

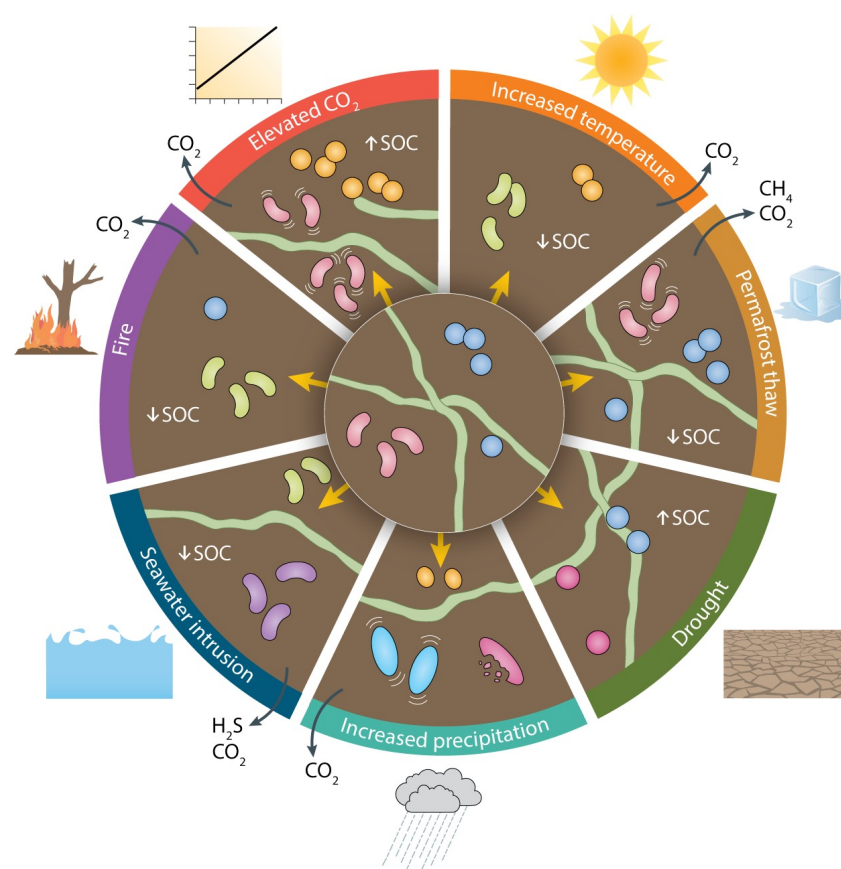


Using ^{13}C to Unravel the Molecular Mechanisms of Microbiome Response to Rewetting in Soil

