

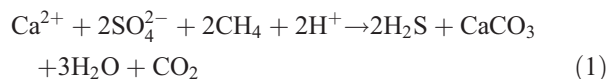
ISOTOPIC EVIDENCE FOR THE MIGRATION OF THERMOGENIC METHANE INTO A SULFIDIC CAVE, CUEVA DE VILLA LUZ, TABASCO, MEXICO

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Abstract: Methane (CH₄) is an economic resource and a greenhouse gas, but its migration through rocks is not immediately associated with speleogenesis. Sulfuric-acid speleogenesis is a cave-forming mechanism that has produced a variety of economically important oil fields and aquifers, and is theorized to be related to the oxidation of CH₄ and hydrocarbons. Despite hypotheses that the oxidation of CH₄ may provide a basis for the generation of sulfides during sulfuric-acid speleogenesis, evidence from active systems has not yet been obtained. In this study, we address how CH₄ influences the development of sulfidic cave systems by sampling the CH₄, H₂S, and CO₂ concentrations, as well as $\delta^{13}\text{C}_{\text{CH}_4}$, $\delta^2\text{H}_{\text{CH}_4}$, and $\delta^{13}\text{C}_{\text{CO}_2}$ values, in a cave currently forming by sulfuric-acid speleogenesis, Cueva de Villa Luz. CH₄, H₂S, and CO₂ concentrations were highest directly above springs in the cave, showing that all three gases enter by means of the spring water. The $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ in the air of CVL ranged from -47.92 ± 0.15 to -35.47 ± 0.12 ‰ (VPDB) and -117 to -83 ‰ (VSMOW), respectively. Keeling plots suggest that CH₄ with $\delta^{13}\text{C}_{\text{CH}_4} = -24 \pm 3$ ‰ and $\delta^2\text{H}_{\text{CH}_4} = -40 \pm 50$ ‰ was outgassing from spring water. This stable-isotope signature does not fall within traditional published $\delta^{13}\text{C}_{\text{CH}_4}$ versus $\delta^2\text{H}_{\text{CH}_4}$ fields. Our data suggest that the CH₄ entering Cueva de Villa Luz is the remnant of a larger thermogenic CH₄ flux that is incompletely oxidized in the subsurface as it travels to Cueva de Villa Luz. Our data support links between the processes forming Cueva de Villa Luz and the proposed mechanisms for other caves associated with sulfuric acid.

INTRODUCTION

The migration of subsurface methane (CH₄) through rock units toward the earth's surface creates natural gas deposits, contributes to the atmospheric CH₄ burden, and supports microbial communities, but is rarely thought to alter the encountered rock units. However, CH₄ and other hydrocarbons may enhance the porosity and permeability of limestones by fostering sulfuric-acid speleogenesis (Davis, 1980; Egemeier, 1981; Kirkland and Evans, 1976; Hill, 1990, 1995). As CH₄-rich fluids migrate within the subsurface and interact with evaporites, such as anhydrite or gypsum, they obtain sulfate (SO₄²⁻) that may react with CH₄ to form hydrogen sulfide (H₂S):



The introduction of sulfide to oxygen in near-surface environments results in the formation of sulfuric acid (H₂SO₄), a strong acid capable of rapidly dissolving limestone.

With some of the world's largest hydrocarbon deposits and aquifers hosted in sulfuric-acid dissolution-influenced limestones (Hill, 1995), a better understanding of how these caves form is important for finding new oil fields and drinking water. The suggestion that sulfuric-acid speleogenesis is associated with hydrocarbons originated from studies of Lower Kane Cave in Wyoming (Davis, 1980; Egemeier, 1981). Later, the

morphologies and mineral assemblages of caves in the Guadalupe Mountains spurred hypotheses that these caves formed through sulfuric-acid speleogenesis (Hill, 1990, 1995; Peryt et al., 2012). The negative $\delta^{34}\text{S}$ values of hydrogen sulfide from springs and sulfide minerals in the caves suggested that microorganisms were responsible for the generation of the sulfides. Additionally, $\delta^{13}\text{C}_{\text{CO}_2}$ values from carbonates in these and other settings suggested that CH₄ and hydrocarbons were oxidized in tandem with SO₄²⁻ reduction. Although the migration of CH₄ is hypothesized to be important for the formation of sulfidic caves, to our knowledge no studies of these caves have measured CH₄ concentrations in multiple locations from their entrances to their interiors, nor have measurements of both $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values been made in a cave currently undergoing sulfuric-acid speleogenesis.

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Stable-isotope ratios of carbon and hydrogen in CH₄ provide a tool for understanding the origins and reactions of CH₄ in the environment. For example, atmospheric CH₄ has $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values that are roughly -47.5‰ (VPDB) and -100‰ (VSMOW), respectively (Thom et al., 1993). Thermogenic CH₄, which forms from the breakdown of organic matter by heat, has $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values that generally range from -56 to -20‰ and from -300 to -70‰ , respectively (Schoell, 1988; Whiticar, 1999). The original $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values are altered during both biotic and abiotic CH₄ oxidation; the former causes an increase of 8.5‰ in $\delta^2\text{H}_{\text{CH}_4}$ for each ‰ increase in $\delta^{13}\text{C}_{\text{CH}_4}$ (Feisthauer et al., 2011). Thermochemical sulfate reduction, a form of abiotic methane oxidation, shows $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values of roughly -30 and -120‰ respectively (Liu et al., 2014). Furthermore, the mixing of CH₄ from different sources can create CH₄ with unusual isotopic ratios. For example, the Homorod mud volcano of Romania exhibits variations in $\delta^2\text{H}_{\text{CH}_4}$ values from -25 to $+30\text{‰}$ that result from mixing between microbially produced CH₄ and CH₄ with a $\delta^2\text{H}_{\text{CH}_4}$ of $+124\text{‰}$ generated from incomplete abiotic oxidation (Etiopie et al., 2011).

CH₄ has been measured from only two caves undergoing sulfuric-acid speleogenesis. The caves showed different characteristics. In Movile Cave in Romania, the CH₄ in an isolated pocket of air between the cave's roof and a water body had a concentration of 10,000 ppmv and a $\delta^{13}\text{C}_{\text{CH}_4}$ value of -60‰ , indicating the presence of microbially generated CH₄ (Sarbu et al., 1996; Hutchens et al., 2004). CH₄ concentrations in Frasassi Cave in Italy ranged from 1.9 to 2.2 ppmv at a single collection site (Jones et al., 2012), although isotopic compositions were not reported. The current CH₄ data set from sulfidic caves must be augmented in order to rigorously test current theories about hydrocarbon-driven sulfuric-acid speleogenesis.

