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Identifying Active Microbial Communities during In Situ Hydrocarbon Degradation in Cold Soils Using Heavy Phosphate

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Background/Objectives. As part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), novel forms of phosphorus fertilizer are being developed to stimulate residual hydrocarbon degradation in the clay rich, cold, calcareous soils of Western Canada. However, identifying the microbial community interacting with these novel forms of phosphorus is a challenge. Stable isotope probing of microbial communities involves the labelling of microbial DNA by a heavy isotope of C, N or O. This labelled DNA can then be separated from non-labelled DNA using a density gradient centrifugation and then this labelled DNA interrogated using a variety of molecular approaches, e.g., sequencing, quantitative PCR analysis, etc. While methods to label microbes using nitrogen fertilizers have been reported, no methods exist for phosphorus fertilizers. The objective of this project is to develop and validate the use of PO_4^{18} as a means of labelling a wide variety of anaerobic and aerobic microorganisms. Our overall objective, as part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), was to develop new methods to track P fertilizer use in hydrocarbon degrading soils.

Approach/Activities. Tracking active bacterial taxa in microbial communities remains a key undertaking in microbial ecology. Advances in DNA stable isotope probing (SIP) allow investigators to track C, N, and H_2O use in diverse microbial ecosystems. Phosphorus (P) is also a key nutrient in microbial ecosystems, though with the exception for radioactive tracer studies, tracing P in microbial ecosystems has not been possible. Here we introduce a new DNA SIP technique using heavy oxygen-labelled phosphate ($P^{18}O_4$) and assess its effectiveness in pure cultures, biodegrading consortia, and intact field soil ecosystems. First, we compared unlabelled and $P^{18}O_4$-labelled pure bacterial cultures to determine the extent of isopycnic separation of isotopically light and heavy bacteria. Second, we explored two potential applications of $P^{18}O_4$-SIP: 1) tracking benzene degradation in a denitrifying consortia, and 2) separation of inactive and active microbial communities in frost boils in a polar desert. We then used high-throughput amplicon sequencing of 16S rRNA genes to characterize active bacterial taxa in microbial consortia and polar desert soils.

Results/Lessons Learned. We successfully separated active and inactive taxa in both benzene-contaminated and polar desert soils, effectively demonstrating $P^{18}O_4$-DNA SIP as a viable method for identifying bacterial taxa that respond to phosphorus fertilization. In addition to remediation and nutrient cycling studies, $P^{18}O_4$-DNA SIP may be used in combination with C and N SIP to track major nutrient use in microbial ecosystems.