New Quadrupole Mass Spectrometer Method for the Quantification of ^{18}O-Stable Isotope Probing Inorganic and Organic Phosphate Species

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Background/Objectives. Phosphorus-phosphate is an essential macronutrient for microbial development and stimulates the biodegradation of petroleum hydrocarbons. However, phosphorus is often in limiting concentrations within the calcareous clayey soils of Western Canada. Accordingly, phosphate fertilizers such as orthophosphate, tri-ethylphosphate or tri-polyphosphate are added to promote remediation. However, issues arise as anthropogenic phosphate reacts rapidly, either adsorbing to mineral surfaces or precipitating into sparingly available inorganic complexes. These reactions limit phosphate mobilization and assimilation by microbial communities. Therefore, tracking the movement of P fertilizer by quantifying available inorganic phosphate and the amount of microbial organic phosphate will aid in understanding soil dynamics. Using heavy phosphate ($P^{18}O_4$) stable isotope probing (SIP), one can track the compound as it cycles through the soil and microbial populations. A common method in C and N SIP to determine remaining labelled species is with an isotope ratio mass spectrometer (IRMS); however, the instrument is not suitable for phosphate as the compound does not volatilize and co-extractants with phosphate interfere with reactions to create volatile species. Therefore, the objective of this project, as part of the Sustainable In-Situ Remediation Cooperative Alliance (SIRCA), is to develop a quadrupole mass spectrometer method that quantifies both the amount of inorganic and organic phosphate within an ecosystem. We hypothesize that the amount of labelling within DNA and phosphate will provide information into microbial activity in order to collect best representative samples of active microbial communities.

Approach/Activities. Contaminated soil was obtained from three former bulk gasoline and diesel fuel transfer stations, in Saskatchewan, Canada. Duplicates cores from a Phase II assessment were used to construct anaerobic microcosms. Microcosms contained 10 grams of soil and filled with one of three treatments: (i) ^{18}O labelled orthophosphate or (ii) unlabelled phosphate in a bio-stimulatory solution and (iii) Milli-Q control. Following a time-series experiment, microcosms are destructed and samples are collected for BTEX/F1, available P and DNA. DNA samples are digested using enzymes to their respective 3'-deoxynucleoside 5'-monophosphates (dNMP). A QTRAP® 4000 HPLC/MS/MS method for identification of both labelled and unlabelled available P and dNMP is used. The DNA samples are separated using density gradient centrifugation before sequencing via high-throughput amplicon sequencing of 16S rRNA genes.

Results/Lessons Learned. The quadrupole mass spectrometer methods are developed for both ^{18}O-labelled and unlabeled available phosphate and dNMP, validating the instrument as viable for identifying the bacterial taxa response to P fertilization. Results from inorganic P quantification show a decrease for both the labelled and unlabelled orthophosphate microcosms during the time series. The largest reduction in available orthophosphate concentration follows microcosm construction. Control microcosms had low ^{16}O-orthophosphate concentrations whereas no ^{18}O-orthophosphate was measured outside of labelled microcosms. Density gradient centrifugations using cesium chloride gradients for microbial community analysis are in the process and results will be presented at the conference.