We tested the hypothesis that hydrocarbons are fundamental in sulfuric-acid speleogenesis in the active Cueva de Villa Luz (CVL) system by measuring CH₄, H₂S, and CO₂ concentrations and $\delta^{13}\text{C}_{\text{CH}_4}$, $\delta^2\text{H}_{\text{CH}_4}$, and $\delta^{13}\text{C}_{\text{CO}_2}$ values. If CH₄ oxidation is responsible for the generation of H₂S in sulfidic caves, then abundances of CH₄, H₂S, and CO₂ should be correlated in cave air as the gases follow the same pathway into the cave unless there are additional destruction or production mechanisms along the way. Additionally, the $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values should show signs of oxidation, and $\delta^{13}\text{C}_{\text{CO}_2}$ values should be related to oxidized CH₄. This study presents the first observations of CH₄ concentrations along gradients from cave entrance to interior and of the stable-isotope ratios of hydrogen in CH₄ in the air of an actively forming sulfidic cave.

GEOLOGICAL SETTING

REGIONAL GEOLOGICAL SETTING

CVL is a shallow cave, having several natural openings, and is located in the Sierra de Chiapas 2 km south of Tapijulapa,

Tabasco, Mexico. It is hosted within a carbonate shelf of the middle Cretaceous Sierra Madre Formation (Rosales Lagarde et al., 2006; Fig. 1). The sedimentary record of the region spans from the Jurassic to the Tertiary and includes hydrocarbon formation (Aguilar Rodríguez, 2007; Clara Valdéz et al., 2009). Regional hydrocarbons, such as oils located about 50 km north of CVL, are presumably derived from Cretaceous marine limestones (Guzman-Vega and Mello, 1999).

Several potential sources of hydrogen sulfide (H₂S) are present in the CVL region. Possible sources of H₂S for CVL include the volcano El Chichón located 50 km west of CVL; petroleum basins located 50 km north of CVL such as the Macuspana and the Comalcalco basins; petroleum deposits within the Sierra de Chiapas; and economic sulfide deposits 25 km west of CVL (Pantoja-Alor, 1989; Hose et al., 2000; Rosales Lagarde et al., 2006, 2014; Fig. 1). However, sulfur-isotope ratios of dissolved sulfide in the H₂S-rich brackish springs of CVL range from -24 to -6‰ Vienna Canyon Diablo Troilite (VCDT) and indicate that biological sulfate reduction of anhydrite-derived sulfate is the main mechanism producing the hydrogen sulfide in the groundwater column feeding the cave (Rosales Lagarde, 2013).

The regional tectonic history includes periods of extension and compression resulting in large scale faulting, fracturing, and folding (Meneses-Rocha, 2001; Alcántara García et al., 2004; García Palomo et al., 2006). The main passage of CVL follows the strike of east-northeast bedding planes of Cretaceous limestone. This limestone is folded into a northwest plunging anticline and cut by a thrust fault (Rosales Lagarde, 2013; Fig. 1). Faulting and fracturing seem to facilitate the upward migration of deeper fluids. Additionally, hydrocarbon seeps in the region are common (Aguilar Rodríguez, 2007; PEMEX, personal communication).

GEOMORPHOLOGY AND GEOCHEMISTRY OF CUEVA DE VILLA LUZ

The cave's main entrance and twenty-four skylights connect the air of CVL with the atmosphere (Fig. 2) (Hose and Pisarowicz, 1999). CVL is a weakly hydrothermal system and has a slightly elevated mean annual cave-air temperature of 28°C compared to the regional mean annual atmospheric temperature of 27°C . Sulfide-rich springs release H₂S into the cave air, where it ranges in concentration from 1 to 40 ppmv depending on location (Hose and Pisarowicz, 1999). CVL is known to experience strong H₂S and carbon monoxide outgassing events when the H₂S concentration may increase from < 12 ppmv to > 240 ppmv in less than 3 minutes (Kleina, and Hose, pers. comm.; Boston, unpub. results). The cause of these outgassing events is unknown.

CVL's twenty-six known internal springs broadly fall into two categories: (i) springs with $< 4.3\text{ mg L}^{-1}$ dissolved O₂ and $< 0.1\text{ mg L}^{-1}$ dissolved H₂S and (ii) springs with $< 0.1\text{ mg L}^{-1}$ dissolved O₂ and 300 to 500 mg L^{-1} dissolved H₂S (Hose et al., 2000). Non-sulfidic springs have a higher temperature than

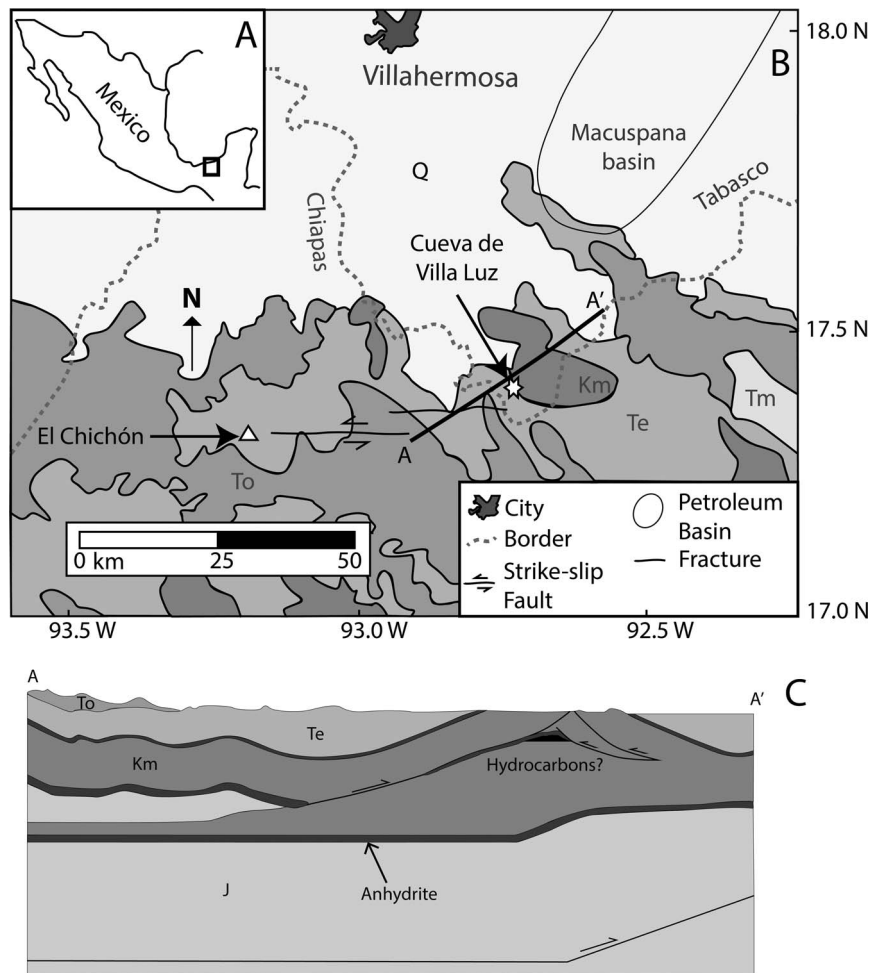


Figure 1. The regional setting of the study area showing A) the location of the study area in Mexico and B) geological features and units that may be influencing the CH_4 concentrations in Cueva de Villa Luz. Q: Quaternary sediments; Te: Eocene units; To: Oligocene units; Tm: Miocene units; Km: Cretaceous units; J: Jurassic units. C) A cross-section from A to A' showing lithological units in the region. This map has been redrawn from Alcántara García et al. (2004) and the U.S. Geological Survey World Energy Assessment Team (2000).

those with sulfide (Rosales Lagarde, 2013). All springs are brackish, and the water chemistry is influenced by dissolution of limestone, dolomite, halite, and gypsum (Rosales Lagarde et al., 2014; Hose et al., 2000, Spilde et al., 2004).

