Spatially resolved carbon isotope measurements for tracing root exudates into the rhizosphere

JAMES MORAN¹, TIMOTHY LINLEY¹, JASON KRIESEL², ELIZABETH DENIS¹, PETER ILHARDT¹, JAMES KELLY²

¹Pacific Northwest National Labortory, Richland, WA ²Opti-Knolwedge Systems, Inc., Torrance, CA (*correspondence: james.moran@pnnl.gov)

Root exudation provides a primary carbon (C) source to soil microbial communities which helps drive high rates of microbial activity within the rhizosphere. In turn, microbial activity supported by organic carbon in root exudates provides a wide range of processes linked to nutrient biogeochemistry, plant health, and overall C cycling within soil. Despite its importance, however, tracking the localization of root exudation into the rhizosphere is severly challenged by the rhizosphere's small size (mm-scale, adjacent to the root). Understanding the C dynamics of the rhizosphere is essential to developing models describing soil C cycling, better understanding the interactions of plants with soil microbial populations, and for informing emerging efforts in rhizosphere engineering.

To provide the needed spatial resolution for detailed analysis of root exudation, we are developing two new tools for examining the rhizosphere. Both approaches leverage a ¹³CO₂ tracer introduced to the host plant and then apply spatially resolved sampling and $\delta^{13}C$ analysis to enable tracking the extent and localization of resulting exudates in the rhizosphere. In each case, laser ablation sampling is used for sample selection and harvesting from the rhizosphere. A helium carrier flow is used to pass the ablation particulates through a combustion reactor to convert all C to CO2 for isotope analysis by either: 1) an isotope ratio mass spectrometry (IRMS) or 2) capillary absorption spectroscopy (CAS). When using IRMS, we achieve $50 - 100 \ \mu m$ spatial resolution; sufficient for tracking the decrease of root exudate at increasing distances from the root surface and for evaluating variation in the amount of fresh photosynthate within different roots. We designed the CAS system for increased sensitivity, and it currently requires approximately three orders of magnitude less CO₂ (versus IRMS) for individual $\delta^{13}C$ measurement. As a result, we can use smaller laser ablation spot sizes for analysis and achieve as much as 5µm spatial resolution. Together, these two systems provide a tool for spatially tracking root exudate within the rhizosphere and can help improve our understanding of C biogeochemistry within the rhizosphere and and surrounding soil.