

Temporal Changes in Microbial Metagenomic Profiles after Hydraulic Fracturing

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Energy extraction using horizontal drilling and hydraulic fracturing technologies in deep hydrocarbon-bearing shales significantly alters biogeochemical conditions and microbial ecological function at depth. We tracked changes in microbial community dynamics and functional potential via genome reconstruction over an 11-month period in fluids produced from three wells drilled and fractured in the Marcellus shale. A marked shift in 16S rRNA sequences occurred over time; communities shifted from low-salt tolerance, mesophilic aerobic bacteria to dominance by halophilic, thermotolerant anaerobic bacteria and archaea. Genomes from halotolerant microorganisms, including *Halolactibacillus*, *Vibrio*, *Marinobacter*, *Halanaerobium* and *Halomonas*, encoded the potential for fermentation, hydrocarbon oxidation, and sulfur-cycling metabolisms, while *Arcobacter* contained potential for chemoautotrophic sulfide oxidation metabolism. Relative to earlier samples, the 11-month samples were enriched in genes for the acquisition and degradation of aromatic compounds, sulfur, iron, and nitrogen, supporting the importance of these processes in later produced fluids when anaerobic conditions prevail. In addition, enzymes responsible for taurine reduction were detected several months after fracturing, suggesting this metabolism may be important when oxidized sulfur species (e.g. sulfate) are depleted from injected fluids via abiotic reactions or microbial consumption within the shale. Later time points also show the enrichment of microorganisms closely related to *Methanohalophilus* and *Methanolobus*. These methanogens are known to disproportionate methylamines, and the recovery of genes associated with the fermentation of trimethylamine to dimethylamine suggests a pathway for methane production exist within detected genomes. These data provide preliminary insight into the microbial changes that occur from hydraulic fracturing in currently understudied deep shale ecosystems.