

Understanding Soil Metabolism from Carbon Release And Carbon Incorporation from Position-Specific ¹³C-Labeled Substrates

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Project Goals

- To better understand microbial population dynamics and its relationship to microbial energy metabolism and C cycling
- To link processes of individual microbial species to whole system C and element cycling and their responses to temperature
- To discover global patterns in microbial population dynamics across ecosystems

Abstract

Our knowledge of microbial biochemistry is mostly obtained in pure culture laboratory experiments, but little is known about the regulation of cell metabolism in real, intact, and complex microbial communities. An improved understanding of metabolic processes under realistic conditions of temperature, moisture and substrate quality and availability has important implications for our ability to predict how soil carbon (C) cycling processes respond to changes in the environment.

We have developed a method to measure the microbial community C use efficiency (CUE) by measuring CO₂ production from individual C-atoms in position-specific ¹³C-labeled compounds and, based on this, model the flux distribution over the central C metabolic network pathways and determine C use efficiency. However, more information on microbial physiology and ecology can be obtained by additionally studying the position-specific ¹³C-incorporation into microbial products, such as lipids, DNA, amino acids, or the entire microbial biomass. Here we report on the ¹³C-incorporation from position-specific labeled glucose and pyruvate into entire microbial cells using nano-SIMS and compare it to measurements of ¹³CO₂ release.

We incubated soil from a meadow in the mixed conifer forest near Flagstaff, Arizona at 25 °C for 10 days. At the end of the incubation, we added glucose (1-¹³C and U-¹³C) and pyruvate (1-

^{13}C and 2,3- ^{13}C) isotopomers in parallel incubations. We measured $^{13}\text{CO}_2$ production 0, 20, 40 and 60 minutes after addition of the substrate. Immediately afterwards, we stored the soil at 5°C until fixing of microbial cells with formaldehyde. Cells in the soil were extracted using the Nicodenz method, and captured on $0.2\ \mu\text{m}$ polycarbonate membranes. Isotope incorporation of cells on the filter was then quantified with NanoSIMS analysis. Only a small fraction of the cells exhibited significant isotope incorporation from the short exposure to low concentrations of substrate. The largest percentage of labeled cells, as well as the highest labeling on a per-cell basis, was measured in the treatment with U- ^{13}C glucose. Incubations with 1- ^{13}C and 2,3- ^{13}C pyruvate exhibited intermediate levels of isotope incorporation, while no incorporation was detected in the 1- ^{13}C glucose incubation. These results qualitatively correspond to the results from simultaneous $^{13}\text{CO}_2$ measurements and modeling that showed that much of atom 1 of glucose and atom 1 of pyruvate was released as CO_2 in the first step of the pentose phosphate pathway and by pyruvate dehydrogenase respectively. These results suggest that it is possible to quantitatively link C losses as CO_2 and C incorporation into microbial biomass to further detail microbial energy and carbon metabolism.