Spatially resolved proteomic analysis to reveal controls on rhizosphere microbial recruitment and track carbon exchange between plants and microorganisms

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Project Goals: This project seeks to elucidate key microbiological and geochemical controls on nutrient exchange within the rhizosphere and the role that spatial organization within the root-rhizosphere-soil continuum plays in directing nutrient acquisition by the host plant. Spatially resolved understanding of nutrient exchange through this dynamic zone will identify key variables that may form part of an effective rhizosphere management program targeting enhanced plant productivity. Our aims are directed towards identifying the microbial and geochemical factors that stimulate enhanced plant investment (in the form of root exudation) into specific regions of the rhizosphere and assessing the implications of this carbon input on the microbial and geochemical response.

While small in physical stature, rhizosphere embodies the dynamic interface between plants and soil and can impart profound impacts on overall plant performance. High diversity and microbial activity within rhizosphere (especially relative to bulk soil) reflect the tight inter-Kingdom metabolic coupling present in this zone where interactions are linked to increased nutrient mobilization, enhanced pathogen resistance, and improved drought tolerance. Soil, as a precursor to rhizosphere development, is extremely heterogeneous with large spatiotemporal changes in biological and geochemical conditions over short distances. Rhizosphere displays comparable variability with rates and composition of root exudation, recruitment of microbial members, and overall growth or constriction reflecting shifting environmental conditions, seasonal cycles, and plant growth stage. Efforts to both improve biomass production on marginal soils and increase subsurface C storage are motivating a need to better understand and engineer specific rhizosphere interactions with a suite of resulting spatially resolved techniques being developed to better interrogate this system. *Here, we describe and demonstrate a spatially specific, non-destructive proteomic technique that enables evaluation of microorganisms present and metabolically integrated with a rhizosphere.* For instance, we use this approach to identify microbial recruitment associated with *de novo* rhizosphere microbial community development by comparing nascent rhizosphere associated with root tip to communities aligned with more mature root segments.

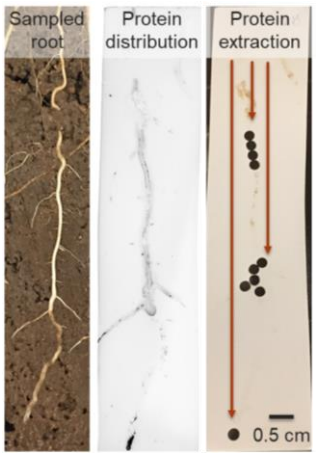

Figure 1: Sample selection method involved direct blotting of rhizosphere with a membrane for spatially resolved, nondestructive protein extraction followed by staining to map protein distribution and excision of manually selected regions for analysis.

Our method employs a membrane blotting technique for nondestructive removal of mobile proteins from rhizosphere

(Figure 1). The proteins are largely immobilized when bound to the membrane, enabling retention of their two-dimensional distribution in the sample and can then be imaged/mapped using a fluorescent, general protein stain (SYPRO Ruby). Membrane sections correlating to sample areas of interest (e.g., high protein abundance, specific root morphology, alignment with rhizosphere geochemical gradient, etc.) are manually excised from the membrane and bound proteins are digested and purified in preparation for proteomic analysis using Orbitrap mass spectrometry. Importantly, the approach is nondestructive in that, while mobile proteins are removed from the system, the host plant, rhizosphere, and soil are not removed, making both timeseries investigations and analysis using additional tools feasible (e.g., imaging, stable isotope analysis, nutrient quantification, etc.). The demonstrations we performed all used switchgrass (*Panicum virgatum*, Cave-in-rock) cultured in rhizoboxes containing soil harvested from the Kellogg Biological Station (Hickory Corners, MI).

We used comparison of proteomic assessment of rhizosphere at the tip of a growing root with that associated with a more mature rhizosphere as an initial demonstration of this technique. The relatively rapid growth rate of root tips can result in them spatially outpacing microbial colonization. Yet, due to multiple interactions between growing root tips and soil, these areas are also hotspots for deposition of plant derived C which can help fuel establishment of rhizosphere microbial communities. Our analysis (Figure 2) demonstrated dominance of switchgrass associated proteins at the root tip with observation of a relatively smaller composition of bacterial proteins. In contrast, rhizosphere associated with the mid root had overall increase alpha diversity and a dominance of bacterial taxa at the expense of switchgrass related proteins.

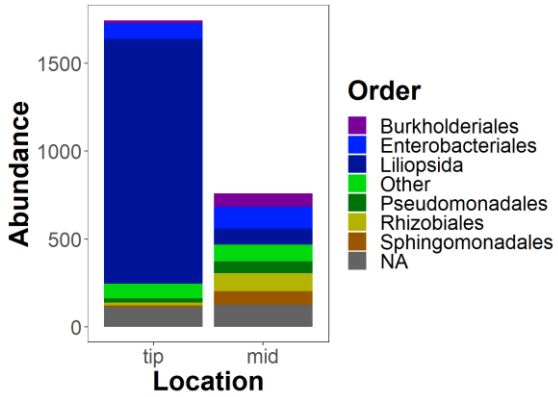

Figure 2: Proteins associated with switchgrass dominated samples from the root tip while microbial diversity in the mid root samples is indicative of establishment of the rhizosphere community.

We further used this approach to track differential protein signatures associated with changes in P availability in a series of split root rhizoboxes where roots from individual plants could grow into both P replete and deplete conditions. We incorporated a $^{13}CO_2$ stable isotope tracer into this experiment to better evaluate the exchange of plant derived carbon to the microbial community. Surprisingly, there was no large-scale preferential incorporation of ^{13}C under either set of conditions; roughly twelve percent of observed proteins were identified as containing tracer in each condition. This is consistent with bulk scale measurements suggesting commensurate levels of root exudation in each condition. However, all observed ^{13}C labeled switchgrass associated phosphatases were in P replete environments. Thus, while these results do not suggest a wholesale shift in release of plant root exudates based on P availability, we did observe indications of a spatial physiological response in switchgrass. In contrast, we identified a larger number of bacterial phosphate transporters in P deplete conditions. While switchgrass had access to ample P in one portion of its root network, the bacterial members in the P deplete environment had limited P availability which may account for the observed increased frequency of associated transporters.