METHODS

AIR SAMPLING

The geochemistry of cave air is affected by atmospheric conditions, which may dominate near entrances or skylights, and internal cave processes, which may dominate the chemistry in cave interiors (Peyraube et al., 2016). We sampled air in CVL using *in-situ* and discrete techniques. We used *in-situ* sampling to measure H_2S concentrations and obtained discrete air samples in 1 L Tedlar bags for later analysis of CH_4 and CO_2 . We selected sampling locations based on their proximity to springs and entrances and measured

the distances from each sampling location to the nearest entrance or spring based on the map of Hose and Pisarowicz (1999). Samples were defined as “near a spring” if they were obtained < 2 m from a spring and samples were defined as “near an entrance” if they were < 10 m from an entrance.

We measured H_2S concentrations with a GasBadge Pro H_2S instrument (Industrial Scientific, Pasadena, Texas) and a Ventis MX4 device (Industrial Scientific, Pasadena, Texas) from nine locations in CVL (Fig. 2) (Table 1). We measured H_2S concentrations at several elevations above the stream to assess the extent to which exsolution of H_2S from spring waters altered the concentration of H_2S in cave air. When data were measured with both the GasBadge and the Ventis (Table 1), we took the average of the H_2S concentrations (average difference, Ventis – Gasbadge = -2 ppmv). At locations 1 through 12 we obtained discrete air samples in the cave, and we also obtained a discrete air sample from the local atmosphere 20 m from the main entrance of CVL using

Table 1. H₂S concentrations in Cueva de Villa Luz. Dates are listed as mm/dd/yyyy.

Site	Name	Date Collected	Closest Entrance (m)		Closest Spring (m)		[H ₂ S] 20 cm		[H ₂ S] 50 cm		[H ₂ S] 100 cm		[H ₂ S] 150 cm	
			(m)	(m)	(m)	(m)	(ppmv)	e (2SD) ^a	(ppmv)	e (2SD) ^a	(ppmv)	e (2SD) ^a	(ppmv)	e (2SD) ^a
Site 4	Midway Springs	12/19/2013	9	0	7	4	3	5	5.1	0.9	4.85	0.15		
Site 5	RBCSG ^b	12/16/2013	23	27	11.6	1.0	6.2 ^c	1.0	4.3 ^d	1.0e ^e		
Site 7	Slot Spring 2	12/16/2013	23	0	23.05	0.05	13.8	1.2	7.6	1.8	6.9	0.9		
Site 9	Behind Spring	12/16/2013	15	0	11.8	0.6	8.7	0.4	9.5	0.5	5.1	0.6		
Site 13	Opp ^e	12/16/2013	14	3	5.9	0.6	8 ^c	5 ^c	8	2	6.6	1.5		
Site 14	Sala Grande 1	12/16/2013	20	6	1.5	1.1	2.5	1.3	1.5	1.1	2.9	0.9		
Site 15	Sala Grande 2	12/16/2013	20	13	3.6	0.7	4.2	0.4	3	0.8	2.9	1.0		
Site 16	Sala Grande 3	12/16/2013	25	16	2.4	1.0	1.9	0.8	1.2	1.2	1.1	1.1		
Site 17	BYR ^f	12/16/2013	24	25	2.7	1.0	0.9	0.8	0.9	0.8		

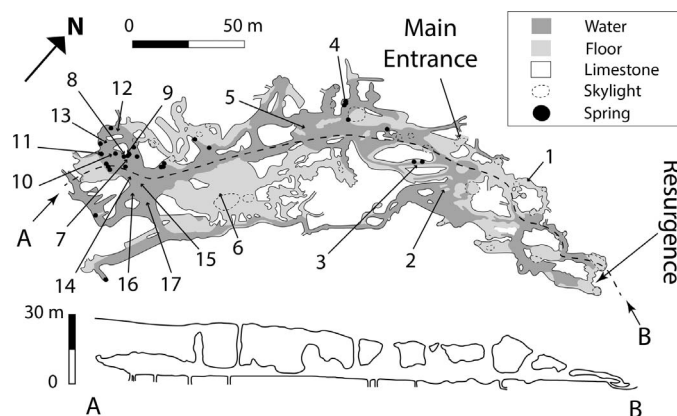
^a SD = Standard Deviation.^b Room Before the Crawl to Sala Grande.^c Sample was obtained at 40 cm above the spring.^d Sample was obtained at 60 cm above the spring.^e Outside of Perched Pool.

Figure 2. A simplified map of Cueva de Villa Luz in Mexico redrawn from Hose and Pisarowicz (1999). A cross-section of the cave's main passage is shown from A to B. Numerous skylights and springs are present in the cave. Arrows indicate air sample locations. Discrete air samples were obtained from sites 1 to 12.

standard air-sampling techniques (Fig. 2) (Webster et al., 2016). All discrete cave-air samples from locations near springs were obtained 30 cm above the springs.

LABORATORY ANALYSES

We measured CH₄ and CO₂ concentrations from discrete samples with a Varian 450 gas chromatograph (GC) (see Webster et al., 2016). CH₄ and CO₂ calibration curves for concentration and uncertainty estimates were calculated following the methods of Webster et al. (2016).

We measured the $\delta^{13}\text{C}_{\text{CH}_4}$, $\delta^2\text{H}_{\text{CH}_4}$ and $\delta^{13}\text{C}_{\text{CO}_2}$ values to help determine the sources of CH₄ and CO₂ entering CVL. $\delta^{13}\text{C}_{\text{CH}_4}$, $\delta^2\text{H}_{\text{CH}_4}$ and $\delta^{13}\text{C}_{\text{CO}_2}$ values were measured on a Thermo Finnigan Delta Plus XP isotope-ratio mass-spectrometer (IRMS). $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values were measured in continuous-flow mode using a gas chromatography-oxidation/pyrolysis-IRMS (GC-ox/pyr-IRMS) interface following previously published methods (Miller et al., 2002; Webster et al., 2016). $\delta^{13}\text{C}_{\text{CO}_2}$ was measured with a Thermo Finnigan GasBench II inlet connected to the IRMS.

