Measurement of Isotope Assimilation Rates into Microbial DNA Through Quantitative Stable Isotope Probing with Internal Standards.

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Project Goals: This project aims to develop and apply new methods to understand the ecology of soil microorganisms using stable isotope tracers and genomics. This new suite of techniques will investigate and describe the microbial ecology of nutrient cycling in soil environments as microorganisms grow and die. The work will focus on particular soil microorganisms, bacteria and fungi, that make up the majority of life in soil, and which are responsible for most of the nutrient transformations in soil that are vital to ecosystems, and to people. This project will also evaluate how soil microorganisms and the nutrient cycling processes they catalyze are sensitive to shifts in temperature, a major driver of biological processes.

In DNA-based quantitative Stable Isotope Probing (qSIP) a tracer enriched in heavy stable isotopes is added to a microbial community. Microbes that assimilate the tracer and replicate their genome incorporate the heavy isotopes into their DNA which can be separated from DNA with natural abundance levels of isotopes along a cesium chloride gradient generated in an ultracentrifuge. By analyzing the DNA in each of the 20-30 fractions taken from the gradient it is feasible to calculate the atom percent enrichment of each bacterial taxon's DNA. However, it has proven challenging to compare qSIP results among different experiments and laboratories. This may be because different types of tracers are used or because the equipment used for qSIP is not the same in all labs. We propose that using internal standards in qSIP will improve comparisons, decrease variance within a qSIP experiment and the amount of labor required for qSIP analysis. We constructed 2 different plasmids, approximately 9 kb in size, which contain bacterial 16S V4 primer sites commonly used to characterize a fragment of the bacterial 16S rRNA gene via Illumina based amplicon sequencing. The sequence in between the primer sites was taken upstream from the E. coli 16S rRNA gene and is not highly homologous to known 16S rRNA gene sequences, so that it is possible to distinguish between the internal standards and the 16S rRNA gene sequences derived from the microbial community. We used one of the plasmids as a template in PCR with nucleotides that contained natural abundance levels of ^{13}C and ^{15}N while the other plasmid served as a template in PCR with nucleotides that contained 98 atom% ^{13}C and ^{15}N. As a result the PCR product from one plasmid was approximately 26.5 neutrons heavier than the other PCR product. This resulted in a 0.05 g/mL difference in density as measured through isopycnic centrifugation on a cesium chloride gradient. We will present a strategy for

standardizing the difference in density between the two standards so that DNA-qSIP results from different laboratories and experiments can be compared.

This work was supported by the US Department of Energy, Program in Genomic Sciences, Award Numbers: DE-SC0016207 and DE-SC0020172. Work at LLNL was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.