



## Evaluating biogeochemical processes within microenvironments along the root-rhizosphere-soil continuum

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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. To test this hypothesis, we are developing and employing a suite of tools to enable evaluation of the microenvironments within the rhizosphere to identify spatial heterogeneity in root exudation, soil geochemistry, and microbial activity. We are using microcosms constructed from natural soil (Kellogg Biological Station, Hickory Corners, Michigan, USA) and planted with switchgrass as a platform for developing the techniques and providing a preliminary evaluation of our central hypothesis.

First, we adapted laser ablation-isotope ratio mass spectrometry (LA-IRMS) for analysis of rhizosphere samples and used the approach coupled with a  $^{13}\text{C}$  tracer to track variable rates of photosynthate flow into different roots and subsequently into the rhizosphere. We are also exploring laser ablation integrated with spectroscopy-based isotopic evaluation for making similar measurements. The greater sensitivity of the capillary absorption spectroscopy (CAS) approach enables smaller sample sizes to provide higher levels of spatial resolution (10 – 20  $\mu\text{m}$ ). Second, we developed a laser-induced breakdown spectroscopy (LIBS) technique to enable mapping of macro- and micro-nutrients in the soil surrounding roots and demonstrated its ability to identify specific elemental foci that may support hotspots of microbial activity. 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 plant root. 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 spatial distribution of the proteins in the soil. This technique is non-destructive to the host plant 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.

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.