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Microbial contributions to subterranean methane sinks

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Abstract

Sources and sinks of methane (CH_4) are critical for understanding global biogeochemical cycles and their role in climate change. A growing number of studies have reported that CH_4 concentrations in cave ecosystems are depleted, leading to the notion that these subterranean environments may act as sinks for atmospheric CH_4. Recently, it was hypothesized that this CH_4 depletion may be caused by radiolysis, an abiotic process whereby CH_4 is oxidized *via* interactions with ionizing radiation derived from radioactive decay. An alternate explanation is that the depletion of CH_4 concentrations in caves could be due to biological processes, specifically oxidation by methanotrophic bacteria. We theoretically explored the radiolysis hypothesis and conclude that it is a kinetically constrained process that is unlikely to lead to the rapid loss of CH_4 in subterranean environments. We present results from a controlled laboratory experiment to support this claim. We then tested the microbial oxidation hypothesis with a set of mesocosm experiments that were conducted in two Vietnamese caves. Our results reveal that methanotrophic bacteria associated with cave rocks consume CH_4 at a rate of 1.3–2.7 mg $CH_4 \cdot m^{-2} \cdot d^{-1}$. These CH_4 oxidation rates equal or exceed what has been reported in other habitats, including agricultural systems, grasslands, deciduous forests, and Arctic tundra. Together, our results suggest that depleted concentrations of CH_4 in caves are most likely due to microbial activity, not radiolysis as has been recently claimed. Microbial methanotrophy has the potential to oxidize CH_4 not only in caves, but also in smaller-size open subterranean spaces, such as cracks, fissures, and other pores that are connected to and rapidly exchange with the atmosphere. Future studies are needed to understand how subterranean CH_4 oxidation scales up to affect local, regional, and global CH_4 cycling.

1 | INTRODUCTION

Atmospheric methane (CH_4) is a potent greenhouse gas with rising concentrations that can mainly be attributed to anthropogenic activities (IPCC, 2013; US EPA, 2015). Credible forecasting of global warming by climate models mandates knowledge about the sources and sinks of atmospheric CH_4. One potentially important, but overlooked sink of CH_4 is the oxidation that occurs in subterranean environments. Recent studies have documented that cave ecosystems sometimes have subatmospheric CH_4 concentrations. For example, in a 4-year study of St. Michael's Cave in Gibraltar, the CH_4 concentrations of cave air were typically 10-fold below atmospheric levels (Mattey et al., 2013). A similar pattern was documented in a set of Spanish caves with some samples having CH_4 concentrations that were below detection limits suggesting near-complete removal of CH_4 from underground air (Fernandez-Cortes et al., 2015).

Two hypotheses have been put forth to explain the pattern of CH_4 depletion in subterranean environments. First, CH_4 is a carbon and energy source that can be used by methanotrophic bacteria. Although methanotrophic bacteria were found in Movile Cave in Romania (Hutchens, Radajewski, Dumont, McDonald, & Murrell, 2004), microbiological surveys of methane oxidizing bacteria in caves are sparse (Jones & Macalady, 2016), and to the best of our knowledge, direct estimates of CH_4 oxidation in caves are non-existent. Instead, inferences about methanotrophy in caves have been made based on evidence from stable isotopes and thermodynamic considerations (Pohlman, 2011). For example, an inverse relationship between CH_4 concentrations and CH_4 carbon stable isotope ratios (i.e., $\delta^{13}C$) was considered a diagnostic signature of methanotrophy in St. Michael's Cave in Gibraltar (Mattey et al., 2013). A second and more recent hypothesis is that the depletion of CH_4 concentrations in subterranean ecosystems is due to radiolysis. This abiotic mechanism of CH_4 oxidation was developed to help explain low CH_4 concentrations in a poorly ventilated cave that had a high density of ions, but no recoverable methanotrophic bacteria (Fernandez-Cortes et al., 2015). An inverse correlation between the concentration of CH_4 and ions in cave air was provided as evidence that α-particles and induced ionization from radioactive decay (*via* radon and daughter nuclides) may contribute to the removal of CH_4 from subterranean environments (Fernandez-Cortes et al., 2015).

In this study, we test the relative importance of biotic and abiotic mechanisms that have been put forward to explain low concentrations of CH_4 observed in cave ecosystems. First, we develop theoretical expectations in an effort to constrain the rates of radiolytic CH_4 oxidation. Second, we present results from a controlled laboratory experiment aimed at quantifying the effect of ionizing radiation on the rate of CH_4 oxidation. Third, we discuss findings from a set of field mesocosm experiments in Vietnamese caves to quantify the methanotrophic potential of cave microbial communities.

