Evaluating Biogeochemical Processes Within Microenvironments Along the Root-rhizosphere-soil Continuum

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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 inorganic nutrients made available by a combination of soil 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 switchgrass seedlings (variety Cave-in-Rock). We are specifically evaluating spatial heterogeneity in 1) root exudation, 2) microbial activity, and 3) soil geochemistry.

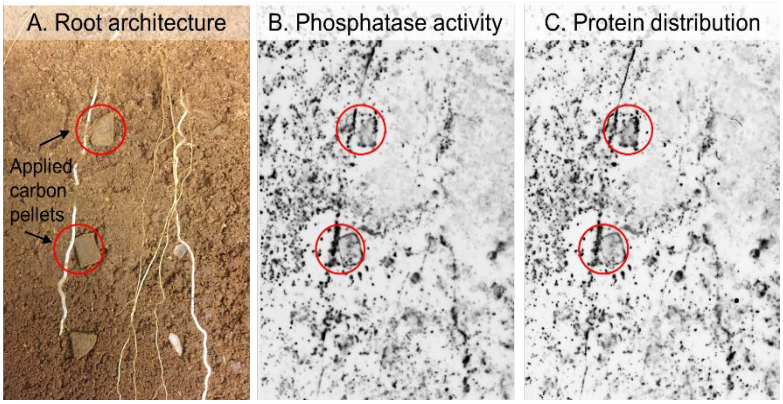

Figure 1: (A) Homogenized and pelleted root biomass was placed within the rooting zone of our soil microcosms. Samples of the mobile proteome were extracted, while maintaining their spatial localization, using a membrane transfer technique and then sequentially stained for (B) phosphatase activity and (C) total protein content. Increased protein content in areas of proximity between root and the applied resource islands suggest stimulation of overall biologic activity in these areas versus control regions: applied resource island with no root exposure, applied quartz (versus organic) inserts, and bulk soil.

Root exudation can provide a valuable carbon resource to subsurface environments which are frequently limited in this key nutrient. We are using a $^{13}CO_2$ tracer combined with laser ablation-isotope ratio mass spectrometry (LA-IRMS) to track variable rates of photosynthate flow into different roots and subsequently into the rhizosphere. We can clearly identify increased allocation of fresh photosynthate to specific roots over

others and are actively seeking to identify factors controlling the observed carbon distribution. We are also evaluating the spatial extent of root exudates into soil to identify locations of the rhizosphere that experience greater carbon investment by the host plant.

To help better understand the localized microbial response to root exudation, we are developing two methods to evaluate the microbial components of the system including 1) spatially resolved proteomics assays and 2) selective activity-based staining of specific enzymatic functions within the system. Our proteomic technique involves transferring mobile phase proteins (mainly exoproteins) onto a membrane while maintaining the native spatial distribution of the proteins. This technique is non-destructive to the system and enables timeseries analysis of the microbial community. Our enzymatic assays are designed to complement this approach to specifically map phosphatase activity onto the spatial distribution of proteins. In an initial experiment, we pelleted homogenized root biomass and dispersed this material into the root zone of our microcosms (Figure 1). The resulting images suggest there is both higher protein production and increased phosphatase activity where the root and the applied resource islands are in spatial proximity. We are working to identify how microbial diversity and protein expression may be stimulated by the combination of root exudation and bioavailable carbon.

Finally, in order to better characterize the geochemical microenvironment within and surrounding the rhizosphere, we developed a laser-induced breakdown spectroscopy (LIBS) technique to enable mapping of macro- and micro-nutrients in the soil and demonstrated its ability to identify specific elemental foci that may support hotspots of microbial activity (Ilhardt et al., 2019). We developed a quantitative image analysis package to identify gradients of nutrient concentration, such as carbon, calcium, potassium, phosphorus, and iron, at increasing distance from a root (Figure 2). Our ongoing work is focused on superimposing elemental gradients with microbial diversity and activity maps within the rhizosphere.

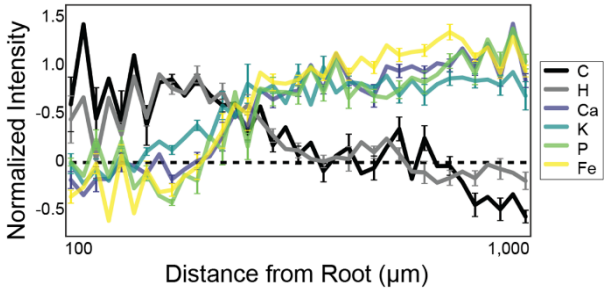

Figure 2: Measuring gradients of key nutrients with increasing distance from a root using LIBS (Ilhardt et al., 2019).

Overall, our developments allow us to track photosynthate into the rhizosphere and surrounding soil with high spatial resolution, and subsequently characterize the elemental and microbial composition of specific locations. Together, this data will reveal how soil geochemical microenvironments and microbial activity relate to the distribution of fresh photosynthate provided by the host plant.

References

1. Ilhardt, P., J. Nuñez, E. Denis, J. Rosnow, E.J. Krogstad, R. Renslow, J. Moran (2019) Rapid, high-resolution elemental mapping of the root-rhizosphere-soil continuum using laser-induced breakdown spectroscopy (LIBS). *Soil Biology and Biochemistry*, **131**, 119-132. doi: 10.1016/j.soilbio.2018.12.029

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