Raw $^{13}\text{C}/^{12}\text{C}$ and $^2\text{H}/\text{H}$ isotope ratios measured by the mass spectrometer were converted to conventional δ -values using two-point calibrations. The in-house CH₄ reference materials were calibrated against international measurement standards NBS 19, LSVEC, VSMOW, and SLAP. Reference materials had $\delta^{13}\text{C}_{\text{CH}_4}$ values of -38.25 ± 0.06 and -58.64 ± 0.15 ‰ and $\delta^2\text{H}_{\text{CH}_4}$ values of -41 ± 3 and -160 ± 4 ‰, respectively. Analytical uncertainties of the $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values of the CH₄ reference materials ranged from ± 0.07 to ± 0.15 ‰ (2 SD), and ± 8 to ± 12 ‰ (2 SD), respectively, depending on the day of measurement, and these were assigned to data from air samples that were measured on the same day.

Raw $^{13}\text{C}/^{12}\text{C}$ ratios of CO₂ were converted to $\delta^{13}\text{C}_{\text{CO}_2}$ values on the VPDB scale using a single standard with

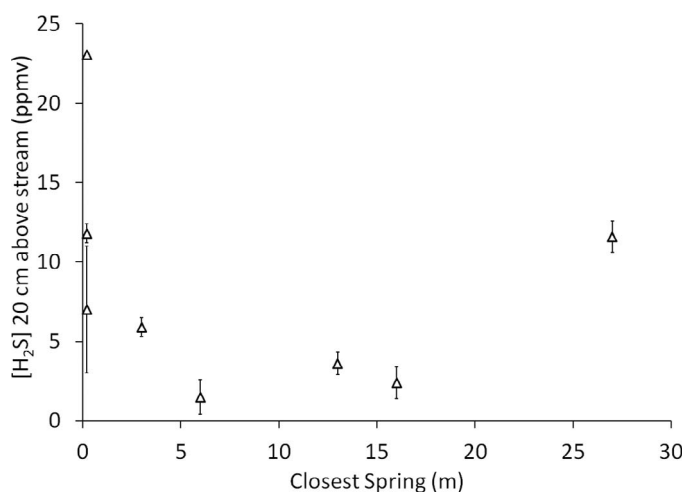


Figure 3. H₂S concentration in air 20 cm above the cave stream versus distance to the nearest spring in Cueva de Villa Luz. The elevated H₂S concentration at a distance of 28 m from a spring may be related to turbulent water entering from an adjacent room.

$\delta^{13}\text{C}_{\text{CO}_2} = -12.0 \pm 0.2$ ‰. Samples and standards were measured ten times. Sample uncertainties were assessed using the methods of Webster et al. (2016).

QUANTITATIVE ANALYSES

Relationships between CH₄, CO₂, and H₂S concentrations and their distances to a spring were assessed through two-sample *t*-tests assuming unequal variance. Additionally, correlations between CH₄ and CO₂ concentrations in the air of CVL were examined through regression analysis. The isotopic composition of the source of CH₄ from cave springs was assessed using Keeling plots, as described in Thom et al. (1993). This approach assumes that gas samples represent varying mixtures of two gas end-members with contrasting CH₄ concentrations and isotopic compositions. The $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values of CH₄ entering from springs were determined by the *y*-intercepts of linear regression analyses of $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values of air samples versus $[\text{CH}_4]^{-1}$ of the same samples (where $[\text{CH}_4]$ is the concentration of CH₄ in ppmv). Keeling plots were also used to determine the potential $\delta^{13}\text{C}_{\text{CO}_2}$ end member value of CO₂ in CVL air following the methods of Rey et al. (2012).

RESULTS

H₂S concentrations in the air of CVL ranged from 0.9 ± 0.8 to 23.05 ± 0.05 ppmv (Table 1) and were highest closest to springs (two-sample *t*-test unequal variance, t_{14} stat = 3.02, $p = 0.005$; Fig. 3). CH₄ concentrations ranged from 1.88 ± 0.10 ppmv to 3.67 ± 0.18 ppmv ($n = 12$), and CO₂ concentrations ranged from 400 ± 20 ppmv to 920 ± 50 ppmv ($n = 12$; Fig. 4; Table 2). The concentrations of CH₄ and CO₂ in the local

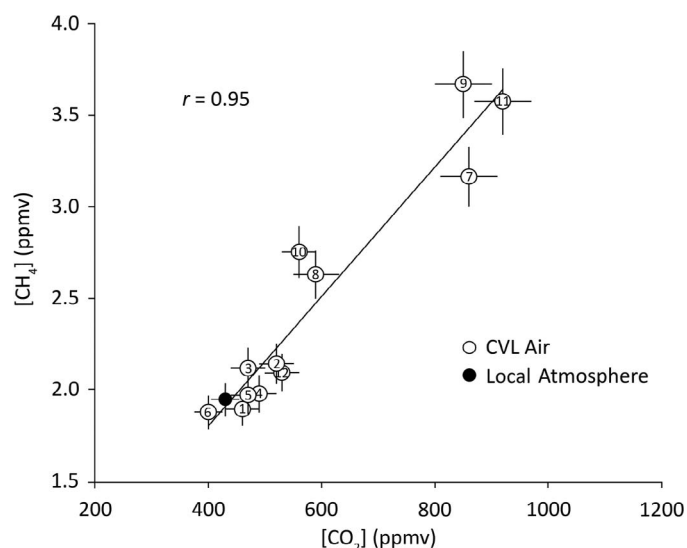


Figure 4. CH₄ versus CO₂ concentrations in Cueva de Villa Luz (CVL) showing a strong positive correlation ($r = 0.95$, $n = 12$, $p < 0.01$). Open circles represent CVL air samples, whereas the black circle represents the open atmosphere outside of the cave.

atmosphere were 1.96 ± 0.10 ppmv and 430 ± 20 ppmv, respectively. CH₄ and CO₂ concentrations were positively correlated in the atmosphere of CVL ($r = 0.95$, $n = 12$, $p < 0.01$; Fig. 4). CH₄ and CO₂ concentrations were higher at locations close to springs than at other locations (CH₄: two-sample *t*-test unequal variance, t_5 stat = 3.57, $p = 0.008$; CO₂: two-sample *t*-test unequal variance, t_6 stat = 3.05, $p = 0.01$; Fig. 5A). CH₄ and CO₂ concentrations were not related to the distance from an entrance (CH₄: two-sample *t*-test unequal variance, t_5 stat = -1.16, $p = 0.15$; CO₂: two-sample *t*-test unequal variance, t_3 stat = -0.75, $p = 0.25$; Fig. 5B).

