Microbial contributions to subterranean methane sinks

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Atmospheric methane (CH_4) is a potent greenhouse gas with rising concentrations that can mainly be attributed to anthropogenic activities [1,2]. Credible forecasting of global warming by climate models mandates knowledge of sources and sinks of atmospheric CH_4. One potentially important, but mostly overlooked sink of CH_4 is the oxidation that occurs in subterranean environments. Based largely on correlative evidence, it was recently hypothesized that radiation-induced ionization was responsible for sub-atmospheric CH_4 concentrations in a set of Spanish caves [3]. This abiotic mechanism of oxidation is reported to deplete CH_4 in caves at a rate faster than it is exchanged with the atmosphere. Here, we present theoretical and experimental evidence suggesting that CH_4 depletion in the air of subterranean ecosystems is more likely due to biological processes, specifically CH_4 oxidation by methanotrophic bacteria.

Weak theoretical support for the importance of radiolytic CH_4 oxidation — The following thought experiments demonstrate that radiation-induced CH_4 oxidation cannot contribute appreciably to CH_4 depletion in subterranean environments over days to weeks. 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 caves.

Ionizing radiation in the air of subterranean limestone-based ecosystems is derived predominantly from α-particles that are generated during radon decay [4, 5]. 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 chain reaction leading to the complete oxidation of that molecule. 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 [3], it would take nearly 50 million years to eliminate 2 ppm 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. Fernandez-Cortes et al. describe how radiolysis of water vapor via radon decay can generate OH^- that act to remove CH_4 [3]. However, if every α-decay at 35,000 Bq m^{-3} generates $4.3 \cdot 10^5$ ions and radicals [3], it would still require more than 100 years to eliminate 2 ppm of CH_4. In fact, this likely overestimates the potential for radiolytic CH_4 oxidation since the calculations unrealistically assume that all OH^- selectively react with CH_4. In sum, kinetic constraints of CH_4 radiolysis and secondary reactions of CH_4 with ions and radicals are inconsistent with the view that CH_4 can be abiotically depleted in subterranean ecosystems on the time scales of days [3]. See Supplementary Information for more detail.

Weak experimental support for the importance of radiolytic CH_4 oxidation — Results from a laboratory experiment confirm our theoretical predictions by demonstrating that ionizing radiation had a minimal effect on CH_4 oxidation rates. We placed 7.08 g uranium metal powder in a Petri dish on the bottom of a closed polyethylene bag containing 43 L of air with an elevated CH_4 concentration (23.5 ppm). The radioactivity inside the bag containing depleted uranium was approximately $2.5 \cdot 10^6$ Bq m^{-3}, which is 70-fold higher than the natural radiation reported in Spanish cave air [3]. 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 t-test: $t_6 = -0.97$, $P = 0.37$, Fig.1). See Supplementary Information for more detail.

Strong support for biotic CH_4 oxidation — The results from our field mesocosm experiments suggest that CH_4 depletion is due to biological activity, specifically methanotrophic bacteria that are commonly associated with rocks, soils, and walls of cave ecosystems. In two separate caves with low radon abundances (≤100 Bq m^{-3}) on the island of Cát Bà in Vietnam, we deployed 200-L polyethylene bags filled with cave air containing local limestone cave rocks. Half of these mesocosms (n = 3) were treated with a 10 % bleach (sodium hypochlorite) solution 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, CH_4 concentrations in the dead mesocosms were indistinguishable from the control mesocosms (no cave rocks) and the cave air (one-sample t-tests, $P > 0.52$, Fig. 2). In contrast, we observed an 87 % ± 0.047 % (mean ± SEM) reduction of CH_4 concentrations in the live mesocosms. From these data, we estimate that CH_4 oxidation associated with cave rocks was between 1.33 and 2.70 mg $CH_4 \cdot m^{-2} \cdot d^{-1}$, which is comparable to rates reported elsewhere [7]. Furthermore, we conducted quantitative PCR assays on DNA extracted from biofilms of rocks that were incubated in the live mesocosms using primers that targeted the particulate methane monoxygenase (*pmo*A) gene, which is responsible for bacterial CH_4 oxidation (see Supplementary Information). 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, we estimate that the relative abundance of methanotrophs in the cave biofilms ranged from 0.16 to 1.48 % of the microbial community.

In methane-depleted Spanish caves, the importance of methanotrophy was ruled out based on the assumption that microbes would not be able to meet their metabolic demands for maintenance and growth [3]. However, this argument overlooks two important ecophysiological

92 features of microorganisms in natural ecosystems. First, growing evidence suggests that some microorganisms can tolerate extreme energy limitation on timescales ranging from centuries to
94 millennia [8] owing to life-history strategies such as dormancy [9]. Second, microorganisms in nature are commonly challenged with "feast or famine" conditions. For example, the supply of
96 CH_4 to cave habitats varies through time depending on the source of CH_4, seasonality, ventilation, microclimatic conditions, and geography. Methanotrophic bacteria in caves are likely
98 adapted to such fluctuations in CH_4 concentrations, which are not captured with synoptic sampling.

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Conclusion — Although ionizing radiation can accumulate in poorly vented, deep recesses of
102 some caves, this is neither necessary nor sufficient to explain the pattern of CH_4 depletion in subterranean ecosystems. Both theoretical and experimental lines of evidence lead us to the
104 conclusion that radiolytically induced CH_4 oxidation cannot serve as a significant mechanism for rapid depletion of CH_4 in cave air as has been recently suggested [3]. Rather, our results support
106 the hypothesis that bacterial methanotrophy alone has the potential to significantly oxidize CH_4 in caves, but also smaller-size open subterranean spaces, such as cracks, fissures, and other pores
108 that are connected to the atmosphere.

