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Out of thin air: tracking trace gas metabolism in cave microorganisms

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Karst terrains cover approximately 20% of Earth's land surface and are home to unique microorganisms. Importantly, cave microorganisms are known to metabolize trace greenhouse gases (i.e. CH_4, CO, N_2O) [1]. This phenomenon has been described mostly by single time point studies of cave microbiomes, yet many karst settings are seasonally dynamic. Parameters such as temperature, humidity, and air circulation—all which can affect microbial growth—differ seasonally in mid-latitude settings [2]. Furthermore, while trace gas oxidation has indeed been identified in caves, relatively few described microorganisms are known to metabolize trace gases at their atmospheric concentrations [1].

To quantify the microbially-mediated cycling of greenhouse gases in caves, time-resolved study is necessary. To this end, we combine seasonal field expeditions and observations with molecular methods and geochemical analysis. We target Mammoth Cave, the longest mapped cave system in the world [3], collecting biofilms, sediments, and gases. Our data reveal subtle differences between the microbial communities in biofilms and sediments, although a notable lack of canonical methanotrophs. Instead, biofilms are dominated by Actinomycetota, a clade known for its metabolic diversity and with a known methanotrophic member [4]. Within biofilm Actinomycetota, metagenomes reveal genes responsible for CO- and CH_4-oxidation. Furthermore, biofilm ^{13}C values are significantly depleted relative to sediments, suggesting either carbon fixation or the incorporation of a ^{13}C-depleted substrate. Analysis of the trace gas composition of Mammoth Cave air reveals seasonal differences in composition, particularly in CH_4 and CO_2 concentrations. Ongoing measurements of the isotopic make-up of cave CH_4 and seasonal patterns in microbial community composition and activity, aim to identify key players in greenhouse gas cycling. Ultimately, these data constrain models of greenhouse gas cycling and may highlight novel microorganisms capable of mediating these transitions.

[1] Martin-Pozas, Cuezva, Fernandez-Cortes, Mulec, Hernández (2026), *Current Opinion in Microbiology*, 90:102707.

[2] Olson (2017), Mammoth Cave Meteorology, *Mammoth Cave*.

[3] Toomey, Hobbs, Olson (2017), An Orientation to Mammoth Cave and This Volume, *Mammoth Cave*.

[4] Morin, Dekoninck, Sridhar, Disney-McKeethen, Proctor, Eng, Traxler (2025). *Annual Review Microbiology*, 79(1):753-772.