$\delta^{13}\text{C}_{\text{CO}_2}$ values ranged from -12.0 ± 0.2 to -8.7 ± 0.4 ‰ (Table 3). Air samples near cave entrances showed the lowest concentrations of CO₂ and had $\delta^{13}\text{C}_{\text{CO}_2}$ values around -9.6 to -9.4 ‰. The $\delta^{13}\text{C}$ of atmospheric CO₂, -10.8 ± 0.6 ‰, did not match the $\delta^{13}\text{C}_{\text{CO}_2}$ values of other samples with near atmospheric CO₂ concentrations, nor samples of atmospheric CO₂ from other studies (Peyraube et al., 2013; Fernandez-Cortes et al., 2015), and was excluded from further analyses. As CO₂ concentrations increased, their $\delta^{13}\text{C}_{\text{CO}_2}$ values diverged from the $\delta^{13}\text{C}_{\text{CO}_2}$ of low-CO₂ samples (Table 3). The slope of $\delta^{13}\text{C}_{\text{CO}_2}$ data in a Keeling plot was not different from zero ($r^2 = 0.23$, $n = 12$, $p = 0.11$).

The relationships between CH₄ concentration and $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values in CVL air fit a two end-member mixing model in Keeling plots (Fig. 6). $\delta^{13}\text{C}_{\text{CH}_4}$ ranged from -47.92 ± 0.15 to -35.47 ± 0.12 ‰ and was linearly related to the inverse CH₄ concentration ($\delta^{13}\text{C}_{\text{CH}_4} = [-45 \pm 7] \times [\text{CH}_4]^{-1} + [-24 \pm 3]$, $r^2 = 0.98$, $n = 9$, $p < 0.01$; Fig. 6A). $\delta^2\text{H}_{\text{CH}_4}$ ranged from -117 ± 9 to -65 ± 12 ‰ and was linearly related to the inverse CH₄ concentration ($\delta^2\text{H}_{\text{CH}_4} = [-140 \pm 80] \times$

Table 2. Sample collection dates and measurement dates for the concentrations of CH₄ and CO₂. Dates are listed as mm/dd/yyyy.

Site	Collection Date	CH ₄ concentration			CO ₂ Concentration		
		Analysis Date	Conc. (ppmv)	e (2SD) ^a	Analysis Date	Conc. (ppmv)	e (2SD) ^a
Site 1	12/17/2013	12/31/2013	1.89	0.09	12/31/2013	460	30
Site 2	12/17/2013	12/31/2013	2.14	0.11	12/31/2013	520	30
Site 3	12/17/2013	12/31/2013	2.12	0.11	12/31/2013	470	30
Site 3	12/17/2013	12/31/2013	2.03	0.10	12/31/2013	450	30
Site 4	12/17/2013	12/31/2013	1.98	0.10	12/31/2013	490	30
Site 5	12/16/2013	12/31/2013	1.97	0.10	12/31/2013	470	30
Site 6	12/16/2013	12/31/2013	1.88	0.09	12/31/2013	400	20
Site 7	12/16/2013	12/31/2013	3.16	0.16	12/31/2013	860	50
Site 7	12/16/2013	12/31/2013	3.03	0.15	12/31/2013	860	50
Site 8	12/16/2013	12/31/2013	2.63	0.13	12/31/2013	590	40
Site 8	12/16/2013	12/31/2013	2.33	0.12	12/31/2013	570	30
Site 9	12/16/2013	12/31/2013	3.67	0.18	12/31/2013	850	50
Site 10	12/16/2013	12/31/2013	2.74	0.14	12/31/2013	560	30
Site 11	12/17/2013	12/31/2013	3.58	0.18	12/31/2013	920	50
Site 12	12/17/2013	12/31/2013	2.09	0.10	12/31/2013	530	30
Outside Cave	12/17/2013	12/31/2013	1.96	0.10	12/31/2013	430	30

^a SD = Standard deviation.[CH₄]⁻¹ + [-40 ± 50], $r^2 = 0.62$, $n = 8$, $p = 0.02$; Fig. 6B).

Air samples from locations nearest to entrances define one end-member of CH₄ entering the cave with a $\delta^{13}\text{C}_{\text{CH}_4}$ of -47.6 ± 0.8 ‰ and a $\delta^2\text{H}_{\text{CH}_4}$ of -112 ± 14 ‰. Samples obtained near springs suggest a second source of CH₄ entering CVL with a $\delta^{13}\text{C}_{\text{CH}_4}$ of -24 ± 3 ‰ and a $\delta^2\text{H}_{\text{CH}_4}$ of -40 ± 50 ‰ (Figs. 6, 7).

DISCUSSION

HYDROGEN SULFIDE, METHANE, AND CARBON DIOXIDE ENTER CVL FROM SPRINGS

The observed concentrations in CVL of H₂S, CH₄, and CO₂, and the stable-isotope compositions of CH₄ and CO₂ result from a combination of airflow through the cave,

Table 3. Sample stable isotopic compositions of CH₄ and CO₂. Dates are listed as mm/dd/yyyy.

Site	Collection Date	$\delta^{13}\text{C}_{\text{CH}_4}$			$\delta^2\text{H}_{\text{CH}_4}$			$\delta^{13}\text{C}_{\text{CO}_2}$		
		Analysis Dates	‰ (VPDB)	e (2SD) ^a	Analysis Dates	‰ (VSMOW)	e (2SD) ^a	Analysis Date	‰ (VPDB)	e (2SD) ^a
Site 1	12/17/2013	4/9/2014	-47.26	0.12	LSV ^b	1/9/2014	-9.4	0.2
Site 2	12/17/2013	4/3/2014	-45.71	0.15	3/18/2014	-96	10	1/9/2014	-10	0.2
Site 3	12/17/2013	4/3/2014	-46.51	0.15	3/19/2014	-97	12	1/9/2014	-9.6	0.3
Site 4	12/17/2013	SL ^c	LSV ^b	1/9/2014	-9.6	0.3
Site 5	12/16/2013	4/4/2014	-45.73	0.07	3/18/2014	-116	10	1/9/2014	-9.6	0.7
Site 6	12/16/2013	4/3/2014	-47.92	0.15	3/21/2014	-117	9	1/9/2014	-9.5	0.3
Site 7	12/16/2013	4/9/2014	-38.28	0.12	3/18/2014	-104	10	1/9/2014	-12	0.2
Site 8	12/16/2013	4/3/2014	-42.45	0.15	3/19/2014	-87	12	1/9/2014	-10.6	0.2
Site 9	12/16/2013	4/9/2014	-35.47	0.12	3/19/2014	-65	12	1/9/2014	-10.4	0.2
Site 9	12/16/2013	10/9/2014	-39	3
Site 10	12/16/2013	4/3/2014	-40.63	0.15	3/21/2014	-83	8	1/9/2014	-9.7	0.3
Site 11	12/17/2013	SL ^c	LSV ^b	1/9/2014	-9.1	0.3
Site 12	12/17/2013	SL ^c	LSV ^b	1/9/2014	-8.7	0.4
Outside Cave	12/17/2013	4/3/2014	-47.34	0.15	3/18/2014	-107	10	1/9/2014	-10.8 ^d	0.6 ^d

^a SD = Standard deviation.^b Sample was not measured due to low sample volume (LSV = low sample volume).^c The sample had leaked at the time of measurement (SL = sample leakage).^d This measurement did not agree with other measurements of low CO₂ concentration air.

