Chemolithoautotrophy in the Rifle subsurface relevant to C, S, and Fe cycling

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The Sustainable Systems Scientific Focus Area (SFA) 2.0 aims to develop a predictive understanding of how climate-induced changes in hydrology and vegetation can affect overall watershed biogeochemical functioning. At the Rifle (CO) study site, this scope includes changes in water-table elevation due to reduced precipitation and recharge, and the resulting influx of dissolved oxygen (DO) 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 microbial communities and to use these data to inform the project's modeling effort (GEWaSC or Genome-Enabled Watershed Simulation Capability).

Although chemolitho(auto)trophic metabolism has not been extensively investigated in the subsurface several lines of evidence from early data in SFA 2.0 indicate that it can play a significant role in Rifle subsurface biogeochemistry. For example phylogenetic (16S rRNA) analyses of planktonic biomass collected during a DO release/perturbation experiment revealed the emergence of bacterial taxa known for their chemolithotrophic lifestyles [specifically Fe(II) and/or S oxidation] including Sulfuricurvum spp. (up to ~65% relative abundance) Thiobacillus denitrificans (up to ~25%) and Gallionella spp. (up to ~25%). In an analogous nitrate-release experiment Fe(II)-oxidizing Gallionella spp. were dominant in groundwater samples (14-50% throughout the study). Metagenome and metatranscriptome sequencing of DO and nitrate release samples is underway. Further evidence of chemolithoautotrophic metabolism in the Rifle subsurface came from shotgun metaproteomic analysis of background groundwater samples. Notably there was strong evidence for the activity of members of the order Gallionellales. Within each sample peptides matching more than 250 Gallionellales proteins were detected including RubisCO (for autotrophy). These findings are supported by metagenomic reconstructions from groundwater communities which revealed a broad diversity and abundance of RubisCO sequences from Archaea and Bacteria including form II RubisCO sequences most closely related to Gallionella and Thiobacillus spp. sequences. Based on results of the DO perturbation study and a recent non-SFA metatranscriptomic study by Methe and co-workers indicating the possible importance of the obligately chemolithoautotrophic S- and Fe(II)-oxidizing bacterium Thiobacillus denitrificans in the Rifle subsurface we isolated and sequenced two strains of T. denitrificans from Rifle groundwater. Comparisons of 46 selected genes associated with S oxidation CO_2 fixation (form I and II RubisCO) denitrification H2 oxidation and U(IV) oxidation in these Rifle strains relative to a well-characterized soil-derived strain (ATCC 25259) revealed a very high level of synteny and protein sequence identity (mean =97%).