

## Metatranscriptomic evidence of diverse chemolithoautotrophy in the Rifle (CO) subsurface relevant to C, S, N, and Fe cycling

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The Genomes-to-Watershed Scientific Focus Area (SFA) 2.0 aims to develop a predictive understanding of how climate or land-use induced changes can affect overall watershed biogeochemical functioning. At the Rifle (CO) study site, this scope includes influx of dissolved oxygen or nitrate into a perennially suboxic/anoxic aquifer containing a large reservoir of reduced Fe- and S-containing compounds. The role of the "Metabolic Potential" component of SFA 2.0 is to characterize prevalent metabolic pathways in subsurface microbes and to use these data to inform the next generation of trait-based microbial models (GEWaSC).

Although there is only a limited understanding of the chemolithoautotrophic activity of aquifer microorganisms, such subsurface microbial activity could greatly influence the cycling of elements such as C, S, N, and Fe. Here, we present transcriptional (RNA-Seq) evidence of the occurrence of such chemolithoautotrophic activities in groundwater filter samples from a 2-month experiment in which up to 1.5 mM nitrate (a native electron acceptor) was injected into the Rifle aquifer. Illumina sequence data from rRNA-subtracted cDNA libraries was mapped to metagenome-derived genomes.

Metatranscriptomic analysis revealed pervasive and diverse chemolithoautotrophic bacterial activity in the Rifle subsurface. More specifically, chemolithoautotrophic bacteria with very high-ranking genes in the metatranscriptomes of at least some samples included the following: (1) member(s) of the Fe(II)-oxidizing Gallionellaceae family, (2) anammox bacteria, i.e., planctomycetes capable of anaerobic ammonia oxidation, (3) the S- and Fe(II)-oxidizing *Thiobacillus denitrificans* and (4) member(s) of the facultatively anaerobic S-oxidizing *Sulfuricurvum* genus. High-abundance transcripts belonging to these bacteria had very high sequence identity to known representatives (e.g., a cold-shock protein with 98% sequence identity to that in *Gallionella capsiferriformans*; a hydrazine oxidoreductase gene with 93% identity to that of the anammox planctomycete KSU-1; and an APS reductase with 99% identity to that of a *T. denitrificans* strain that we isolated from the Rifle aquifer). These four groups of chemolithoautotrophs had different temporal patterns of expression throughout the experiment. Surprisingly, anammox transcripts maximized in the pre-release sample (even though nitrite, the electron acceptor for anammox, is not detectable in native groundwater) and Gallionellaceae transcript abundance correlated very strongly with nitrate consumption (even though reported isolates are microaerophiles). A reconstructed Rifle *Gallionella* genome encodes the nitrate reductase *napA* and associated genes, suggesting an explanation for the strong correlation of Gallionellaceae transcripts with nitrate. These data provide key insights into the important role of chemolithoautotrophy in the Rifle subsurface and are being used to inform modeling efforts.