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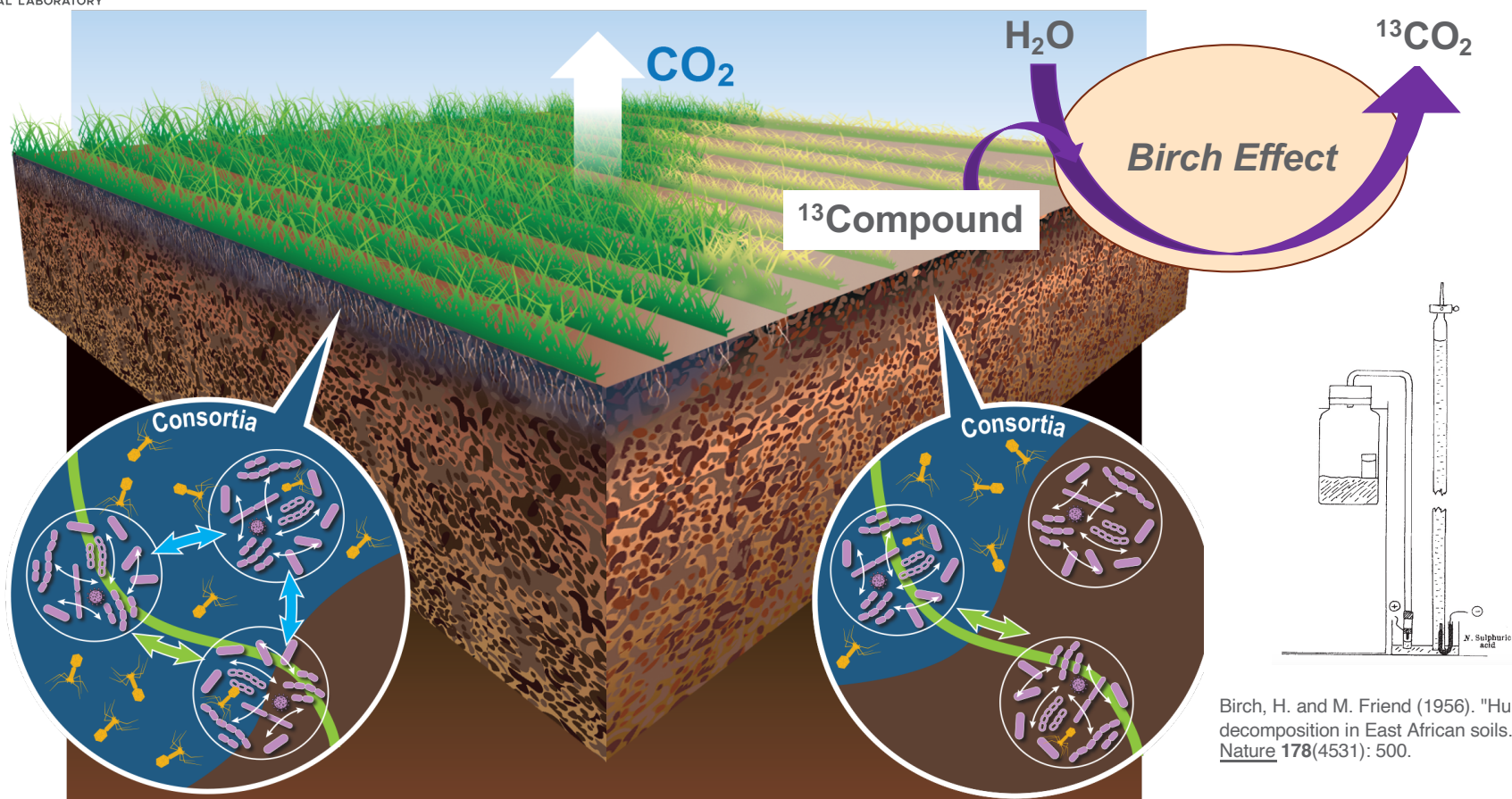
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Jansson & Hofmockel. Nature Microbiology Reviews. 2019

How will changes in soil moisture impact the metaphenome of grassland soils?



Birch, H. and M. Friend (1956). "Humus decomposition in East African soils." *Nature* 178(4531): 500.

What microbial mechanisms encompass the nutrient and carbon pulses?

Nutrient and carbon pulses are due to a hypo-osmotic stress response of the soil microbial community after sudden changes in soil water status

- 1. Microbe rapidly metabolize osmotic biomolecules to reduce their intracellular concentration as well as provide energy for cell growth and division**
- 2. Microbes dump osmotic biomolecules into the extracellular space to mitigate the rapid flow of water into the cell and avoid bursting**

