



## **Clarifying the interpretation of carbon use efficiency estimates in soil through methods comparison**

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Accurate estimates of microbial carbon use efficiency (CUE) are required to predict how global change will impact microbially-mediated ecosystem functions such as organic matter formation, decomposition, and C storage. An understanding of the genetic, environmental, and methodological sources of variability in CUE estimates is needed before we can confidently cross-compare published values or apply them in biogeochemical models. Here we compared five common and current methods under three substrate amendment scenarios (0.0, 0.05, and 2.0 mg glucose-C g<sup>-1</sup> soil) to assess how environmental change and choice of method influence the estimation of microbial growth and CUE in a common soil. The microbial response to these treatments was examined using: <sup>13</sup>C and <sup>18</sup>O isotope tracing approaches which directly estimate microbial growth and CUE; calorimetry which infers growth and CUE from heat flux and respiration; metabolic flux analysis which determines CUE from the balance between biosynthesis and respiration using position-specific CO<sub>2</sub> production of added substrates; and stoichiometric modeling which derives CUE from elemental ratios of biomass, substrate, and exoenzyme activity. The CUE estimates obtained were dependent on glucose treatment and choice of method, ranging from ~0.35 (substrate-independent methods of <sup>18</sup>O and stoichiometric modeling) to ~0.70 (<sup>13</sup>C method, metabolic flux analysis) without labile C addition. Because methods incorporate some non-overlapping aspects of microbial metabolism, direct comparison of CUE estimates obtained from multiple approaches may not be tenable. Instead, these methods should be considered complimentary rather than interchangeable. Simultaneous application could generate more integrated perspectives on microbial functioning than any single method can provide alone.