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Figures

Fig. 1. Rates of methane (CH_4) oxidation were not 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 (grey lines, n = 7). We attribute the slow loss of CH_4 in all trials to diffusion through polyethylene.

Fig. 2. 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 % 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, Vietnam.

Fig. 1.

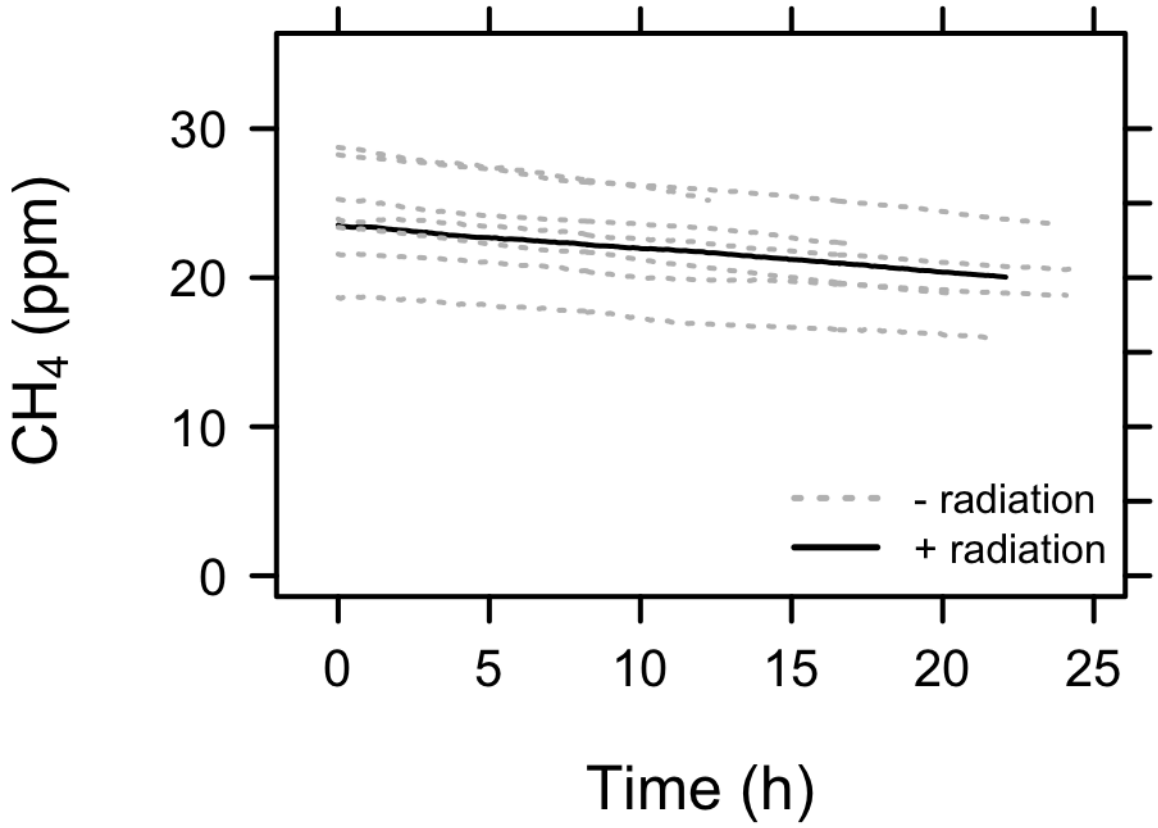

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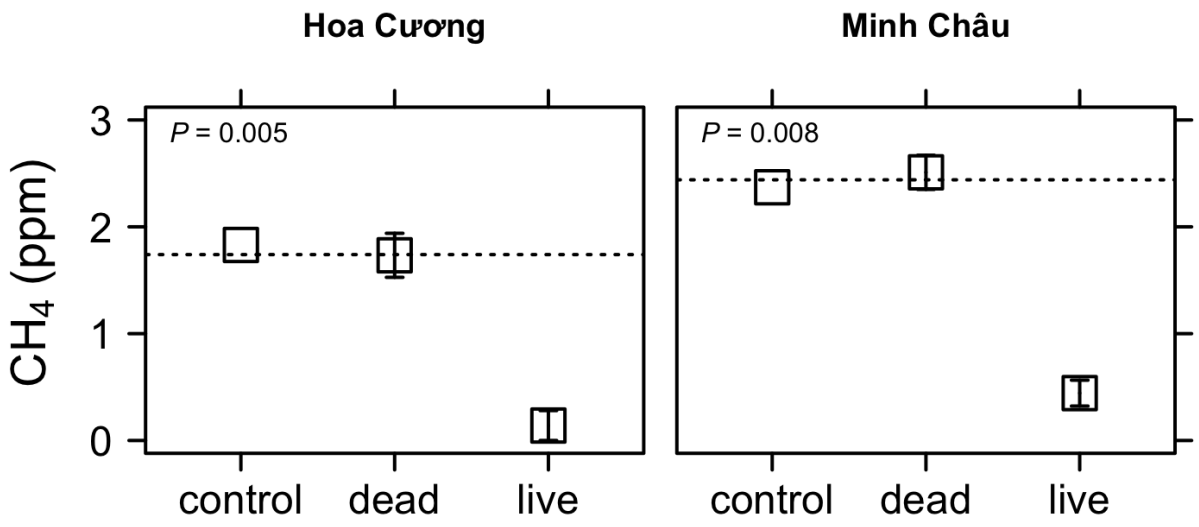
Fig. 2.



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