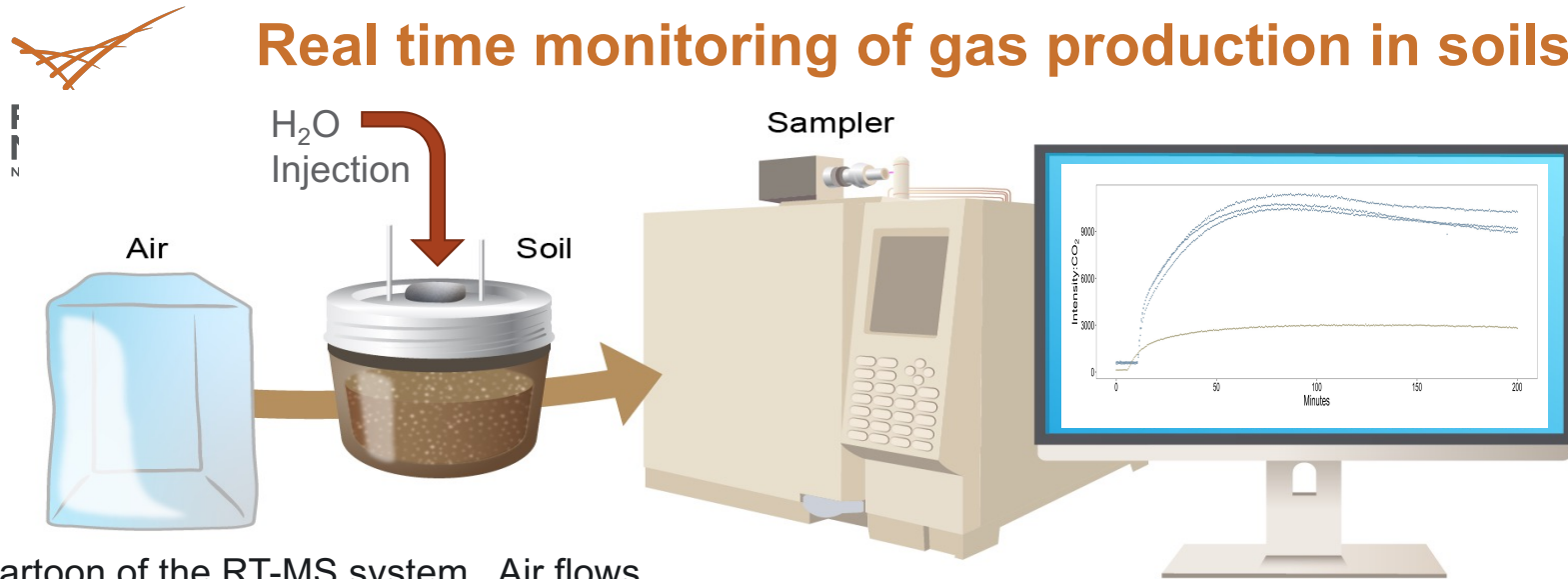


Field site

- Marginal soil
- USDA Texture- Sandy Loam
 - 2.5% Calcium Carbonate
 - Texture: 50% Texture, 5% Clay, 45% Silt
- Seasonal Temperatures
 - Average: 53.7F
 - Average high: 82-90 in summer
 - Average low: 28-33F in the winter
- Average Rainfall: 9.03 in
- Average Snowfall: 3 in