2 | RESULTS AND DISCUSSION

2.1 | Weak theoretical support for the importance of radiolytic CH_4 oxidation

The following thought experiments reveal that radiolysis is a process that should contribute minimally to CH_4 oxidation in subterranean environments on short (i.e., daily) timescales as has been recently claimed (Fernandez-Cortes et al., 2015). We arrive at this conclusion based on the imbalance between the large number of CH_4 molecules and the comparatively small number of radioactive decay events that are typical in cave air.

Ionizing radiation in the air of subterranean limestone-based ecosystems is derived predominantly from α-particles that are associated with radon decay (Alvarez-Gallego, Garcia-Anton, Fernandez-Cortes, Cuezva, & Sanchez-Moral, 2005; Cigna, 2005). These α-particles could lead to the oxidation of CH_4 *via* different mechanisms. For example, radiolysis could result from the direct collision of α-particles with CH_4 molecules. In this case, an α-particle splits a CH_4 molecule, which triggers a subsequent exothermic oxidation reaction of ions and radicals with atmospheric oxygen. However, with a decay rate of ~35,000 ^{222}Rn atoms per second in a cubic meter of air, as measured in a Spanish cave (Fernandez-Cortes et al., 2015), it would take nearly 50 million years to eliminate 2 ppmv of CH_4 as a result of direct collision between α-particles and CH_4 molecules.

A more likely mechanism occurs when radiogenic energy interacts with water molecules and other major chemical constituents of cave air and thus produces ions and radicals that enter secondary chemical reactions with CH_4. For example, radiolysis of water vapor *via* radon decay could generate hydroxyl radicals ($^{\cdot}OH$) that act to remove CH_4. However, if every α-decay at 35,000 Bq/m^3 generates $4.3 \cdot 10^5$ ions and radicals (Fernandez-Cortes et al., 2015), it would still require more than 100 years to eliminate 2 ppmv of CH_4. While there are reports of ionizing radiation reaching extremely high levels (155,000 Bq/m^3; Hyland & Gunn, 1994; Field, 2007), radon concentrations in caves are generally much lower. A global survey of caves revealed that radon concentrations are lognormally distributed with an average of about 2,500 Bq/m^3 (Cigna, 2005). Based on this value, we calculate that it would take almost 20,000 years to oxidize 2 ppmv of CH_4. Likely, this overestimates the potential for radiolytic CH_4 oxidation as the calculations unrealistically assume that all •OH selectively react with CH_4. It is worth noting that our calculations do not take into account non-atmospheric sources of CH_4 (geologically sourced natural gas ascending along faults into caves, or methanogenesis within or above caves), nor do we attempt to model other complexities such as atmosphere–cave air exchange. Rather, our goal was to generate first-order approximations for the potential rates of radiolytic CH_4 oxidation. Based on this, we conclude that radiolysis is a kinetically constrained process that is unlikely to act as a daily CH_4 sink in subterranean ecosystems. More detail regarding the assumptions and calculations that were used to arrive at our predictions can be found in the Supplementary Information.

2.2 | Weak experimental support for the importance of radiolytic CH_4 oxidation

We conducted a laboratory experiment to test the predictions from our theoretical calculations regarding radiolytic CH_4 oxidation. Briefly, we placed 7.08 g uranium metal powder in a Petri dish on the bottom of a humid polyethylene bag containing 43 L of air with an elevated CH_4 concentration (23.5 ppmv). The radioactivity inside the closed bag containing depleted uranium was approximately $2.5 \cdot 10^6$ Bq/m^3. This level is 70-fold higher than the natural radiation reported in Spanish cave air (Fernandez-Cortes et al., 2015) and exceeds the radiation that would be found in caves having the highest reported radon concentrations in the world (Cigna, 2005; Field, 2007; Hyland & Gunn, 1994). Yet, in the presence of strong ionizing radiation, CH_4 was lost from the system at the slow rate of 0.197 ± 0.0005 (± standard error) ng $CH_4 \cdot m^{-3} \cdot d^{-1}$, which was indistinguishable from the diffusive loss of CH_4 from polyethylene control bags lacking uranium (one-sample

