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Energy Landscapes Drive Microbial Metabolic Function in a Brine-Dominated Hydrothermal Vent System

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Brine-dominated deep-sea hydrothermal vents form when phase separation in the lithosphere generates high-salinity brines that can discharge rapidly or accumulate in subseafloor reservoirs for decades before venting. Elevated chlorinity in hydrothermal fluids results in high dissolved iron concentrations that may precipitate as pyrite upon cooling, generating H2. Despite their distinctive geochemistry, the biogeochemical fluxes and microbial communities at brine-dominated vents remain poorly constrained.

We investigated brine-dominated venting along the Northern and Southern Cleft Segment of the Juan de Fuca Ridge (Northeast Pacific Ocean), where two vent fields with distinct source fluids and geochemical histories provide a natural experiment across spatial and temporal scales. Across nine hydrothermal chimneys and nine diffuse-flow vents, we integrated a comprehensive geochemical dataset with 16S rRNA gene sequencing, metagenomics, metaproteomics, and thermodynamic modeling to link microbial community structure, metabolic potential, realized activity, and environmental energetics. We hypothesize that differences between the two vent fields in dissolved metal enrichment and subsequent volatile generation alter the energetic favorability of redox reactions, driving differences in microbial community composition and metabolism.

Analysis of 16S rRNA gene data indicated that microbial communities in chimney samples clustered distinctly between the Northern and Southern segments. Chimney exteriors hosted sulfur-disproportionating *Dissulfuribacteraceae* and iron-oxidizing *Zetaproteobacteria* and *Gallionellaceae*, while compelling geochemical evidence of H2 generated by pyrite formation in the hot inner chimney supported putative methanogenic and sulfate-reducing taxa. Diffuse-flow vent samples also contained iron oxidizers and were dominated by sulfur- and nitrogen-cycling taxa, particularly *Thioglobaceae* (SUP05) and *Nitrosopumilaceae.*

After selecting samples for metagenomic sequencing, analyses are focused on genes encoding metabolic enzymes—e.g., *cyc2* for iron oxidation, *dsrAB* for sulfate reduction, and *mcr* for methanogenesis—and integrating their prevalence with environmental energetics by calculating redox reaction favorability for diffuse-flow fluids and modeled fluid mixing in chimneys. To link genetic potential with realized function under *in situ* geochemical constraints, we use metaproteomics to identify proteins expressed at the time of sampling and quantify the concentration of key metabolic enzymes, which directly constrain the magnitude of biogeochemical processes.

This integrated dataset links thermodynamic calculations to microbial metabolic activity, establishing brine-dominated vent microbiomes as distinct contributors to deep-sea biogeochemical cycling.