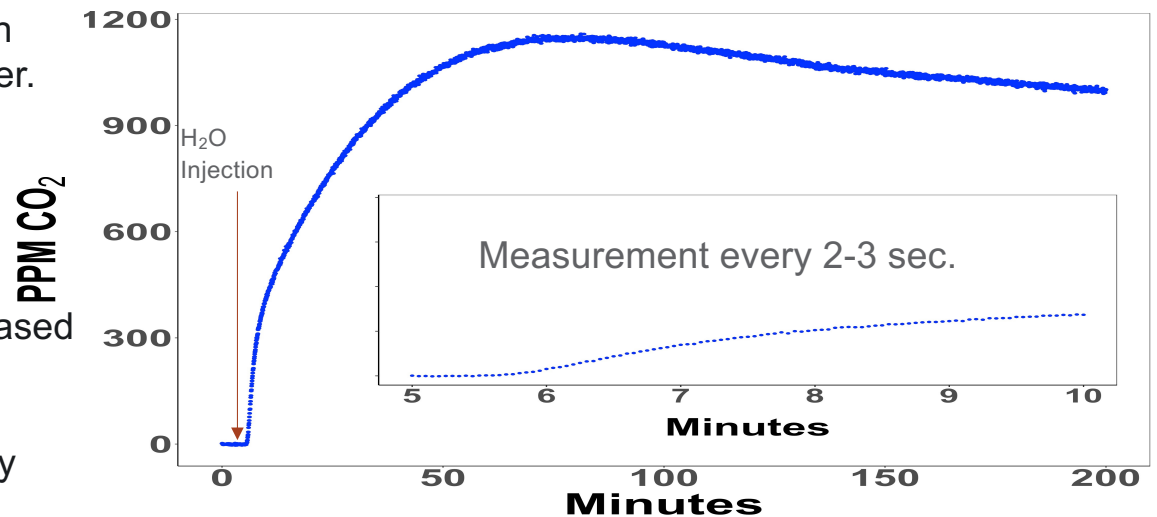


Real time monitoring of gas production in soils

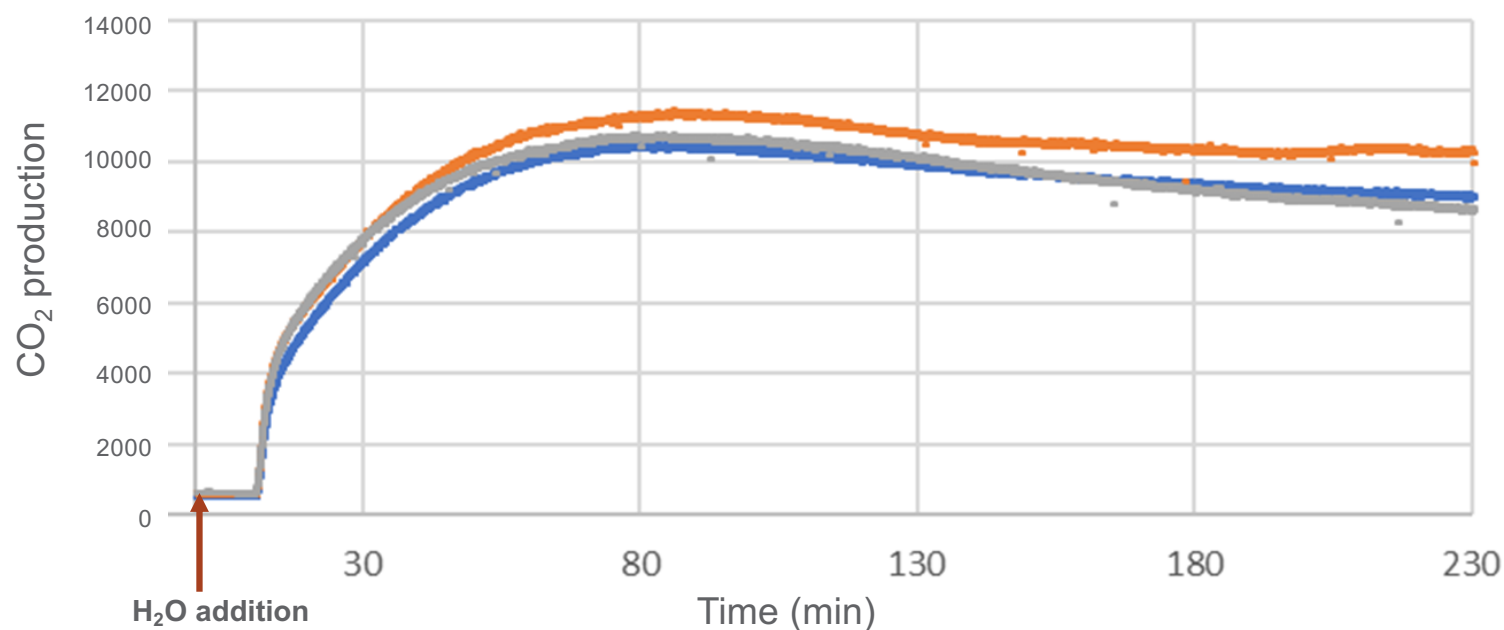


Cartoon of the RT-MS system. Air flows from the air source, through the reaction chamber and into the mass spectrometer. Data is collected in real time.

Output of the RT-MS system. After injection of the H_2O into the soil, increased production of CO_2 is observed. Measurements are taken every 2 to 3 seconds for a high temporal granularity

