Sensitivity Analysis and Methodological Improvements for Quantitative Stable Isotope Probing

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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.***

Stable Isotope Probing (SIP) is a highly effective method for linking diversity to functionality within naturally-occurring communities. Microbial substrates enriched in a stable isotope are added to a microbial community and organisms that utilize that substrate will present an increase in DNA buoyant density due to the incorporation of the stable isotope into their DNA. Quantitative SIP (qSIP) enhances the conclusions we can draw from SIP by adding statistical methods to determine the atom % enrichment of those organisms using the density shift in their DNA buoyant density, allowing us to estimate their effect on substrate assimilation or *in situ* growth rate.

Although the SIP approach for environmental studies was developed in the early 2000's[1] there has been very little assessment of reproducibility and sensitivity. As SIP becomes more quantitative and statistically robust, and transitions towards the use of genomes from metagenomes rather than marker genes, we can now take advantage of new and well-replicated studies in order to improve the sensitivity and detection limit of the method[2,3].

Using three existing datasets reflecting utilization of different labeled carbon substrates and labeled water in various environments we assessed two key aspects of qSIP variability: (1) using buoyant density to predict the GC content of a genome and (2) the effect of density gradient resolution on detection of density shifts. We confirmed that the correlation between GC content and buoyant density using pure cultures varies by up to 0.004 g/ml, which stems from method limitations. In addition, the mean buoyant density of a genome within a community has a 95% confidence interval of ±0.005 g/ml. We have been unable to improve this sensitivity by automation as opposed to manual processing but feel that it could potentially be improved via

use of an internal standard. While there has long been a presumption that collecting more density fractions might lead to more precise results, our analysis suggests otherwise, and that the ability to detect a density shift remains comparable while decreasing the resolution of gradient fractionation (i.e. fewer fractions) as long as the individual fraction size is < 0.01 g/ml (roughly 9 fractions collected per 5 ml gradient). This is an important consideration, since collecting fewer fractions renders qSIP less labor-intensive as well as less computationally complex, which has important implications for the transition to SIP-metagenomics. Even a fraction size threshold of ~0.024 g/ml (three fractions) was sufficient to quantify ^{13}C enrichment in half of all metagenome-assembled genomes above a conservatively estimated detection limit of 26 atom percent excess.

Our analysis is a first step towards enabling genome-informed SIP-metagenomics, in which instead of amplifying a marker gene from each fraction we are using shotgun sequencing and genomes assembled from metagenomes to detect DNA density shifts of entire genomes. Critically, this approach will enable us to move beyond simply identifying active organisms, and instead will identify their full metabolic potential.

References

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