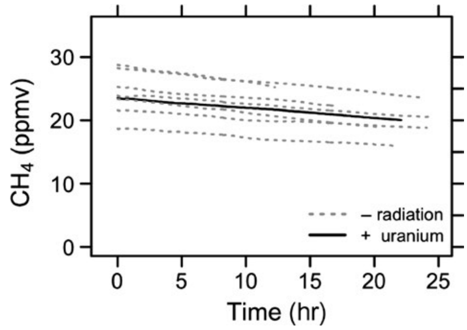

FIGURE 1 Rates of methane (CH_4) oxidation were not significantly affected by ionizing radiation. We conducted a laboratory experiment where we tracked the concentration of CH_4 in a polyethylene bag containing air and ionizing radiation from a source of uranium metal powder (black line, $n = 1$) to the concentration of CH_4 in control bags without an added source of ionizing radiation (gray lines, $n = 7$). We attribute the slow loss of CH_4 in all trials to gas diffusion through polyethylene bags

t-test: $t_6 = -0.97$, $p = 0.37$, Figure 1). Qualitatively, these findings support our theoretical predictions and provide experimental evidence that ionizing radiation has a minimal effect on CH_4 oxidation rates over short (i.e., daily) timescales. More detail concerning experimental procedures can be found in Supplementary Information.

2.3 | Strong experimental support for the importance of biotic CH_4 oxidation

We conducted a field mesocosm experiment to test whether or not microbial methanotrophy has the potential to act as a daily sink for CH_4 in caves. Our experiments were conducted in two caves located on low-altitude, coastal karst of Cát Bà Island in Northern Vietnam. Hoa Cương cave is on the north end of Cát Bà Island and in limestone of the Carboniferous–lower Permian Bắc Sơn (or Đá Mài) Formation, while Minh Châu cave is located on the southern part of Cát Bà Island in siliceous limestone of the late Devonian–early Carboniferous Phố Hàn Formation (Tong-Dzuy & Vu, 2011; Figure 2). At the time of sampling, these fairly well ventilated caves had low radon concentrations (75–115 Bq/m^3), temperatures of 19–21°C, and relative humidities ranging between 85 and 95% depending on the airflow and location within the cave. In both caves, we deployed 200-L polyethylene bags filled with cave air and containing limestone rocks that were collected from inside the cave. Half of these mesocosms ($n = 3$) were treated with a 10 wt% bleach solution (sodium hypochlorite) to inhibit microbial activity ("dead") while the other mesocosms ("live") were treated with an equal volume of water ($n = 3$). After incubating *in situ* overnight, we measured CH_4 concentrations with a Gasmet DX-4030 FTIR analyzer. CH_4 concentrations in the dead mesocosms were indistinguishable from the control mesocosms (no cave rocks) and the cave air (one-sample *t*-tests, $p > 0.52$, Figure 3). In contrast, we observed an 87% ± 0.047% (mean ± SEM) reduction of CH_4 concentrations. Our results suggest that biological processes have the potential to deplete atmospheric levels of CH_4 (2 ppmv) via methanotrophy on a daily basis, while radiolysis could take hundreds or millions of years to do the same.

From our experimental data, we estimate that the rate of CH_4 oxidation associated with cave rocks was between 1.3 and 2.7 mg $CH_4 \cdot m^{-2} \cdot d^{-1}$. To the best of our knowledge, these are the first direct measurements of biological CH_4 oxidation in a cave ecosystem. The magnitude of these rates equals or exceeds the rates of CH_4 oxidation that have been reported in soils from agricultural systems, grasslands, mature forests, and Arctic tundra (von Fischer, Butters, Duchateau, Thelwell, & Siller, 2009; Suwanwaree & Robertson, 2005; Whalen & Reeburgh, 1990). This comparison is noteworthy, because caves maintain relatively constant temperatures throughout the year, while soils in mid-to-high latitudes often experience lower temperatures during the winter season, which results in reduced rates of CH_4 oxidation (e.g., Groffman, Hardy, Driscoll, & Fahey, 2006). As such, future studies should integrate methanotrophic activity over annual timescales to better assess the magnitude and stability of subterranean ecosystems as CH_4 sinks.

