ABSTRACT: The subsurface is a massive reservoir of carbon compounds and hosts a large fraction of the microbial life on earth. Despite this, relatively little is known about sediment communities and their roles in carbon, nitrogen, and hydrogen cycling in the subsurface. We metagenomically sequenced unstimulated (background) sediment samples, as well as acetate stimulated sediment and planktonic communities from Rifle, CO to document microbial composition and metabolic potential. Background aquifer sediments are highly diverse; no single organism constitutes more than 1% of the community. Many of the lineages in sediment are novel, at least fifteen at the phylum level. We reconstructed a complete genome from the dominant background sediment organism, RBG-1, a representative of a previously undescribed bacterial phylum. This organism is metabolically versatile, with a large complement of respiratory machinery that likely enables dominance in sediments subjected to fluctuating redox conditions. Chloroflexi are surprisingly diverse and relatively abundant in background sediments. We reconstructed draft genomes for genomically unsampled members of the Chloroflexi, some of which are capable of carbon fixation and other biogeochemical processes. Archaea are also relatively abundant in background sediment. Among the archaea genomically characterized, we recovered a few Thaumarchaeota, and a vast clade of novel Halobacterales.

We documented very different sediment-associated and planktonic communities, both with and without acetate amendment. From acetate-stimulated sediment, we recovered nearly 100 high quality complete and draft genomes and reconstructed organism abundance patterns within the geochemical context. The results indicate the importance of Bacteroidetes, Betaproteobacteria, and bacteria from phyla lacking (e.g., SR1, OP11, OD1, WWE3) or with very few (e.g., Ignavibacteria, Elusimicrobia) cultivated representatives. Metagenomic sequencing of acetate-stimulated groundwater filtered through a 0.2 µm filter revealed the predominance of bacteria from OP11, OD1, and WWE3 on the 0.1 µm filter. Cryo-TEM characterization demonstrated cell sizes below the theoretical minimum size for life. We also reconstructed over 1,000 complete phage genomes. In a majority of cases phage can be linked to their hosts via CRISPR spacer targeting. The planktonic community composition shifts during the biostimulation process. Notably, genomes were reconstructed for TM6 lineage bacteria from samples on the 0.2 µm filter were collected only late in biostimulation. From the same experiment, methanogens were notable by their near-absence, despite evidence for methane production.