Molecular Composition of Field Derived Microbial Necromass

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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.

Abstract text. Crop selection and soil texture influence the physicochemical attributes of the soil, which structures microbial communities and influences soil organic matter formation, cycling and long-term storage. At the molecular scale, microbial metabolites and necromass alter the soil environment, which creates feedbacks that influence ecosystem functions, including soil organic matter accumulation. Yet the generalizable mechanisms regulating the accrual and long term stabilization of soil organic matter are still unclear. By integrating lab to field studies we aim to identify the molecules, organisms and metabolic pathways that control the formation of molecules that contribute to long term organic matter stabilization in bioenergy soils.

Microbial residues accumulate in soil and thus are an important contributor to the formation of soil organic matter. Plant-derived inputs undergo microbial decomposition, some of the resulting organic residues are incorporated into microbial biomass, and a significant proportion of soil organic matter is attributed to the resulting microbially derived residues, or necromass. This includes biomass residues (lipids, proteins, amino sugars) and microbial exudates (enzymes, exopolysaccharides, lipids, glycoproteins). Yet little empirical evidence is available to support this conceptual model and inform management decisions that aim to amplify biological processes that enhance soil organic matter formation and persistence. To address this knowledge gap, we investigated the chemical signatures of microbial biomass separated into intracellular and extracellular aqueous, and water insoluble fraction from seven taxonomically different cultures. The cultures were enriched from switchgrass (*Panicum virgatum*) plots from the DOE the Great Lakes Bioenergy Research Center (GLBRC) Intensive Biofuel Cropping System Experiments in MI and WI, USA. Microbial communities were selectively cultured from sandy loams of the Kellogg Biological Station (KBS) in MI, and silty loams from the Arlington Agricultural Research Station (AARS) in WI. Microbial communities cultivated from the MI and WI field trials were used to test the hypothesis that microbial communities differ in morphology and biochemistry and therefore in their potential contributions microbial necromass.

Media was chosen to represent a diversity of microbial morphology and taxonomy by broadly enriching for the following groups: (1) Gram+ bacteria, (2) G+ *Bacillus*, (3) G+ *Firmicutes*, (4) *Rhizobium* spp., (5) slow growers, (6) saprophytic fungi including *Aspergillus* spp and *Penicillium* spp and (7) diverse mycelial fungi. Soil diversity and complexity was captured in our experiment with average richness of 34 bacterial, 1 archaeal and 47 fungal genera across the selective media. Using identical culturing conditions, different communities were cultivated from the sandy and silty loams, allowing us to capture the phylogenetic diversity of the field within constrained enrichment cultures.

Results from 16S and ITS revealed unique variation in taxon representation across the seven nutrient-dependent enrichment techniques for both soils. Some enrichments differed in dominant genera depending on the originating soil. For example the media selecting for *Rhizobium* spp. was dominated by *Acinetobacter* in the sandy loam and by *Planococcaceae* in the silty loam. The two fungal enrichment media highlighted the most unique chemical signatures, driven by differing fungal composition between the sites, though the bacterial communities were similar. Within these enrichment cultures 16S bacterial composition for both sandy and silty loams were dominated primarily by *Firmicutes* (specifically *Burkholdriales*). However, ITS results reveal distinct fungal variation despite all taxa belonging to the same phylogenetic division. The saprophytic fungi enrichment in the sandy loam was highly dominated by *Pleosporales*, while in the silt loam, the mycelial fungi enrichment was mainly representative of *Hypocreales*. Although the gram positive selective media also had distinctive insoluble chemical signatures, this difference was not driven by differing fungal communities, as this media showed nearly no fungal growth.

Community differences were reflected in biomarkers specific to the extracellular and intracellular aqueous fraction of microbial biomass, identified using GC-MS and liquid state 1D ^{1}H-NMR. Metabolic profiles for both intracellular and extracellular fractions reveal site specific differences among the selective media. Intracellular metabolic pathways identified consistently revealed elevated levels in the sandy loam compared to the silt loam. Highly represented intracellular pathways were in secondary metabolite biosynthesis (antibiotic biosynthesis, nitrogen-containing secondary compound biosynthesis, and siderophore biosynthesis), amino acid degradation (Arg, Phe, and Thr degradation were of higher levels than others), and fermentation (acetate and glycerol being main metabolites of contribution). Intracellular metabolism was representative of metabolites with high turnover value, including substrates essential for core metabolic pathways. The opposite was true for extracellular metabolic profiles in which primary activity was elevated in silty loams over sandy loam. Pathways represented were secondary metabolite biosynthesis, carbohydrate degradation (carbohydrate, carboxylate, and hormone degradation), and fermentation and CO_2 fixation (formate being a main metabolite of contribution). These results show how differences in community composition among sites can induce intracellular and extracellular shifts that influence the production of microbial derived residues. Even under the same culturing conditions, differences in community composition revealed greater levels of intracellular metabolites in the sandy loam communities and greater extracellular metabolites in silty loam communities. These metabolic profiles influence the

microbial residues available for SOM formation and accumulation, resulting in distinct necromass signatures as evidenced in cell wall chemistry.

Cell walls are presumed to be the most persistent portion of microbial necromass. Our analysis of cell wall residues via solid state ^{13}C-NMR revealed unique lipid and terminal methyl chemical profiles between microbes cultivated from the two soils. Specifically the two fungal and the gram positive enrichments from the sandy loam revealed increased profiles of lipids and terminal methyl groups. By contrast, the silty loam communities increased in a distinctly different profile of lipids and terminal methyl groups that was evident for communities grown on the bacterial selective media. Together these insoluble chemical signatures suggest that fungal and bacterial communities both contribute to necromass accumulation via lipid and fatty acid production and the relative contribution appears to vary with community membership and metabolism. Lipid signals were greatest in bacterial communities enriched from the silt loams and fungal terminal methyl and fatty acid groups were greatest in fungal cultures derived from the sand loam. The hydrophobicity and complexity of these molecules make them good candidates for persistent necromass. Our results suggest that biochemical differences in fungal and bacterial communities may be important for the quantity and quality of necromass production. If lab results translate to field conditions, we expect that differences in community membership between sites will strongly influence the microbial interactions that lead to the formation and persistence of SOM.

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