Our experiments revealed that methanotrophic bacteria were abundant in the biofilms that were associated with Vietnamese cave rocks. We conducted quantitative PCR assays on DNA extracted from rocks

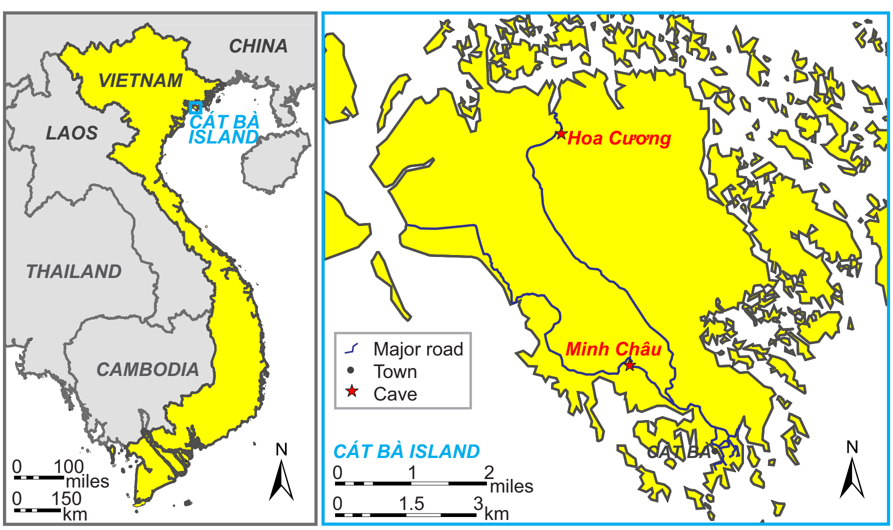

FIGURE 2 Map of Vietnam (left) and Cát Bà Island (right) indicating the location of Hoa Cương and Minh Châu caves where the field mesocosm experiments were conducted to evaluate the importance of biological CH_4 oxidation

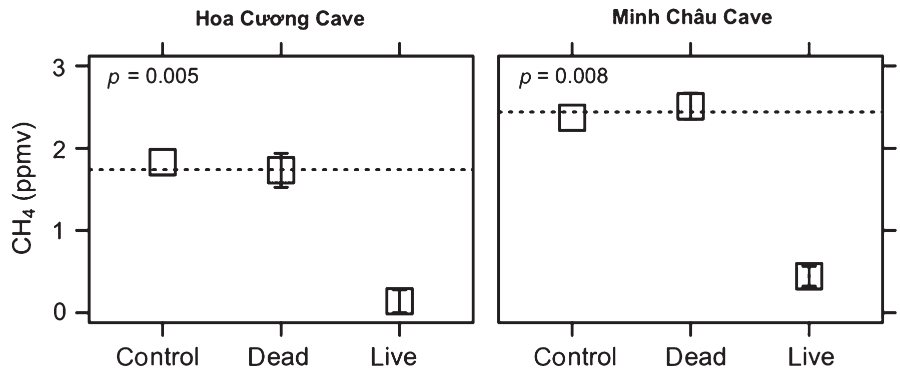

FIGURE 3 Field mesocosm experiments in two Vietnamese caves support the biological methane (CH_4) oxidation hypothesis. Control mesocosms contained no cave rocks and provided an estimate for the diffusive loss of CH_4; "dead" mesocosms contained cave rocks that were treated with a 10 wt% bleach solution; "live" mesocosms contained cave rocks and a volume of water (150 ml) equivalent to the volume of bleach used in the "dead" treatment. The dashed horizontal lines correspond to the CH_4 concentrations in the Hoa Cương and Minh Châu caves on Cát Bà Island, Northern Vietnam

that were incubated in the live mesocosms using primers that targeted the particulate methane monooxygenase (*pmo*A) gene, which is responsible for bacterial CH_4 oxidation (see Supplementary Information for more detail). From this, we recovered $1.0 \cdot 10^4$ to $1.5 \cdot 10^4$ *pmo*A gene copies per gram of rock biofilm. When standardized by 16S rRNA gene copy number, we estimate that the relative abundance of methanotrophs in the cave biofilms ranged from 0.16 to 1.48% of the microbial community. Despite recent global-scale efforts to survey the diversity of microbial communities from a wide range of habitats, reports of methane oxidizing bacteria from cave ecosystems are scarce. For example, using cultivation-independent approaches, no sequences closely matching known methanotrophs were recovered from the Frasassi Cave complex in central Italy (Macalady et al., 2006). Methanotrophs were recovered from some, but not all Spanish caves (Fernandez-Cortes et al., 2015). In limestone caves of Kartchner Caverns, Arizona (USA), a single sequence was recovered that was closely related to *Methylocella*, which is a facultative methanotroph (Ortiz et al., 2013). Similarly, only one sequence from the walls of a karst cave in Slovenia was closely related to *Methylococcus*, which is an obligate methanotroph (Pašič, Kovče, Sket, & Herzog-Velikonja, 2010). In contrast, the presence and activity of methanotrophs were documented in water and mat samples collected from Movile Cave using stable isotope probing (SIP), but this cave system is unique because it is supplied with CH_4 from an underground anoxic water body. Nevertheless, researchers tracked ^{13}C-labeled CH_4 into the DNA of bacteria that were closely related to known methanotrophs such as *Methylomonas*, *Methylococcus*, and *Methylocystis*/*Methylosinus* (Hutchens et al., 2004). In a recent study of the semiarid Wellington Caves in Australia, up to 16% of the 16S rRNA gene sequences recovered from cave soils belonged to groups of known methanotrophs (McDonough et al., 2016). The high relative abundance of methanotrophs in these systems suggests that microbially mediated CH_4 oxidation should be important in at least some caves. Given their potential role in consuming subterranean CH_4, more studies are needed to characterize the diversity and activity of methanotrophs in a wider range of cave ecosystems.