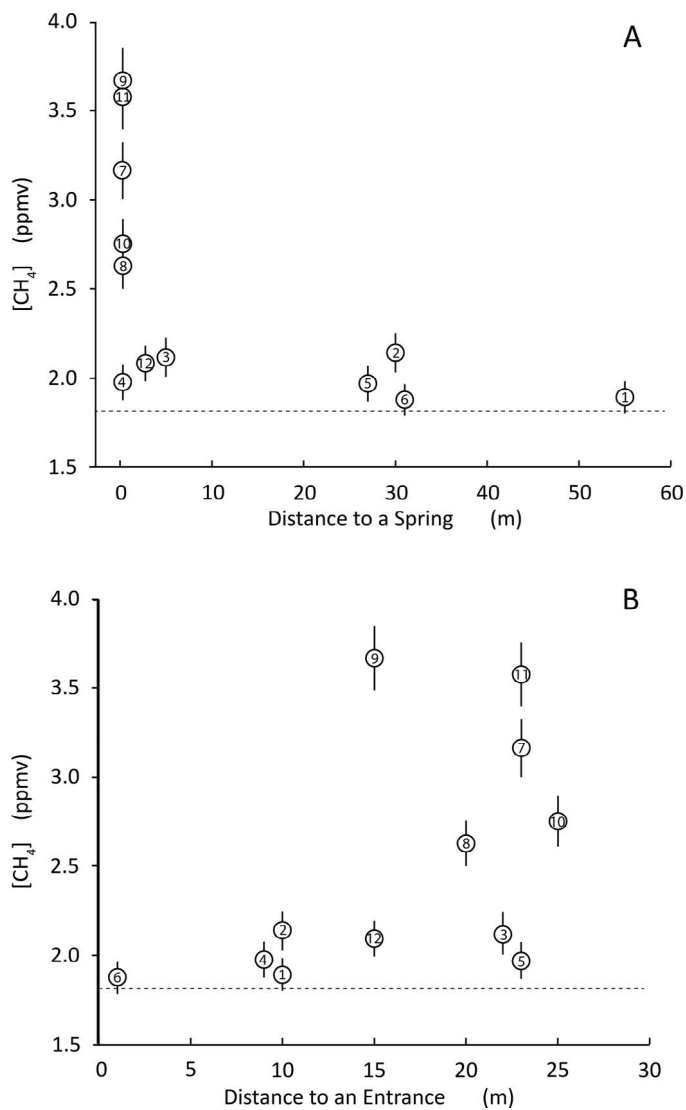


Figure 5. A) CH₄ concentrations in cave air at different distances from springs. B) CH₄ concentrations in cave air at different distances from an entrance. Dashed lines indicate the global atmospheric average CH₄ concentration (Adushkin and Kudryavtsev, 2013).

biogeochemical processes operating within the cave, and biogeochemical processes in the waters that feed the cave springs. The temperature of the open atmosphere at the time of sampling was lower than the temperature inside CVL, and cool air was felt entering from the skylights. This type of airflow should result in cave air composition near skylights that resembles the open atmosphere, as was observed in CVL at the time of sampling. Additionally, high rates of atmospheric exchange are indicated by roughly atmospheric CH₄ and CO₂ concentrations at sample sites that were neither close to an entrance nor immediately adjacent to springs.

Elevated CH₄ and CO₂ concentrations at springs imply that CH₄ and CO₂ are exsolving from spring water (Fig. 4). The only site located close to a spring that did not show elevated

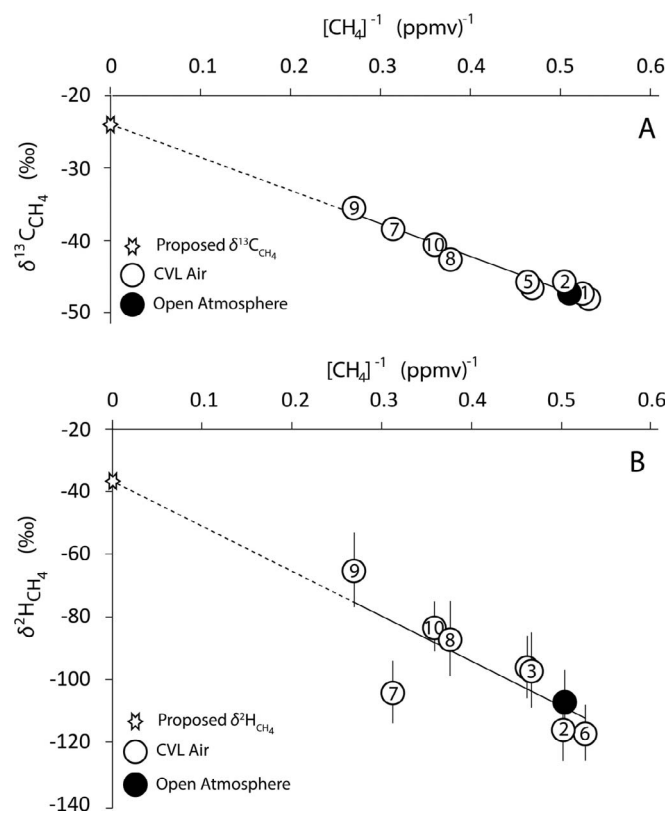


Figure 6. Keeling plot of δ¹³C_{CH₄} from cave air (A, $\delta^{13}\text{C}_{\text{CH}_4} = [-45 \pm 7] \times [\text{CH}_4]^{-1} + [-24 \pm 3]$, $r^2 = 0.98$, $n = 9$, $p < 0.01$) and δ²H_{CH₄} (B, $\delta^2\text{H}_{\text{CH}_4} = [-140 \pm 80] \times [\text{CH}_4]^{-1} + [-40 \pm 50]$, $r^2 = 0.62$, $n = 8$, $p = 0.02$). The error bars are smaller than the data points in A. The star marks the y-intercept that indicates the δ¹³C_{CH₄} and δ²H_{CH₄} of the dissolved CH₄ in the spring water. The open circles represent Cueva de Villa Luz air samples, and black circles represent the outside atmosphere as measured during the study.

