the atmosphere as (~47.5 ‰, −100 ‰, □), acetate fermentation as (−49 ‰, −325 ‰, S1), and carbonate reduction as (−63 ‰, −125 ‰, S2). Thin dotted lines indicate the expected shift in $\delta^{2}H_{CH_{4}}$ and $\delta^{13}C_{CH_{4}}$ caused by partial aerobic methane oxidation adopting a slope of 8.5. Thick dashed lines indicate the expected shift in $\delta^{2}H_{CH_{4}}$ and $\delta^{13}C_{CH_{4}}$ caused by oxidation with ∙OH adopting a slope 75 (Saueressig et al., 2001; Feisthauer et al., 2011). Mixing in $\delta^{2}H_{CH_{4}}$ vs $\delta^{13}C_{CH_{4}}$ space plots as a straight line, dark-red dot-
dashed line. Numbers represent individual caves. Note the all of the data points can be described by a source of CH₄ from the atmosphere, a source from acetoclastic fermentation (S1), a source from carbonate reduction (S2), mixing, and methanotrophic oxidation and do not plot within the bounds of -OH oxidization (gray and blue fields). For clarity, some points are shown as black dots with a line pointing to the cave number of the sample. Error bars are not included with black circles for clarity. In other locations, if error bars are not visible, they are smaller than the data points.

Table 1: Overview of collected data. ‘Discrete’ measurements refer to the laboratory.
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\[ a \text{ Dates are formatted yyyy-mm-dd} \]
\[ b \text{ NA = not applicable where only 1 sample was obtained.} \]
\[ c \text{ NZ = New Zealand} \]
\[ d \text{ NA = Only CH}_4\text{ was measured} \]

**Table 2: In-situ instrumentation used in this study.**

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