In the methane-depleted Castañar Cave in Spain, the importance of methanotrophy was ruled out based on the assumption that bacteria would not be able to meet their metabolic demands for maintenance and growth (Fernandez-Cortes et al., 2015). However, this critical argument overlooks important ecophysiological features of microorganisms in natural ecosystems. First, many caves are considered oligotrophic habitats that are characterized by energy limitation (Jones & Macalady, 2016). However, growing evidence suggests that many microorganisms can tolerate extreme energy limitation on timescales ranging from centuries to millennia (Hoehler & Jørgensen, 2013) owing to life history strategies such as dormancy (Lennon & Jones, 2011). Second, microorganisms in nature are commonly challenged with "feast or famine" conditions. For example, the supply of CH_4 to cave habitats varies through time depending on the source of CH_4, seasonality, ventilation, microclimatic conditions, and geography. It is well documented that there are high-affinity methanotrophs that are adapted to living on trace concentrations of CH_4 (Bull, Parekh, Hall, Ineson, & Evershed, 2000). It is also likely that there are methanotrophic bacteria in caves that are adapted to fluctuations in CH_4 concentrations, which are not captured with synoptic sampling.

3 | CONCLUSION

Although ionizing radiation can accumulate in poorly vented, deep recesses of some caves, this is neither necessary nor sufficient to explain the observation of CH_4 depletion in cave ecosystems (e.g., Fernandez-Cortes et al., 2015; Mattey et al., 2013). In this study, we present theoretical and experimental lines of evidence suggesting it is unlikely that radiolytically induced CH_4 oxidation serves as a significant mechanism for rapid depletion of CH_4 in cave air as has recently been reported (Fernandez-Cortes et al., 2015). Rather, our results support the hypothesis that bacterial methanotrophy alone has the potential to significantly oxidize CH_4 in caves, and perhaps other smaller-size open subterranean spaces, such as cracks, fissures, and other pores that are connected to the atmosphere. Rapid rates of CH_4 oxidation have led to speculation that subterranean habitats could be managed in cost-effective ways to mitigate industrial emissions of CH_4 (Fernandez-Cortes et al., 2015) especially as karst landforms make up 10–20% of the continental landforms (Palmer, 1991). However, our understanding of CH_4 dynamics in subterranean ecosystems is limited. Only a small number of caves in a handful of locations have been studied thus far. More information is needed from diverse geographical, geological, and biological settings before the importance of subterranean CH_4 sinks can be assessed on local, regional, and global scales as has been performed in other ecosystems (e.g., Oh et al., 2016).

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REFERENCES

Alvarez-Gallego, M., Garcia-Anton, E., Fernandez-Cortes, A., Cuezva, S., & Sanchez-Moral, S. (2005). High radon levels in subterranean environments: monitoring and technical criteria to ensure human safety (case of Castañar cave, Spain). *Journal of Environmental Radioactivity*, *145*, 19–29.

Bull, I. D., Parekh, N. R., Hall, G. H., Ineson, P., & Evershed, R. P. (2000). Detection and classification of atmospheric methane oxidizing bacteria in soil. *Nature*, *405*, 175–178.

Cigna, A. A. (2005). Radon in caves. *International Journal of Speleology*, *34*, 1–18.

Fernandez-Cortes, A., Cuezva, S., Alvarez-Gallego, M., Garcia-Anton, E., Concepcion, P., Benavente, D., …. Sanchez-Moral, S. (2015). Subterranean atmospheres may act as daily methane sinks. *Nature Communications*, *6*, 7003.

