

# Identifying Putative Subsurface Microbial Drivers of Methane Flux on Earth and Mars

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## Abstract

On Earth microorganisms are critical drivers of the methane cycle, both producing and consuming methane (Boetius et al. 2000, Knittel and Boetius 2009, Orphan et al. 2001). Molecular and isotopic-based investigations of archaeal-bacterial consortia catalyzing the anaerobic oxidation of methane (AOM) in marine methane seeps identified the pivotal role of these microorganisms in mitigating the release of methane into the atmosphere (Knittel and Boetius 2009, Orphan et al. 2001). In the marine environment, AOM is predominantly carried out by closely associated consortia of methanotrophic archaea (ANME) and sulfate reducing bacteria (SRB) coupling methane oxidation to sulfate reduction in the absence of oxygen.

Wolf Spring (WS), Axel Heiberg Island, Nunavut is a hypersaline cold spring methane seep and the only known terrestrial permafrost hosted methane seep known to host ANME-1 archaea associated with AOM (Niederberger et al. 2010, Magnuson et al. 2022). Wolf Spring is an unparalleled analogue for putative subsurface brines and sites of methane release on Mars. Enigmatic observations of methane in the near-surface Martian atmosphere remain a tantalizing potential biosignature.

The combination of field site characterization, microbial microcosm experiments, and *in situ* methane monitoring represents a coordinated interdisciplinary effort to identify methane driven microbial metabolisms not only critical to understanding methane flux in the Arctic, but also as possible drivers to the methane cycle on Mars. Detailed microbial characterization of these springs has identified a chemotrophic community dominated by sulfur cycling (Altshuler et al. 2022, Niederberger et al. 2010). To date, microbial and geochemical characterization has been carried out on sediment samples to a few centimeters depth. This study expands on these initial studies, with the successful collection and analysis of deeper sediment cores at WS focusing on AOM activity to better understand the microorganisms involved and the methane cycling capacity at depth.

Two decades of observing methane on Mars (Mumma et al. 2009) have generated data indicative of a dynamic, geochemical system characterized by a profile similar to the release of methane from seeps on Earth (Etioppe and Oehler 2019) producing both distinct pulses known as plumes and slow background seepage. These observations suggest as of yet unknown geochemical and potentially geobiological methane sources and sinks.

While methane can be produced abiotically (Etioppe and Lollar 2013), on Earth most methane is biogenic. Determining the biogenicity of CH<sub>4</sub> is non-trivial and requires a correlated approach including determination of carbon isotopes. In terrestrial systems, biogenic CH<sub>4</sub> is <sup>13</sup>C depleted. To characterize methane sources and sinks on Mars, near surface measurements at a frequency not possible with existing instrumentation are required.

We are currently developing off-axis integrated cavity-enhanced output (OA-ICOS) spectrometry as a portable trace gas analyzer capable of obtaining high frequency measurements of methane at the sub-ppb level (Sapers et al. 2021). Optimizing OA-ICOS trace methane measurements at WS will help refine sensitivity and measurement cadence in a Mars-like environment as well as providing new remote methane monitoring capabilities for Arctic methane emissions. We are currently developing in situ <sup>12</sup>CH<sub>4</sub>:<sup>13</sup>CH<sub>4</sub> capabilities using OA-ICOS technology. The importance of δ <sup>13</sup>C as a biosignature is summarized in Fig. 1.

## **Keywords**

methane, anaerobic oxidation of methane, Arctic, hypersaline spring, cold spring, Mars, methane seep, ANME-1

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## **Conflicts of interest**

The authors have declared that no competing interests exist.

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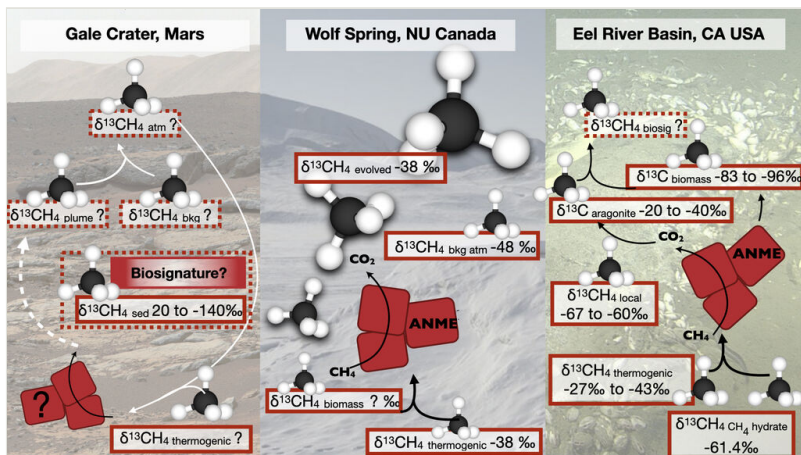


Figure 1.

Right: Submarine methane cold seep, Eel River Basin, California USA. Source methane is thermogenic characterized by light  $\delta^{13}\text{C}$  values up to  $-27\text{‰}$ . Variable contributions by more depleted gas hydrates and a local methane pool with a biogenic signature down to  $-67\text{‰}$ .  $^{13}\text{C}$  values from ANME biomass is significantly  $^{13}\text{C}$  depleted (as low as  $-96\text{‰}$ ).  $\delta^{13}\text{C}$  values from authigenic aragonite are significantly more depleted than that of normal marine carbonate indicating in situ mineralization of  $\text{CO}_2$  produced via AOM. Data from (Orphan et al. 2004).

Center: Wolf Spring, Axel Heiberg Island, Nunavut, Canada. Source methane has an isotopic signature indicative of a predominately thermogenic source ( $\delta^{13}\text{CH}_4$   $-38\text{‰}$ ). Data from (Niederberger et al. 2010).  $\delta^{13}\text{C}$  measurements from biomass collected at depth is currently planned. Representative  $\delta^{13}\text{CH}_4$  for background atmosphere taken from  $\sim 7$  km altitude during a stratospheric balloon flight launched from Kiruna, Sweden sampling Arctic air mass during the Arctic summer (Röckmann et al. 2011).

Left: Methane evolved from mudstones in Gale Crater, Mars. There are two main methane sources, background seepage and periodic plumes, contributing to the methane pool in the near surface atmosphere with unknown  $\delta^{13}\text{C}$  values. Recently,  $^{13}\text{CH}_4$  was measured from a mudstone collected in Gale crater with an extremely wide range of values (House et al. 2022). These highly  $^{13}\text{C}$ -depleted values are reminiscent of the authigenic carbonates produced via mineralization of biogenically produced  $\text{CO}_2$  during AOM in submarine methane seeps. While the carbon isotopic reservoirs on Mars are not well constrained, on Earth highly  $^{13}\text{C}$  depleted value are consistent with methane derived through microbial methanogenesis. All  $\delta^{13}\text{C}$  values compared to V-PDB.