Quantitative Stable Isotope Probing to Measure Microbial Growth and Activity

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Project Goals: Microorganisms play key roles in soil carbon turnover and stabilization of persistent organic matter via their metabolic activities, cellular biochemistry, and extracellular products. Microbial residues are the primary ingredients in soil organic matter (SOM), a pool critical to Earth's soil health and climate. We hypothesize that microbial cellular-chemistry, functional potential, and ecophysiology fundamentally shape soil carbon persistence, and we are characterizing this via stable isotope probing (SIP) of genome-resolved metagenomes. We focus on soil moisture as a 'master controller' of microbial activity and mortality, since altered precipitation regimes are predicted across the temperate U.S. *Our SFA's ultimate goal is to determine how microbial soil ecophysiology, population dynamics, and microbe-mineral-organic matter interactions regulate the persistence of microbial residues under changing moisture regimes.*

Quantitative stable isotope probing, or qSIP, measures the concentration of rare, stable, isotopes in nucleic acids with taxon-specific resolution, enabling quantitative measurements of population-specific, *in-situ* growth (DNA) and activity (RNA) in complex microbial communities[1]. qSIP is promising for its potential to resolve taxon-specific element fluxes, which is one way to link microbial biodiversity with ecosystem processes[2]. The technique relies on stable isotope tracers whose concentrations are resolved by physical density separation. Here, we highlight recent advances and new frontiers for DNA-qSIP, including new developments in sample processing and mathematical analysis.

Addition of Isotope Tracers

qSIP begins with the addition of a tracer enriched in a rare, stable, isotope, paired with the addition of a non-labeled control, otherwise chemically identical to the tracer. For a qSIP experiment with ^{18}O-labeled water, for example, the tracer sample would receive water at 97 atom % ^{18}O (the highest enrichment typically available), and a paired control sample would receive water with natural abundance ^{18}O composition (approximately 0.2 atom % ^{18}O). While the vast majority of soil experiments have added tracers in laboratory incubations, applications to soils in the field may also be possible.

Sequencing and qPCR

Amplicon sequencing measures taxon-specific relative abundance of the target marker gene in each density fraction. A broad-coverage qPCR technique measures the total (pan-bacterial) copies of the marker gene in every fraction. Together, relative abundance data from sequencing and quantification of the marker gene allow computation of the absolute abundance of each taxon's gene copies in across fractions. We are working to expand the qSIP pipeline to enable analysis of SIP-targeted metagenomes in order to characterize the ecophysiological potential of

in-situ microorganisms. In these experiments, we are using ^{18}O and ^{13}C to target the metagenomes of specific microbial populations: $H_2{}^{18}O$ to target growing, dormant, and dying organisms; and ^{13}C organic matter (exudates, necromass, litter) to target specific heterotrophic populations.

High-throughput SIP (HT-SIP)

Recently, LLNL partnered with the Join Genome Institute to build a semi-automated SIP-metagenomics pipeline that uses robotics to speed processing of SIP samples by an order of magnitude, making well-replicated SIP experiments far more tractable. HT-SIP can be fully integrated with quantitative SIP *(*qSIP*)* coupled to metagenomic or amplicon data.

Modelling Incorporation of the Rare Isotope into DNA

The observed difference in DNA density, calculated as a weighted average, between the labeled and control treatments reflects isotopic assimilation and can be used to quantify the concentration of the rare isotope in DNA. For any given taxon, the molecular weight of DNA in the unlabeled control is estimated from the relationship between GC content and buoyant density. The theoretical heavy weight of fully-labeled DNA can be calculated based on the expected increase in weight from each additional neutron if 100% replacement by the rare isotope is expected. The molecular weight in the labeled treatment is estimated based on the molecular weight of unlabeled DNA and the density shift between treatments due to isotopic incorporation alone. Knowing the background fractional abundance of the rare isotope in DNA, the difference in molecular weight between the labeled and control treatments, relative to the theoretical maximal change in molecular weight, can be used to quantify atom fraction excess of the rare isotope.

References

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