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Using C stable isotopes to infer shifting metabolism in response to variable environmental conditions

Ford Ballantyne (1), Sharon Billings (2), Christoph Lehmeier (3), and Kyungjin Min (2)

(1) Odum School of Ecology, University of Georgia, USA, (2) Ecology and Evolutionary Biology, University of Kansas, USA, (3) Animal and Plant Sciences, University of Sheffield, UK

The flow of carbon (C) from organic matter substrates through microbial biomass and into CO_2 comprises a complex suite of processes. Organic matter compounds are modified by extracellular enzyme activity, potentially taken up by microbes, and can either remain as altered organic compounds in the soil matrix, or are transformed into inorganic C forms, including CO_2. During these transformations, discrimination between ^{12}C and ^{13}C occurs. The net result of all fractionations is what we observe in the $\delta^{13}C$ of respired CO_2. However, our understanding of fractionations associated with soil organic matter (SOM) transformations is far from complete, especially for biologically-mediated transformations. To make proper inference from $\delta^{13}C$ values of respired CO_2, we need a more comprehensive understanding of what governs isotopic fractionation along the path from SOM to CO_2 release. Here, we present equations for ^{12}C and ^{13}C dynamics in a chemostat system, with which C flux data coupled to isotopic ratios can be used to infer the degree of fractionation associated with functionally distinct processes. Using patterns in the fractionation between substrate and biomass and between biomass and respired CO_2 observed for *Pseudomonas fluorescens* in the experimental chemostat system, we argue that a single mechanism cannot be responsible for temperature-induced changes in the flow rates of ^{12}C and ^{13}C from a single substrate, cellobiose, into respired CO_2. We further describe how changing C availability can influence fractionation among C pools and compare predictions to chemostat runs for which C availability varied. Our modeling applied to observed C isotope fluxes strongly suggests that significant discrimination against ^{13}C occurs during cellobiose uptake by *P. fluorescens*, and that apparently smooth changes in specific respiration rates and associated C use efficiency are actually the result of discontinuous shifts in C flow through anabolic and catabolic pathways. Accounting for such isotopic effects is critical for a better interpretation of $\delta^{13}C$ of soil respiration. Finally, we emphasize that performing controlled experiments, such as in chemostats, is critical for identifying and interrogating mechanisms responsible for the genesis of patterns in stable isotopes.