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Active microbial nitrogen cycling in a subsurface serpentinizing system

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Subsurface serpentinization generates heat, hydrogen, methane, simple organic compounds, and establishes redox and pH gradients that could support early life on Earth [1]. While nitrogen (N) is essential for life, how early life accessed it remains unclear. Here, we conducted ^{15}N-isotope tracer experiments and transcriptomic analyses to explore active microbial N-transformations within the Samail Ophiolite, one of the world's largest serpentinizing systems. We found multiple N-species coexisting in borehole fluids, with diverse N-cycling processes sustaining the subsurface microbiome by occupying distinct niches. Ammonium, likely sourced from rocks and minerals, predominated in hyperalkaline, reduced fluids (median 53 µM), while nitrate was enriched in oxidised, slightly alkaline conditions (median 141 µM). Under reduced conditions, N_2 fixation and ammonium assimilation contributed to 44% and 54%, respectively, of the N needed for microbial biomass, while N_2 fixation alone supplied 74% of N in oxidised conditions. Energy-yielding nitrification and nitrate reduction were enhanced in oxidised fluids, whereas dissimilatory nitrate reduction to ammonium occurred only in reduced settings. Moreover, methane stimulated nitrous oxide production from nitrite sixfold, indicating a link between methane oxidation and incomplete denitrification. These findings reveal active, differential microbial N-cycling along pH/redox gradients within this serpentinizing system, with significant implications for the biogeochemical processes that may have supported early life on Earth and perhaps also other celestial bodies.

Reference: [1] Russell, M., Hall, A. & Martin, W. Serpentinization as a source of energy at the origin of life. *Geobiology* **8**, 355-371 (2010).