Spatial plasticity in plant-microbe interactions in response to applied nutrient heterogeneity in soil

James Moran[1,*] (james.moran@pnnl.gov), Vivian Lin[1], Elias Zegeye[1], Jamie Nuñez[1], Sam Purvine[1], Nikola Tolic[1], Kim Hixson[1], Montana Smith[1], Nicholas Sconzo[1], Nicholas Huggett[1], Young-Mo Kim[1], Mary Lipton[1]

[1]Pacific Northwest National Laboratory, Richland, Washington
https://science.pnl.gov/staff/staff_info.asp?staff_num=8559

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. Rhizosphere development is spurred by carbon delivery into the subsurface through a variety of plant processes collectively termed rhizodeposition. In this work, we are probing the ability of plants to spatial focus their subsurface carbon delivery in response to the heterogenous distribution of nutrients typical of soils. Rhizodeposition can be viewed as an investment by the plant, used to establish beneficial microbial and geochemical relationships to enable more effective nutrient extraction from soil and to help maintain defenses against pathogens and environmental stresses. The intense spatial heterogeneity in nutrient distributions in soil exemplifies the potential advantage conferred to a plant by spatially focusing its carbon investment at specific location conducive to highest nutrient extraction/return. Previous studies demonstrated a phenomenon of root proliferation in even small zones of increased nutrient availability within soil. Here, we are applying a multi-faceted approach to attempt capturing the functional plasticity of roots themselves, the resulting implications on the rhizosphere microbiome, and the propagation of these effects on plant growth. As a test platform, we cultured switchgrass (*Panicum virgatum*, var. Cave-in-rock) under marginal conditions in rhizoboxes containing a mixture of sand (as a low-nutrient growth medium to establish marginal growth conditions) and soil harvested from the Kellogg Biological Station (Hickory Corners, MI; to serve as a microbial inoculum and nutrient source). Within these rhizoboxes, we established a thin (approximately 1 cm in vertical thickness) horizon of soil amended with various nutrient resources included organic/inorganic

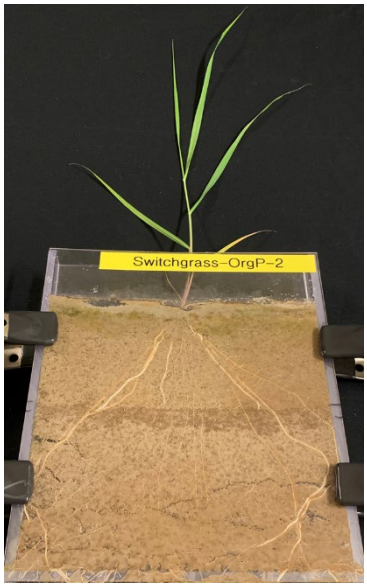

Figure 1: Sample set up utilized a series of rhizoboxes having a horizontal resource amendment midway through the rooting zone as seen above in the darkened soil band.

phosphorus, chitin, or a combination of organic phosphorus and chitin. We applied a a stable isotope tracer ($^{13}CO_2$) in concert with bulk isotope analysis, leveraged proteomic analysis to identify microbial uptake of plant-derived carbon, applied a chitinase enzyme mapping technique to spatially map enzyme activity, and tracked carbon transformations. Combined, we attempted to quantify the resulting plant-microbe adaptation to the applied nutrient heterogeneity.

We observed increased plant biomass in the experimental replicates containing a thin horizon of chitin-amended soil as recorded through larger aboveground biomass production. While we leveraged our $^{13}CO_2$ tracer to observe root exudation in all systems containing a plant, we observed the highest rates of root exudation specifically in the chitin-amended samples. Most importantly, in agreement with our hypothesis related to the ability of plants to spatially focus their carbon deposition, the increased root exudation was spatially focused within the chitin amendment zone and dropped off above and below this layer. The spatial focusing of root exudation was not observed in our controls nor in systems amended with various forms of phosphorus, suggesting this phenomenon was motivated by the presence of chitin, likely as a source of nitrogen.

To leverage increased carbon supply toward improved nutrient extraction from the amended chitin, it needs to first be broken down to release its organic nitrogen. We developed a fluorescent-based mapping approach for tracking chitinase activity in these systems. Briefly, we removed the rhizobox side panel and performed a membrane blotting technique to extract chitinases from the soil. We synthesized and applied a molecular chitin surrogate containing a fluorescent probe which becomes activated upon reaction with chitinase. Importantly, this approach more closely tracks chitinase potential activity versus quantifying in situ rates. Still, we observed a statistical increase in chitinase activity spatially focused within the amended band. We are following up on these observations by performing a more thorough proteomic analysis with specific attention to the exchange of plant derived carbon (here containing a ^{13}C tracer) into proteins associated with various microbial taxa to assess the impact of the observed changes on the rhizosphere microbiome.

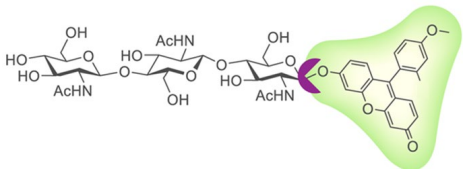

Figure 2: We developed a molecular chitin surrogate that, when cleaved by a chitinase enzyme, activates a fluorescent tag for spatially tracking chitinase activity.

Taken together, we sought to test the hypothesis that plant roots can spatially regulate their interactions with soil to target regions with higher potential for resource extraction. When grown in simulated marginal growth media, we observed spatial focusing of switchgrass root exudates and associated chitinase activity within a thin band of soil amended with chitin and that these plants outperformed experimental control cohorts. We are furthering this study to explore selective shifts in plant-microbe carbon transfers accompanying these spatially applied nutrient amendments to gain insights to the mechanisms supporting spatially driven subsurface responses. Understanding the adaptability and drivers that guide subsurface carbon flow can help contribute to better plant performance in marginal soils and inform future efforts to direct carbon deposition in soil systems to specific locations or horizons.

This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER). This contribution originates from an Early Career Research Award granted at the Pacific Northwest National Laboratory (PNNL).