

Multi-Year Analysis of the Microbiome of an Aged Subsurface Petroleum Spill

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The 35-year old subsurface petroleum spill site in Bemidji, Minnesota, has been subjected to a multi-year long microbial community analysis. We have used high-throughput DNA sequencing of the 16S rRNA gene to characterize the microbial populations from sediment cores retrieved from the center of the free-phase petroleum plume over a five-year period. Our results show that the free-phase petroleum, which is fluctuating with the seasonal shifting water table, is characterized by methanogenic conditions. The syntrophic bacterial genus *Smithella* and the methanogenic archaeon *Methanoregula* exhibited the highest abundance in the oil plume. The major microbial populations stayed constant over the five-year period; however, the methanogenic microbial community shifted up and down with the fluctuating ground water, following the oil plume. The vadose zone was dominated by iron-reducing bacteria such as *Albidiferax* and methylotrophs such as *Methylocystis*. In addition, the *Peptococcaceae* of the Firmicutes phylum seem to play a major role in hydrocarbon degradation in and above the plume. We also have studied the influence of the microbial hydrocarbon-degradation activities on the geophysical characteristics of the surrounding sediments and observed changes in the magnetic susceptibility. These changes also seem to be related to the fluctuating water table.