

# Metagenomic and isotope geochemical characterization of deep subsurface microbial methanogenesis at the Siljan impact structure, Sweden

FEMKE VAN DAM<sup>1</sup>, RIIKKA KIETÄVÄINEN<sup>2</sup>, GEORGE WESTMEIJER<sup>1</sup>, SHUHEI ONO<sup>3</sup>, MARK DOPSON<sup>4</sup>, MARCELO KETZER<sup>1</sup>, JENNIFER MCINTOSH<sup>5</sup> AND HENRIK DRAKE<sup>1</sup>

<sup>1</sup>Linnaeus University

<sup>2</sup>University of Helsinki

<sup>3</sup>Massachusetts Institute of Technology

<sup>4</sup>Centre for Ecology and Evolution in Microbial Model Systems, Linnaeus University, Kalmar

<sup>5</sup>University of Arizona

Presenting Author: femke.vandam@lnu.se

Deep fracture networks in crystalline bedrock can be important habitats for microorganisms that can influence geochemical cycles. Accumulations of methane have been found in the Siljan impact crater, a meteorite impact structure of Devonian age and the largest impact crater in Europe, located in Sweden [1]. Previous investigations showed that ancient microbial methanogenesis occurred in the fractures of the impact structure [2]. In this study, modern methanogenesis was investigated using gas, water, and microbial analyses on samples taken from 400 m depth at the ring-shaped depression of the impact crater, below the contact between downfaulted sedimentary rocks and the crystalline basement. Isotopic compositions, such as strong <sup>13</sup>C-depletion of the methane in free gas confirmed a dominantly microbial origin, and presence of C<sub>2</sub> to C<sub>5</sub> hydrocarbons indicated a minor thermogenic methane mixing fraction. Multiply substituted isotopologues of methane ( $\Delta^{13}\text{CH}_3\text{D}$ ) for both the free gas and the incubation head space indicated disequilibrium fractionation due to kinetic isotope effects caused by microbial methanogenesis. Groundwater incubations supplemented with indigenous oil from Siljan boreholes successfully produced methane. Gas analysis showed a positive isotopic relationship between  $\delta^{13}\text{C}$  values of methane and the CO<sub>2</sub> in the headspace, indicative of carbon fixation pathway, which was also indicated by positive  $\delta^{13}\text{C}_{\text{-CO}_2}$  values of the free gas. Substrate experiments showed fastest production of methane with methanol as the electron donor. Metagenomic data from groundwater samples and transcriptomic data from incubations further elucidated which metabolic pathway the methanogens were utilizing and their possible syntrophic relationships. These results showed that methanogens were actively producing methane in the Siljan impact structure fracture network and gave insights into microbial methanogenesis in the deep biosphere in general, and in highly fractured and porous rocks of meteorite impact craters in particular.

[1] Drake et al. (2019), *Nature Communications*, 1-14, 10(1)

[2] Drake et al. (2021), *Communications Earth &*