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Deciphering taxonomic carbon exchange between plants and microorganisms using proteomics coupled with 13C tracers and spatially resolved protein extraction

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Clear elucidation of plant-microbe interactions within the rhizosphere and how these relationships change over time can be confounded by the large microbial biodiversity, shifting microenvironmental conditions, and extensive spatial constraints within these complex systems. Proteomics analysis of root or soil samples, when linked with metagenomic interpretation, can provide key insights to both the taxonomy and functional capability of microbial populations within a sample. Yet, existing proteomic approaches may not always be able to provide the needed temporal and spatial resolution to capture fine-scale and short-term interactions between plants and microorganisms. To remedy this limitation, we are developing a suite of methodological adaptations intended to leverage proteomic analysis to help identify key interactions between rhizosphere microorganisms and their host plant.

First, we are employing ^{13}C tracers coupled with automated data analysis to identify specific organisms consuming both simulated and natural root exudates. We are specifically exploring microcosms constructed from natural soil (Kellogg Biological Station, Hickory Corners, Michigan, USA) and planted with switchgrass as a platform for developing the techniques. Multiple previous studies have linked key interactions between both free-living and epiphytic microbial members with improved performance of a switchgrass host under nutrient-depleted, natural field conditions. Providing evaluation of the amount and taxonomic recipient of switchgrass-supplied carbon under varying conditions may help link key taxonomic groups with improved plant performance and biomass production.

Second, we are leveraging a membrane extraction technique coupled with specialized sample digestion, purification, and analysis to enable non-destructive, spatially-resolved protein extraction from the root-soil interface within our constructed microcosms. Through its non-destructive nature, this approach permits timeseries analysis for tracking specific taxa and, in some cases, functions associated with rhizosphere processes both before and after a system perturbation as well as variations over plant growth phases during a growing season. The high sensitivity of this system enables spatial analysis at the one to two mm scale where samples can be manually

selected based on proximity to specific root structure, metabolic hotspots in the system, or other parameter of choice. Spatial analysis can be leveraged to track taxonomic distribution within the rhizospheres associated with roots at different growth stages or levels of maturity.