Evaluating Biogeochemical Processes Facilitated by Plant and Microbial Interactions within the Rhizosphere

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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.

We hypothesize that localized regions within the rhizosphere act as foci for exchanging root-derived organic carbon with soil-derived nutrients made available by a combination of microbial activity and inherent soil resource availability. Further, we hypothesize that the locations of these resulting nutrient exchange hotspots are not stochastically distributed throughout the rhizosphere but are controlled by microenvironmental conditions resulting from a combination of plant-derived carbon, microbiological activity, and soil geochemistry. To test these hypotheses, we are applying a suite of tools to evaluate the rhizosphere within a series of microcosms constructed with natural soil (Kellogg Biological Station, Hickory Corners, Michigan, USA) and *Panicum virgatum*, (switchgrass, variety Cave-in-Rock) seedlings. We are using these investigations to evaluate drivers of spatial heterogeneity in root exudation rates, rhizosphere microbial membership and activity, and localized shifts in soil geochemistry and nutrient availability.

In the first investigation, we are imposing spatially resolved variation in phosphorus availability to track the degree of plasticity in root physiology and how this can be leveraged to improve plant fitness. We applied spatially constrained phosphorus resources [either inorganic (calcium triphosphate) or organic (phytic acid) sources] with and without the addition of a known plant growth promoting organism implicated in phosphorus mobilization (*Flavobacterium johnsoniae*) to assess localized plant response. Monitoring levels of soluble phosphorus in pore water extracted both proximal and distal to roots allowed us to demonstrate an increase in orthophosphorus availability at the interface of plant roots and the applied resource, suggesting plant linkage to enhancement of solubilization. We further observed the greatest levels of phosphorus solubilization under experimental conditions supplemented with the *Flavobacterium*, highlighting the potential bacterial role in phosphorus cycling within the system which is consistent with previous studies. We are currently pushing beyond this association by assessing the ability of plant roots to preferentially direct organic carbon exudation into areas of higher phosphorus ability to help stimulate further solubilization of this resource. We are also assessing the variability in the composition of soluble organic compounds extracted from pore waters at various locations in the

rhizobox microcosm to provide insight to the mechanism of interaction between plant host and phosphorus solubilizing bacteria which is facilitated by plant root exudation.

Secondly, we developed a suite of spatially resolved techniques designed to elucidate microbial activity and distribution. We are spatially mapping phosphatase activity within our rhizobox microcosms using fluorescent, substrate-specific activity probes. This approach enabled us to identify increased phosphatase activity in the spatial vicinity of a root growing proximal to an applied organic phosphorus sources in comparison to a control root (Figure 1). A key feature of the approach is its non-destructive nature which allows for tracking shifts in phosphatase activity during plant growth and at different phases of root development. We are currently working to link this phosphatase approach with a similar probe targeting chitinase activity to help spatially map intersections of phosphorus, carbon, and nitrogen cycling within rhizosphere systems. These hotspots of nutrient cycling are hypothesized to be key locations of nutrient transfer to plant roots.

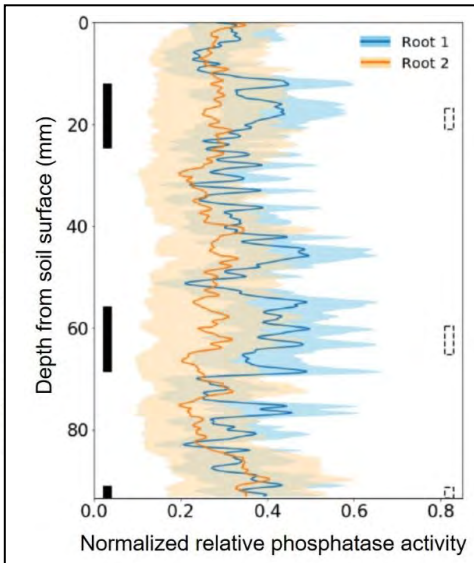

Figure 1: Vertical variation in phosphatase activity surrounding two roots was tracked as the roots grew through soil sections supplemented with an organic phosphorus source (Root 1, approximate areas of enrichment depicted by the shaded box) and a control (Root 2, approximate area depicted by dashed box).

In recognition of the dominant role of microbial processes in nutrient cycling, we are developing protein extraction approaches coupled with proteomic analysis to permit spatially resolved assessment of taxonomic and, in some cases, functional distribution within this complex system. This approach is non-destructive when applied in our rhizobox systems and enables us to track variation in the observed metaproteomes associated with root development as well as spatially focused variation arising from microenvironments within the rhizobox. We are currently employing the approach to differentiate proteomic expression in the rhizospheres associated with roots grown under two different nutrient conditions – phosphorus replete and deplete. We are using a split root experimental design for these experiments where we allow roots from a single plant to grow into these controlled nutrient conditions and are just beginning to assess resulting adaptation of the plant and microbial proteome in response to shifted nutrient conditions.

Our developments are enabling us to interrogate the high spatial heterogeneity of the rhizosphere system to begin elucidating key interactions being leveraged to facilitate nutrient transfer to a host plant. This knowledge will provide insights on how to strengthen nutrient transfer processes to increase plant performance under varied and challenging growth conditions.

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