

Geologic Constraints On Microbial Dynamics In A Subsurface Serpentinite Ecosystem K. R. Rempfert¹, H. M. Miller¹, N. Bompard², D. Nothhaft¹, J. M. Matter², P. Kelemen³, N. Fierer⁴ and A. S. Templeton¹, ¹Department of Geological Sciences, University of Colorado, Boulder, CO, USA, ²National Oceanography Centre, University of Southampton, U.K., ³Lamont Doherty Earth Observatory, Columbia University, Palisades, NY, U.S.A., ⁴Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, U.S.A.

During serpentinization, or the hydration of peridotite, the reduction of water and DIC also commonly occurs, generating H₂, CH₄, and hydrocarbons that can fuel microbial ecosystems. Ultramafic rocks are common in the Earth's mantle, on Mars, and in the cores of icy satellites. Thus, the potential for serpentinization to provide sufficient energy for metabolism is widespread throughout the solar system and through time. However, the geochemical conditions arising from these water-rock reactions also challenge the known limits of microbial physiology (extreme pH, availability of e⁻ acceptors and DIC). Recent studies have investigated the habitability of surface hyperalkaline seeps hosted in serpentinites [1-5], however, little is known about the microorganisms that may persist in the *subsurface* where DIC and sources of oxidants are likely more limiting. In order to develop models for the habitability of serpentinizing environments on other planetary bodies, these communities must first be characterized in the deep subsurface within the context of the geological parameters that may limit microbial activity.

The Samail Ophiolite in the Sultanate of Oman is an ideal setting to investigate subsurface serpentinite-hosted ecosystems because a series of pre-existing boreholes (up to ~470 m deep) allows for access to deep fluids in contact with the crust/mantle transition. We have recovered 20 biomass and deep fluid samples from 12 boreholes in the ophiolite, which has allowed us to directly study the fluid geochemistry and phylogenetic diversity of subsurface microorganisms. This study [6] provides the first comprehensive data for subsurface microbial communities in the Samail Ophiolite, and significantly expands upon the diversity of geochemical regimes investigated compared to Miller et al. (2016) [7].

We measured highly variable fluid compositions (pH varies from 7.4 to 11.4) across the ophiolite, with potential metabolic substrates (H₂, CH₄, NO₃⁻, SO₄²⁻, DIC) differing in concentration considerably between wells. PCA of fluid geochemistry along with geologic context indicate the presence of at least four fluid types in the Samail Ophiolite (*gabbro*, *alkaline peridotite*, *hyperalkaline peridotite*, and *gabbro/peridotite contact*). Fluid geochemistry likely reflects differences in both host rock composition and extent of water-rock reaction. Notably, even the most reacted hyperalkaline

peridotite fluids contain appreciable concentrations of e⁻ acceptors such as SO₄²⁻ and NO₃⁻.

Microbial community composition is strongly correlated with aqueous geochemistry of fluids, with similar microbial assemblages grouping consistently by fluid type. pH is a good predictor for the richness of microbial communities in this ecosystem. Microbial communities in hyperalkaline peridotite fluids are characterized by low richness. In contrast to previous studies of surficial seeps, we found that taxa affiliated with *Meiothermus*, OP1, and the family Thermodesulfobionaceae dominated the communities. However, we also detect Comamonadaceae, Firmicutes, Pseudomonadaceae, and *Methanobacterium* present in other serpentinizing environments. Gabbro- and alkaline peridotite- aquifers consist of more diverse communities enriched in microbial taxa affiliated with *Nitrospira*, Nitrososphaeraceae, OP3, Parvarcheota, and OP1 order Acetothermales. The presence of phylotypes affiliated with nitrification suggests N-cycling may be especially prevalent in upper aquifer fluids. Wells that sit at the contact between gabbro and peridotite host microbial communities distinct from all other fluid types, characterized by an abundance of betaproteobacterial taxa. We hypothesize these contact zones represent areas of fluid mixing, and thus enhanced chemical disequilibrium that selects for this class. Taxonomic information and geochemical data suggest that several metabolisms may be operative in subsurface fluids including methanogenesis, acetogenesis, and fermentation, as well as the oxidation of CH₄, H₂, and organic acids utilizing NO₃⁻, SO₄²⁻, and Fe³⁺ as e⁻ acceptors.

Altogether, our data shows that differential energy availability and aqueous geochemistry (e.g. pH, Eh, available DIC and e⁻ acceptors) resulting from fundamental differences in host rock lithology and the extent of water-rock reaction strongly influence microbial community structure and habitability in the subsurface.

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