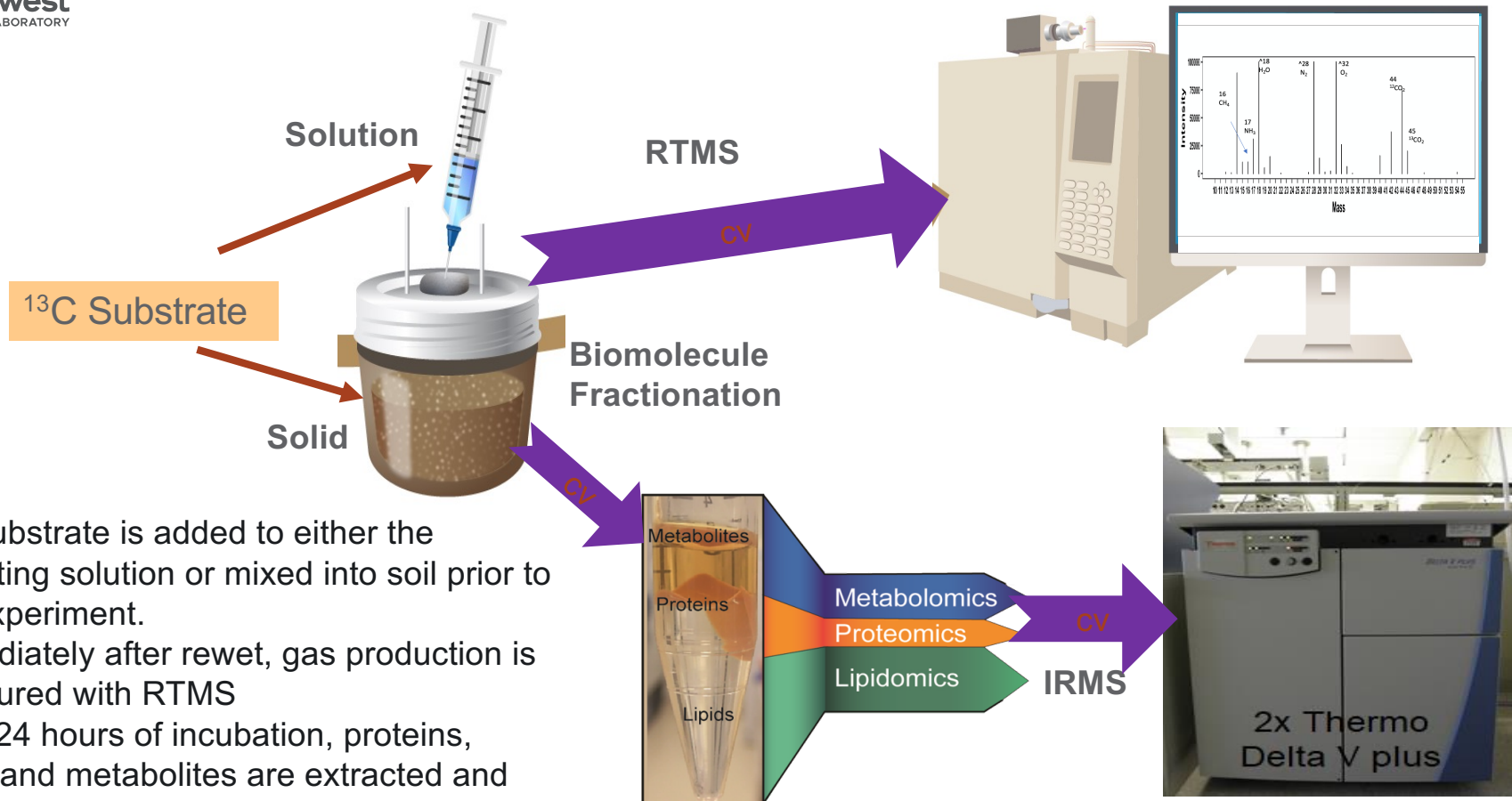


Initial measurements of CO₂ production upon rewetting is reproducible



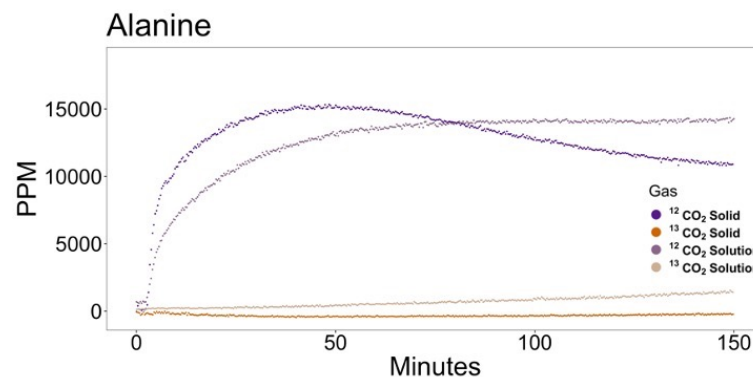
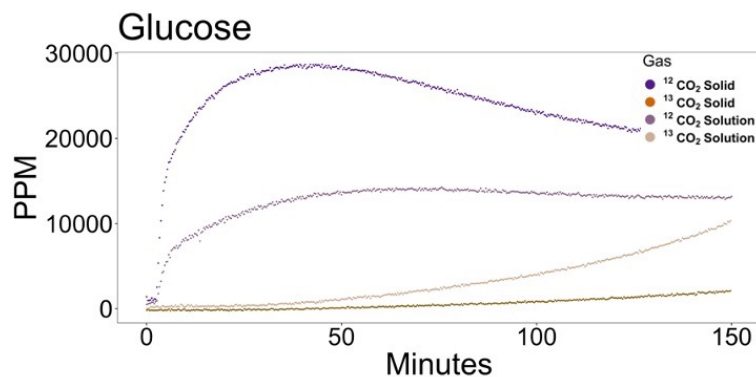
Overlay of three post wetting CO₂ production traces illustrates the reproducibility of the measurement (top). Linear relationship of CO₂ produced with the amount of H₂O added to 50g of soil illustrates that the method is quantitative.

