Heavy, Wet, and Stuck in the Mud: Quantifying the Effect of Violating Common Assumptions of the $^{18}$OH$_2$ Method of Measuring Carbon Use Efficiency

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**Project Goals:**

Our goals are 1. to determine how violating assumptions of the $^{18}$OH$_2$ method affect estimates of microbial growth during soil carbon use efficiency measurements; and 2. to quantify the impact of violating these assumptions on conclusions drawn regarding the sensitivity of carbon use efficiency to biotic and abiotic manipulations.

**Abstract:**

Measuring carbon use efficiency (CUE) – or the fraction of carbon taken up by a cell and incorporated into biomass – is essential to understanding the ability of soil microbial communities to retain carbon on short to intermediate time frames. Historically, CUE was measured by tracing the heavy carbon from single $^{13}$C-labeled compounds into biomass and respiration. However, in response to dual critiques of failure to distinguish between the uptake and anabolism of substrates, and failure to assess growth efficiency on realistic substrates, the $^{18}$OH$_2$ incorporation into DNA method was developed. Accurate use of this heavy water method requires making a number of assumptions, but the consequences of violating these assumptions is unknown.

Here we used a combination of a literature search and numerical simulations to evaluate the effect of violating these assumptions on the degree to which CUE is biased. These include heterogeneous microbial growth rates and DNA contents; DNA and microbial biomass carbon extraction efficiencies; and the use of sources other than exogenous water for oxygen in DNA.

We found that estimates of CUE using the $^{18}$OH$_2$ method are sensitive to both DNA and MBC extraction efficiency biases, particularly when the bulk MBC:DNA ratio used for converting DNA growth to carbon growth is not representative of the actively growing community. We will apply our results to our own $^{18}$OH$_2$-CUE data in order to validate and interpret results in light of the method's assumptions - not in spite of them - and encourage other researchers to do the same. Once complete, R scripts will be made publicly available for this purpose.

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