CH₄ and CO₂ concentrations was Midway Spring (Site 4), which is located 9 m from a skylight (Fig. 4). Elevated CH₄ concentrations in cave air near springs have not been reported from other caves. The CH₄ concentrations in CVL that were not located by springs (1.9 to 2.2 ppmv) are more similar to those measured in the sulfidic Frasassi Cave system (1.9 to 2.2 ppmv) in Italy (Jones et al., 2012) than they are to the sulfidic Movile Cave (10,000 ppmv) in Romania (Hutchens et al., 2004). This difference may be due to the lack of atmospheric connectivity at the site in Movile Cave, which is mostly water-filled. We did not observe CH₄ concentrations below 1.5 ppmv, as have been observed in many epigenic, non-sulfidic caves in Gibraltar, Australia, the United States, and Spain (Mattey et al., 2013; Fernandez-Cortes et al., 2015; McDonough et al., 2016; Webster et al., 2016; Lennon et al., 2016). Our results demonstrate that if *in-situ* CH₄ oxidation processes were operating in CVL, they were not strong enough to react all of the CH₄ in the collected samples. We can therefore

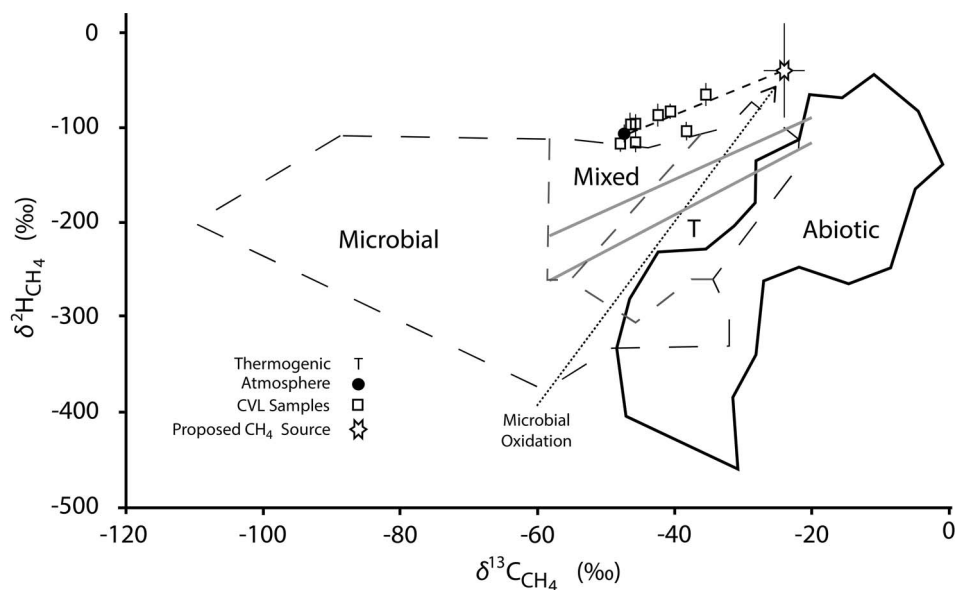


Figure 7. Isotopic characteristics of methane in air samples from Cueva de Villa Luz (CVL) plotted on published occurrence envelopes for microbial, thermogenic (T), and abiotic methanes (Etiope and Sherwood Lollar, 2013). Solid grey lines represent observed isotopic boundaries of methane from terrestrial and marine source rocks (Schoell, 1980). Data from CVL air samples (squares) plot along a bidirectional mixing line between an atmospheric source of CH_4 and a methane entering CVL with a stable isotopic signature of $\delta^{13}\text{C}_{\text{CH}_4} = -24 \pm 3 \text{‰}$, $\delta^2\text{H}_{\text{CH}_4} = -40 \pm 50 \text{‰}$.

conclude that two most likely processes affecting CH_4 in CVL are atmospheric circulation and spring-water outgassing.

STABLE ISOTOPIC COMPOSITION AND SOURCES OF METHANE

The CH_4 concentrations and values of $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ observed in CVL are consistent with mixing between an atmospheric CH_4 source and a CH_4 source entering from the springs. The lowest concentrations of CH_4 in the air of CVL were measured at locations that were both close to entrances and far from springs. The $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values from these locations plot near the respective values of atmospheric CH_4 (Figs. 6, 7). The concentrations and isotopic composition of CH_4 from the local atmosphere and cave locations near skylights agree with other measurements of atmospheric CH_4 (Mikaloff Fletcher et al., 2004). The CH_4 concentrations in CVL suggest that a secondary source of CH_4 with a $\delta^{13}\text{C}_{\text{CH}_4}$ of $-24 \pm 3 \text{‰}$ and a $\delta^2\text{H}_{\text{CH}_4}$ of $-40 \pm 50 \text{‰}$ enters CVL dissolved in spring water. CH_4 with this stable isotopic composition does not closely match known isotopic compositions generated by primary biotic or abiotic processes, and is driven largely by relatively positive $\delta^2\text{H}_{\text{CH}_4}$ values (Fig. 7).

The origin of the CH_4 entering CVL at springs can be constrained in three ways, by comparing the CH_4 isotopically to other CH_4 sources in the region, by taking into account the regional thermal history, and by compensating for shifts in CH_4 isotopic composition caused by oxidation. A possible explanation for $\delta^{13}\text{C}_{\text{CH}_4}$ values in cave air is that the CH_4 entering CVL from the springs has an original isotopic

composition with a $\delta^{13}\text{C}_{\text{CH}_4}$ value of $-24 \pm 3 \text{‰}$ and a $\delta^2\text{H}_{\text{CH}_4}$ value of $-40 \pm 50 \text{‰}$, based on the Keeling analysis. However, this $\delta^{13}\text{C}_{\text{CH}_4}$ of CH_4 from CVL spring water is incompatible with CH_4 from neighboring sedimentary basins, which have $\delta^{13}\text{C}_{\text{CH}_4}$ values of -32 to -70‰ in the Macuspana Basin; -38 to -45‰ in the Papaloapan Basin; -39 to -55‰ in the Cuichapa Basin; and -40 to -52‰ in the Reforma Basin (Clara Valdés et al., 2009). The most similar $\delta^{13}\text{C}_{\text{CH}_4}$ value from a regional natural gas well is about -32‰ from the Macuspana Basin (Talukdar et al., 2002; Clara Valdés et al., 2009). Additionally, the proposed $\delta^2\text{H}_{\text{CH}_4}$ value of CH_4 entering CVL from springs is very positive compared to values from the literature (Schoell, 1980; Etiope et al., 2011; Etiope and Sherwood Lollar et al., 2013). The evidence implies that a parental CH_4 with the proposed $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^2\text{H}_{\text{CH}_4}$ values is unlikely, and supports the idea that CH_4 entering CVL in springs is the remnant of a larger CH_4 flux at depth that has been mostly oxidized to CO_2 on the way to the cave.

