Title: Three Feet Deep: Abiotic and Biotic Drivers of Organic and Mineral Soil Carbon Cycling

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Project Goals: PNNL's Phenotypic Response of Soil Microbiomes SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture. We perform multi-scale examinations of molecular and ecological interactions occurring within and between members of microbial consortia during organic carbon decomposition, using chitin as a model compound. Integrated experiments address spatial and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions throughout the soil profile. Data are used to parametrize individual- and population-based models for predicting interspecies and inter-kingdom interactions. Predictions are tested in lab and field experiments to reveal individual and community microbial phenotypes. Knowledge gained provides fundamental understanding of how soil microbes interact to decompose organic carbon and enable prediction of how biochemical reaction networks shift in response to changing moisture regimes.

Abstract Text: This study focuses on the influence of soil moisture and plant cover on multi-omic, microbial, and chemical indicators of carbon (C) cycling at different depths through the soil profile. Soil C is known to be metabolized through the soil-plant-microbe continuum in interrelated processes that determine the balance of C sequestered in soil or released back into the atmosphere. Long-term storage of soil C is thus dependent upon resource use efficiency by soil microorganisms and the complex interplay with the physiochemical soil matrix of organic and mineral forms of C. In surface soils, soil organic C (SOC) cycling is regulated by soil pH, moisture, and plant cover. Deep soil profiles are characterized by resource gradients of soil pH, moisture, nutrient availability, and mobile plant and microbial residues. A unique opportunity to understand mineral and organic carbon interactions through the soil profile is presented in the case of calcareous soils – characterized by high soil pH and calcium throughout the soil profile with carbonate-containing horizons a few feet deep. Calcium carbonate-containing marginal soils hold the potential for long-term storage of select forms of C as presented in our study.

We hypothesized that soil biochemical diversity would increase with plant cover and decrease with depth into the soil profile. Furthermore, we hypothesized that differences in soil moisture would result in differences in soil C chemistry with depth. We tested our hypotheses using a multi-omics approach to determine the composition and diversity of the soil microbiome, and the metabolome, lipidome, and proteome from 0 to 1 m depth into the soil profile, and how these relate to soil

moisture and C chemistry. We analyzed soils from our tall wheatgrass (*Thinopyrum ponticum*) irrigation experiment located in the Columbia Basin of Washington state. Samples were collected from the drip irrigated plots and adjacent non-irrigated bare soil. Irrigated plots received surface drip irrigation weekly based on crop evapotranspiration, monitored by WSU, at a rate of 18.75% of the recommended application to impose drought stress.

In total, 336 polar metabolite features, 649 lipids, and 19,165 proteins were detected in 48 soil samples. Greater depths were characterized by higher soil pH and calcium, lower SOC and SOM, and higher abundances of select lipids and organic acids. Of the organic acids which responded to depth, calcium was most strongly positively correlated to benzoic acid and most strongly negatively correlated to linoleic acid. SOM was also strongly predicted by linoleic acid and metal micronutrients which suggests that linoleic acid interacts in complexes between metals, cations, and SOM.

Depth's effects on soil chemical and metabolome composition were variable by field treatment. Nonirrigated bare soils contained significantly greater TOC, TN, and trehalose from 0-5 cm and lower carbonate as a polar metabolite, calcium, and magnesium compared to the 48-100 cm horizon. By contrast, in irrigated bare soils, the lower 48-100 cm horizon contained greater soil moisture and benzoic acid, and lower potassium and trehalose compared to the 0-5 cm horizon.

Soil proteome structure was best explained by soil pH compared to all other soil chemical properties measured. Soil proteome composition differed by sampling depth: the top 0-5 and 5-15 cm were characterized by significantly greater di-haem oxidoreductase activity, bacterioferritin activity, bacterial lysR proteins, outer membrane receptor proteins involved in mostly Fe transport, and gram-negative porin protein abundance compared to the lower 48-100 cm horizon. Di-haem oxidoreductase activity was detected in 40 samples. Acetoacetate decarboxylase activity was detected in 39 samples and ranked as one of the most commonly abundant protein signatures in the 15-48 cm and 48-100 cm horizons.

Our results present a novel soil dataset revealing chemimicrobial interactions with SOM and minerals in calcareous, marginal soil systems. This rich dataset and multi-omics perspective highlights the contrasting importance of plant-microbe interactions in the formation of SOM in surface soils and salt-metal-mineral interactions in stabilizing deep soil C in arid marginal soils.