Methods for analyzing substrate fate in desiccated soils after rewetting



- ^{13}C substrate is added to either the rewetting solution or mixed into soil prior to the experiment.
- Immediately after rewet, gas production is measured with RTMS
- After 24 hours of incubation, proteins, lipids and metabolites are extracted and analyzed by IRMS

RTMS data of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ production after rewet



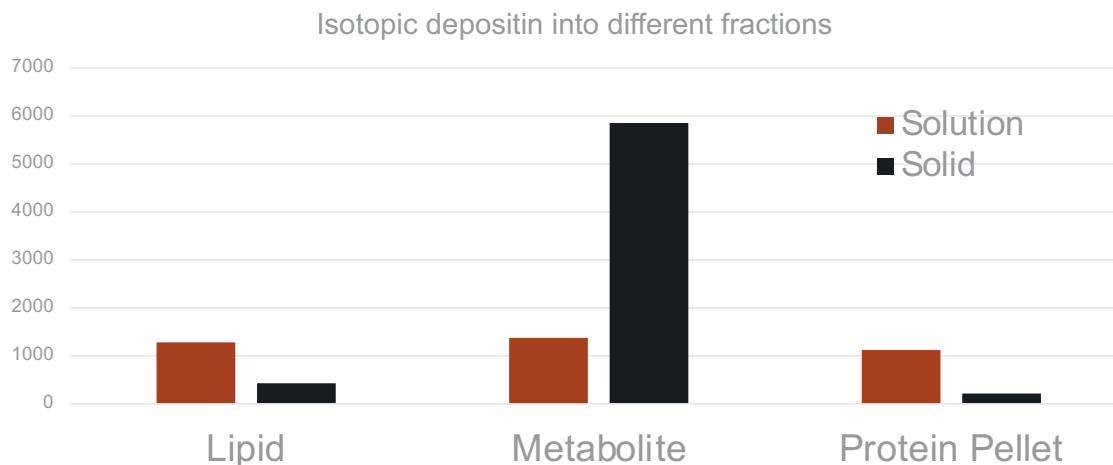
Glucose:

- $^{12}\text{CO}_2$ production is rapid and at a high rate immediately after rewetting regardless of whether the glucose is delivered in solid or liquid form
- $^{13}\text{CO}_2$ production is more rapid if the glucose is in liquid form than if mixed in solid form in the soil

Alanine

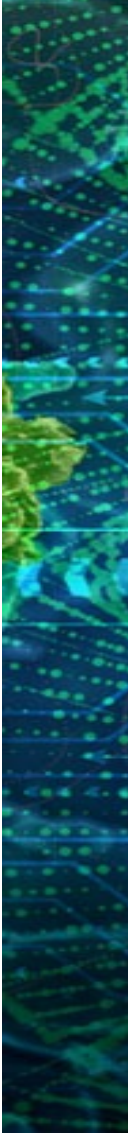
- $^{12}\text{CO}_2$ production is rapid and at a high rate immediately after rewetting regardless of whether the alanine is delivered in solid or liquid form
- $^{13}\text{CO}_2$ production is only slightly more rapid if the alanine is in liquid form than if mixed in solid form in the soil

IRMS data of ^{13}C incorporation into biomolecules after Glucose amendment



Glucose delivered to the soil in the solution phase is partitioned between all fractions equally while glucose mixed in the soil remained in the metabolite fraction

Represents approaches that showcase advanced stable isotope analyses to determine molecular provenance in ecosystems



Summary

- Drought plays a role in terrestrial carbon cycling
 - Developing novel isotope-based techniques to map the interdependence of carbon and nutrient cycling in soils
 - ^{13}C labeled substrates serve as a tracer for nutrient fluxes in desiccated soil microbiomes after rewetting
 - Integration of novel Real Time Mass Spectrometry (RTMS) system, isotope ratio mass spectrometry (IRMS)
 - Resident, cellularly associated carbon is metabolized immediately after rewet
 - Liquid phase glucose is accessed by the cells before dried and solid phase substrate
 - Liquid phase glucose is equally partitioned between proteins, lipids and metabolites after 24 hours subsequent to rewet, however, much of the solid phase glucose remains in the metabolite fraction potentially indicating that it was not utilized by the microbes.
- For more information contact Mary Lipton at mary.lipton@pnnl.gov