Quantitative Analysis of the Fate of Microbial Residues in Biofuel Soils

Kirsten S. Hofmockel,[1*] Sheryl L. Bell[1], and Christopher P. Kasanke[1]
[*] (kirsten.hofmockel@pnnl.gov)
Institutions: [1]Environmental and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA

Project Goals: The overall goal of this project is to test if plant-microbe interactions are limited to influencing the rate of C accrual, while mineralogy regulates the sink capacity of biofuel cropping systems. To accomplish this goal, we are (1) identifying the microbial functions and biopolymers of microbial necromass that contribute to soil C accumulation under controlled conditions, (2) characterizing microbial necromass accumulation in response to crop selection and edaphic factors in situ and (3) generating long-term, cross-site data that can be used to model C cycling in bioenergy cropping systems under different soil conditions.

Molecular-scale biology underpins organismal, community and ecosystem dynamics; yet linking molecular-scale biology to ecosystem outcomes remains a major frontier in deploying sustainable bioenergy feedstock production systems. The overarching objective of this research is to identify how crop selection and soil properties influence the accumulation and stabilization of microbial residues in soil, contributing to soil fertility, reduced CO_2 emissions, and enhanced long-term soil carbon (C) storage. Evidence increasingly supports the concept that most soil organic matter (SOM) is of microbial origin. However, identifying which microbial residues contribute to SOM and what controls their accumulation remains an active area of investigation. Crop selection and soil texture influence the physicochemical attributes of the soil, which structure microbial communities and can influence SOM formation, cycling and long-term storage. To examine the relative importance of biological and geochemical processes, we used a long-term soil incubation and ^{13}C-labeling to test the hypothesis that *microbial community composition and biomass influences microbial derived SOM pool sizes, chemical composition, and ultimately microbial derived SOM accumulation.* By integrating lab to field studies, we aim to identify the molecules and biochemical pathways that contribute to long-term organic matter stabilization in bioenergy soils.

To test this hypothesis, we conducted a long-term lab incubation of soils derived from switchgrass (*Panicum virgatum*) and corn (*Zea mays* L.) plots from the DOE the Great Lakes Bioenergy Research Center (GLBRC) Intensive Biofuel Cropping System Experiments in MI and WI, USA. Soils were collected from silty loams from the Arlington Agricultural Research Station (AARS) in WI and sandy loams from the Kellogg Biological Station (KBS) in MI. Plant-free soil incubations were amended with ^{13}C-labeled glucose, which was rapidly incorporated into microbial biomass; microbial membrane lipids had measurable ^{13}C enrichment after just 24 hours of incubation. The turnover time of microbial cells in soil is estimated to range from 30-50 days; therefore, we harvested the incubation after 2 months to quantify the production and

accumulation of microbial residues. By quantifying ^{13}C accumulation in microbial biomarkers, we can test crop and soil minerology/texture effects on pools of microbial residues.

After 2 months, the incubations were harvested and extracted to measure C concentration and ^{13}C incorporation into microbially derived metabolite, protein and lipid pools as well as unextractable residues associated with solids. We leveraged the sensitivity of PLFA-SIP to quantify the mass and composition of the soil community; microbial DNA was not enriched enough for community profiling DNA-SIP (~1.6 atom % ^{13}C). Microbial biomass was greater in switchgrass compared to corn soils and in the silty compared to sandy soils ($p< 0.001$ for both). Cropping system strongly influenced the profile of the enriched microbial community ($R^2 = 0.33$, $p = 0.002$) and ^{13}C accumulation in microbial biomass ($p < 0.01$). Soils from sandy corn plots had the lowest ^{13}C fungal, G+, and G- bacterial biomass ($p < 0.05$ all). Saprotrophic fungal lipids were the most enriched in ^{13}C, but due to their greater overall biomass, G- bacteria accumulated the most total ^{13}C. Cropping system had the opposite effect on microbial protein, with greater ^{13}C protein in corn compared to switchgrass soils at both sites ($R^2 = 0.82$, $p < 0.001$). The composition of the lipidomes (intact lipids profiles) were markedly different between treatments, with cropping system ($R^2 = 0.28$, $p < 0.001$) having a stronger influence than soil type ($R^2 = 0.11$, $p < 0.001$). Cropping system strongly influenced microbial community and lipidome composition, community biomass and microbial protein concentrations, yet this did not scale to influence overall ^{13}C retention measured in whole soil. Instead, site differences dominated ^{13}C retention, with greater microbial derived ^{13}C in silty (58.7 ± 1.2 μg ^{13}C g soil^{-1}) than sandy soils (49.1 ± 1.8 μg ^{13}C g soil^{-1}). These site effects were largely driven by the small but highly enriched metabolite pool. Although the metabolite pool was the lowest mass pool (< 0.04%) and is generally considered ephemeral, ~13% of the residual ^{13}C accumulated in the metabolite pool after 2 months. Preliminary results suggest the labeled metabolites include a persistent pool of highly ^{13}C enriched trehalose. Our findings reveal that soil texture strongly influences retention of microbial C and cropping systems may enhance organic carbon accumulating pathways indirectly through microbial community selection. Differences in community structure appear to influence the overall metabolic signature, resulting in highly concentrated pools of metabolites and proteins that differ between crop-associated communities. Ongoing efforts include confirming community composition differences using amplicon sequencing, assessing metabolite chemistry differences, and further quantify highly labeled compounds within each pool, in order to understand the mechanisms driving differences in microbial residue retention.

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