Field, M. S. (2007). Risks to cavers and cave workers from exposures to low-level ionizing α radiation from ^{222}Rn decay in caves. *Journal of Cave and Karst Studies*, *1*, 207–228.

von Fischer, J. C., Butters, G., Duchateau, P. C., Thelwell, R. J., & Siller, R. (2009). In situ measures of methanotroph activity in upland soils: a reaction-diffusion model and field observation of water stress. *Journal of Geophysical Research*, *114*, G01015.

Groffman, P. M., Hardy, J. P., Driscoll, C. T., & Fahey, T. J. (2006). Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. *Global Change Biology*, *12*, 1748–1760.

Hoehler, T. M., & Jørgensen, B. B. (2013). Microbial life under extreme energy limitation. *Nature Reviews Microbiology*, *11*, 83–94.

Hutchens, E., Radajewski, S., Dumont, M. G., McDonald, I. R., & Murrell, C. J. (2004). Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing. *Environmental Microbiology*, *6*, 111–120.

Hyland, R., & Gunn, J. (1994). International comparison of cave radon concentrations identifying the potential alpha radiation risks to British cave users. *Health Physics*, *67*, 176–179.

IPCC (2013). Climate change 2013: the physical science basis. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P. M. Midgley (Eds.), *Contribution of working group 1 to the fifth assessment report of the intergovernmental panel on climate change* (pp. 1535). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Jones, D. S., & Macalady, J. J. (2016). The snotty and the stringy: energy for subsurface life in caves. In: C., Hurst (Ed.), *Their world: a diversity of microbial environments advances in environmental microbiology*. vol. *1*, chapter 5 (pp. 203–224). New York: Springer.

Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, *9*, 119–130.

Macalady, J. L., Lyon, E. H., Koffman, B., Albertson, L. K., Meyer, K., Galdenzi, S., & Mariani, S. (2006). Dominant microbial populations in limestone-corroding stream biofilms, Frasassi Cave system, Italy. *Applied and Evironmental Microbiology*, *72*, 5596–5609.

Mattey, D. P., Fisher, R., Atkinson, T. C., Latin, J.-P., Durrel, R., Ainsworth, M., … Fairchild, I. J. (2013). Methane underground air in Gibraltar karst. *Earth and Planetary Science Letters*, *374*, 71–80.

McDonough, L. K., Iverach, C. P., Beckmann, S., Manefield, M., Rau, G. C., Baker, A., & Kelly, B. F. J. (2016). Spatial variability of cave-air carbon dioxide and methane concentrations and isotopic compositions in a semi-arid karst environment. *Environmental Earth Sciences*, *75*, 700.

Oh, Y., Stackhouse, B., Lau, M. C. Y., Xu, X., Trugman, A. T., Moch, J., … Medvigy, D. (2016). A scalable model for methane consumption in arctic mineral soils. *Geophysical Research Letters*, doi: 10.1002/2016GL069049.

Ortiz, M., Neilson, J. W., Nelson, W. M., Legatzki, A., Byrne, A., Yu, Y., … Maier, R. M. (2013). Profiling bacterial diversity and taxonomic composition on speleothem surfaces in Kartchner Caverns, AZ. *Microbial Ecology*, *65*, 371–383.

Palmer, A. N. (1991). Origin and morphology of limestone caves. *Geological Society of America Bulletin*, *103*, 1–21.

Pašič, L., Kovče, B., Sket, B., & Herzog-Velikonja, B. (2010). Diversity of microbial communities colonizing the walls of a Karstic cave in Slovenia. *FEMS Microbiology Ecology*, *71*, 50–60.

Pohlman, J. W. (2011). The biogeochemistry of anchialine caves: progress and possibilities. *Hydrobiologia*, *677*, 33–51.

Suwanwaree, P., & Robertson, G. P. (2005). Methane oxidation in forest, successional, and no-till agricultural ecosystems: effects of nitrogen and soil disturbance. *Soil Science Society of America Journal*, *69*, 1722–1729.

Tong-Dzuy, T., & Vu, K.(Eds.) (2011). *Stratigraphic units of Vietnam (Second Edition - Revised and updated)*. Vietnam National University Publisher, Hanoi. MS: 69-KHTN-2011.

US EPA (US Environmental Protection Agency) (2015). Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2013.

Whalen, S. C., & Reeburgh, W. S. (1990). Consumption of atmospheric methane by tundra soils. *Nature*, *346*, 160–162.

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