

## Using strain-resolved metatranscriptomic analysis to highlight active biogeochemical processes in the subsurface

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Metatranscriptome data represent the *realized* metabolic potential of microbial communities. During the first phase of the Berkeley Lab Subsurface Biogeochemistry Genomes-to-Watershed SFA (SFA 2.0), strain-resolved metatranscriptome data were used to leverage metagenome data to highlight the most active metabolic pathways under conditions of interest at the Rifle (CO) site. Here, we summarize significant and often unexpected metatranscriptomic findings made during two Rifle studies: (1) a nitrate perturbation field experiment that explored subsurface N cycling and (2) a microcosm experiment revealing active metabolic pathways in naturally reduced zones (NRZ), which are biogeochemical hot spots at Rifle. Overall, these data provided key insights into biogeochemical cycling in the Rifle subsurface, including the important role of chemolithoautotrophy, and have been used to inform GEWaSC (Genome-Enabled Watershed Simulation Capability) modeling efforts for SFA 2.0.

In a two-month study of nitrate amendment to the perennially suboxic Rifle aquifer, strain-resolved metatranscriptomic analysis revealed pervasive and diverse chemolithoautotrophic bacterial activity relevant to C, S, N, and Fe cycling (Jewell et al. 2016, *ISME J*). Prior to nitrate injection, anaerobic ammonia-oxidizing (anammox) bacteria accounted for 16% of overall microbial community gene expression, whereas during the nitrate injection, two other groups of chemolithoautotrophic bacteria collectively accounted for 80% of the metatranscriptome: (1) members of the Fe(II)-oxidizing Gallionellaceae family and (2) strains of the S-oxidizing species, *Sulfurimonas denitrificans*. Notably, the proportion of the metatranscriptome accounted for by these three groups was considerably greater than the proportion of the metagenome coverage that they represented. Transcriptional analysis revealed some unexpected metabolic couplings, in particular, putative nitrate-dependent Fe(II) and S oxidation among nominally microaerophilic Gallionellaceae strains, including expression of periplasmic (NapAB) and membrane-bound (NarGHI) nitrate reductases. The three most active groups of chemolithoautotrophic bacteria in this study had overlapping metabolisms that allowed them to occupy different yet related metabolic niches throughout the study.

In a 3-week study of anaerobic microcosms inoculated with Rifle NRZ sediments as the sole source of carbon and microbes, metatranscriptomic data elucidated active metabolic pathways in these biogeochemical hotspots. Successions in biogeochemical activity and microbial community composition were observed throughout the experiment. For example, putative members of the Chloroflexi and chemolithoautotrophic *Sulfuricurvum* taxa were initially active but decreased markedly over time. In contrast, putative members of the *Hydrogenophaga* and *Dechloromonas* genera emerged to be among the most active strains during the study (with one *Hydrogenophaga* strain accounting for up to ~30% of the metatranscriptome). Among the most surprising findings were the following: (1) a relatively small number of bacteria accounted for a substantial proportion of sample metatranscriptomes, (2) active denitrification by *Hydrogenophaga* and *Dechloromonas* strains occurred even though nitrate concentrations were low ( $\leq 120 \mu\text{M}$ ), and (3) the most dominant *Hydrogenophaga* strain catalyzed chemolithoautotrophic activity (S and H<sub>2</sub> oxidation) as well as heterotrophic activity.