The thermal history of the CVL region provides a way to help determine whether abiotic or biotic oxidation is most likely to have altered the CH_4 entering CVL, because the temperature ranges at which they occur do not overlap. The minimum temperature for the onset of abiotic CH_4 oxidation has been estimated to range from 100 to 140 °C, whereas microbial CH_4 oxidation (methanotrophy) generally takes place from 0 to 80 °C (Machel, 1998, 2001; Worden et al., 1995; Holler et al., 2011). Vitrinite reflectance (R_o) data from source rocks in the region range from 0.5 to 0.8 % (Guzman-Vega and Mello, 1999) and correspond to maximum

subsurface temperatures of 60 to 90 °C (Bjørlykke, 1989). Additionally, modern water temperatures from oil wells in the region (30 to about 60 °C) and dissolved silica geothermometers from ground water in the region do not suggest that volcanic heat flow is affecting groundwater temperature (Nencetti et al., 2005). These observations suggest that methanotrophy is responsible for consuming CH₄ in the subsurface before entering CVL.

Correcting for the expected isotopic shifts associated with partial methanotrophic loss of CH₄ can hindcast the original isotopic composition and reveal the origin of CH₄ entering CVL. Microbial oxidation imparts an increase of about 8.5 ‰ in the $\delta^2\text{H}_{\text{CH}_4}$ value for every increase of 1 ‰ in the $\delta^{13}\text{C}_{\text{CH}_4}$ value (Feisthauer et al., 2011). If methanotrophy is assumed to be occurring in the subsurface, then the original stable isotopic composition of the CH₄ entering CVL is hindcast to match a thermogenic source (Fig. 7).

POSSIBLE ELECTRON ACCEPTORS FOR METHANE OXIDATION

The co-occurrence of CH₄ and H₂S supports our claim that sulfate is the most likely electron acceptor, but what about other potential acceptors? Alternative electron acceptors such as O₂, and nitrate (NO₃⁻) are known to be metabolically coupled to CH₄ oxidation in other environments (Hu et al., 2011; Matthey et al., 2013). However, O₂ and NO₃⁻ concentrations in the anoxic springs of CVL were below detection in previous studies, where the concentration of each was < 0.1 mg L⁻¹ (Hose et al., 2000; Rosales Lagarde et al., 2014). Additionally, organoclastic sulfate reduction competes with anaerobic CH₄ oxidation only when organic matter is abundant (~ 1 % by sediment weight) (Malinverno and Pohlman 2011; Pohlman et al., 2013), and the organic carbon concentration in CVL spring water is comparatively low, ranging from 0.5 to 3.5 mg L⁻¹ (Rosales Lagarde, 2013).

STABLE ISOTOPIC COMPOSITION AND SOURCES OF CARBON DIOXIDE

The $\delta^{13}\text{C}_{\text{CO}_2}$ values near springs suggest that oxidized CH₄ is not the primary contributor to dissolved CO₂ in the spring water. The lowest CO₂ concentration in CVL (Site 6, 1 m from an entrance) had a $\delta^{13}\text{C}_{\text{CO}_2}$ value of -9.5 ± 0.3 ‰ which matches known atmospheric CO₂ concentrations (400 ± 20 ppmv) and $\delta^{13}\text{C}_{\text{CO}_2}$ values (-10 ‰) (Peyraube et al., 2013; Fernandez-Cortes et al., 2015). The two highest CO₂ concentrations in CVL at Site 11 (Sulfur Spring) and Site 7 (Slot Spring) were associated with the second-most-positive $\delta^{13}\text{C}_{\text{CO}_2}$ value of -9.1 ± 0.3 ‰ at Site 11 and the most negative $\delta^{13}\text{C}_{\text{CO}_2}$ value of -12.0 ± 0.2 ‰ at Site 7. Processes that may cause diverging $\delta^{13}\text{C}_{\text{CO}_2}$ values of CO₂ near springs include the oxidation of CH₄ and other organic compounds, which are typically ¹³C-depleted, and the dissolution of marine carbonates, like those that host CVL, that have $\delta^{13}\text{C}$ values from 1 to 4 ‰ (Keith and Weber, 1964; Knauth and Kennedy, 2009). $\delta^{13}\text{C}_{\text{CO}_2}$ values of the air above these springs

are similar to the $\delta^{13}\text{C}$ values of -9 to -12 ‰ for dissolved CO₂ measured in sulfidic springs 45 km northwest of CVL (Nencetti et al. 2005). The similarity of $\delta^{13}\text{C}_{\text{CO}_2}$ ranges from both areas demonstrate that processes causing diverging $\delta^{13}\text{C}_{\text{CO}_2}$ values at high CO₂ concentrations are regional rather than specific to CVL.

COMPARISON WITH OTHER SULFIDIC CAVE SYSTEMS

Our proposed model of CH₄ migration into CVL from petroleum basins not only provides sufficient CH₄, but also agrees with proposed pathways of sulfur migration into CVL (Rosales Lagarde et al., 2014). The entrance of thermogenic CH₄ into CVL also agrees with observations of CH₄ and longer-chain hydrocarbons emanating from “Group II” springs of the Sierra de Chiapas region northwest of CVL (Nencetti et al., 2005). The CH₄/ethane molecular ratios from these springs generally match the CH₄/ethane ratios from petroleum basins when the ratios from springs are corrected for the differential solubility of CH₄ and ethane in water (Clara Valdés et al., 2009; Dai et al., 2014). More broadly, a thermogenic source of CH₄ suggests that the processes forming CVL are analogous to those that are thought to have formed Lower Kane Cave and the caves of the Guadalupe Mountains (Kirkland and Evans, 1976; Davis, 1980; Hill, 1995).

CONCLUSIONS

High CH₄, H₂S, and CO₂ concentrations occur in cave air near springs in CVL, demonstrating that the three gases entered in solution via spring water. The poor match of the proposed stable isotopic signature of CH₄ entering CVL from springs ($\delta^{13}\text{C}_{\text{CH}_4} = -24 \pm 3$ ‰, $\delta^2\text{H}_{\text{CH}_4} = -40 \pm 50$ ‰) with known $\delta^{13}\text{C}_{\text{CH}_4}$ values from other studied systems and the thermal history of the region implies that the CH₄ is of a secondary origin, with subterranean anaerobic methanotrophy as the most likely explanation. If methanotrophy is altering the CH₄ as it traveled to CVL, the original source of the CH₄ is mathematically predicted to have a thermogenic origin. The incomplete oxidation of CH₄ in the presence of sulfate appears to be generating the H₂S that enters CVL with spring water. This evidence for CH₄ migrating from regional petroleum basins while being oxidized in the presence of sulfates connects the geochemical processes that are forming CVL to other caves, like those of the Guadalupe Mountains and Lower Kane Cave, whose origins have been attributed to sulfuric acid speleogenesis.

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