

# Multi-omics insights on disproportionate carbon dioxide efflux due to unshared metabolic paths of assimilated organic carbons in soil bacteria

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Partitioning of assimilated organic carbons into biomass relative to carbon dioxide (CO<sub>2</sub>) efflux, termed carbon use efficiency (CUE), is widely used to characterize organic carbon cycling by soil microorganisms. Recent reports challenge earlier proposals of CUE dependence on oxidation state of the substrate carbon and highlight instead microbial metabolic strategies. Still unknown are the metabolic mechanisms underlying variable CUE observed for different organic substrates. Here, using a multi-omics approach, we investigate these mechanisms in *Pseudomonas putida* mt-2, a metabolically versatile soil bacterium, processing a mixture of plant matter-derived substrates. Our <sup>13</sup>C-metabolomics data revealed intracellular co-assimilation of cellulose-derived sugar and lignin-related aromatic substrates. Subsequent <sup>13</sup>C-fluxomics analysis determined disproportionate investment of the sugar towards CO<sub>2</sub> efflux relative to an aromatic substrate due to the specific paths of the different substrates into different metabolic pathways. Proteomics analysis was used to evaluate the extent of enzyme-level regulation on substrate uptake, CO<sub>2</sub>-producing, and biomass-synthesizing metabolic reactions. Using metabolic flux analysis, we further demonstrate that such metabolic partitioning as shown here can explain substrate-dependent CUEs reported for several soil bacteria.

