

Can we measure taxon-specific biochemical efficiency in natural microbial communities?

Bruce A. Hungate^{1,2} (Bruce.Hungate@nau.edu), Paul Dijkstra^{1,2}, Egbert Schwartz^{1,2}, Ember M. Morrissey³, Bram W. Stone¹, Michaela Hayer¹, Steven J. Blazewicz⁴, Xavier Mayali⁴, Peter Weber⁴, Jennifer Pett-Ridge⁴, Kirsten Hofmockel⁵

¹Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ, 86011, USA; ²Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, 86011, USA; ³Department of Biology, West Virginia University, Morgantown, WV, 26506, USA; ⁴Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, 94550, USA; ⁵Environmental and Molecular Sciences Division, Earth Scientist Pacific Northwest National Laboratory, Richland, WA 99352

Project Goals: Short statement of goals. The objectives of the proposed work are to 1) develop a quantitative SIP targeted –omics-based framework to infer taxon-specific rates of growth, mortality, and associated carbon (C) fluxes in soil microbial communities, a framework that will scale from individual microbial taxa to the integrated soil system; 2) develop a new means to quantify taxon-specific C-use and growth efficiency in order to quantify taxon-specific contributions to soil C loss; and 3) apply newly developed quantitative tools to test key hypotheses about responses of the soil microbial community to experimental warming and to latitudinal variation in temperature. This work will push the frontier of –omics enabled techniques by demonstrating their applicability at the ecosystem scale, and by relating taxon-specific isotope assimilation to dissimilatory processes in the C cycle, thereby enabling the identification of organisms especially responsible for soil C loss.

Carbon use efficiency is a key parameter in global biogeochemical models of the carbon cycle, and is usually described as the partitioning of carbon within a cell between allocation to biosynthesis and growth versus allocation to energy production and respiration. Yet, as commonly measured (e.g., by measuring the distribution of ¹³C-tracers between biomass and respiration), carbon use efficiency includes biochemical efficiency, growth efficiency, and biomass turnover (predation, viruses, etc.), creating confusion in the literature about exactly what it is we are measuring (Geyer et al., 2016). Furthermore, carbon use efficiency is almost always considered in aggregate, as a property of the entire microbial community, ignoring potential differences among taxa, despite evidence from pure culture that components of carbon use efficiency vary strongly among taxa, variation sometimes invoked to explain apparent changes in carbon use efficiency observed in nature.

Here, we focus on the use of metabolic flux analysis as a means to measure biochemical efficiency, the instantaneous partitioning between biosynthesis and respiration within the central metabolic network common to the vast majority of life. We chose this focus for two reasons: 1) it provides clarity about the component of carbon use efficiency under investigation, and 2) features of its measurement create the possibility of resolving biochemical efficiency on a taxon-specific basis.

Given the tremendous diversity of microorganisms in evolutionary history and physiological traits, variation in biochemical efficiency among microbial taxa seems very likely. While the evidence is scarce, microbial community composition has frequently been posited as

a potential driver of carbon use efficiency. Biochemical efficiency captures processes that influence growth, survival, and thus fitness, so it should be subject to selective pressure. Ecological strategies may underlie differences in biochemical efficiency. Slow growing organisms, typically present in low nutrient environments, are expected to exhibit high biochemical efficiency, whereas fast growing organisms that are top competitors in high nutrient environments will have lower biochemical efficiency, potentially due to physiological tradeoffs between efficiency and maximum growth rate.

Still, even if it is important, how could we possibly measure it for individual taxa in complex assemblages? The idea is challenging. On the one hand, isotopic tags on the elements assimilated and recovered in nucleic acids provide a way to discern which organisms use what resources at what rates for biomass growth. On the other hand, biochemical efficiency is about the balance of growth and respiration, or dissimilation, where the product, carbon dioxide, cannot be traced back to the organism that produced it.

Metabolic flux analysis can, in principal, be used to develop taxon-specific estimates of biochemical efficiency: using position-specific ^{13}C -labeled substrates such as glucose and pyruvate, monitoring the isotope incorporation into nucleotides and DNA and RNA combines the measurement of metabolic efficiency while retaining information about the organisms that produced them. The atom mapping logic for this approach can be understood from the biochemistry of glucose anabolism and catabolism, in glycolysis, the Krebs cycle, the pentose phosphate pathway, and gluconeogenesis, pathways most important for energy production and biosynthesis, including the biosynthesis of nucleic acids.

Here, we present the model for determining biochemical efficiency of individual bacterial taxa in complex communities, and evaluate the sensitivity with respect to two proposed methods of measuring taxon-specific isotope composition: ChipSIP/NanoSIMS, and quantitative stable isotope probing (qSIP). In this work, the target molecules for the recovery of these isotope tracers are nucleic acids, the very molecules that convey the identities of the organisms. This is critical because it overcomes the problem of assigning responsible taxa to dissimilatory processes. Our analysis indicates that sensitivity with quantitative stable isotope probing is too coarse for this approach, but that NanoSIMS measurements of taxonomically resolved nucleic acid sequences provides sufficient resolution to detect ecologically meaningful changes in biochemical efficiency, suggesting that our proposed technique is a viable way to measure biochemical efficiency for individual taxa growing in a community, a dream of microbial ecologists young and old from around the world.

Reference: Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry* 127, 173-188.

Funding Statement This work was supported by the US Department of Energy, Program in Genomic Sciences, Award Number: DE-SC0016207.