Direct measure of amino acid distribution across *Populus trichocarpa* roots in a rhizosphere-on-a-chip habitat

John F. Cahill[1*] (cahilljf@ornl.gov), Courtney Walton[1], Muneeba Khalid[1], Vilmos Kertesz[1], Scott T. Retterer[2], and Jennifer Morrell-Falvey[1]

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge TN; [2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge TN

Project Goals: The goal of this bioimaging project is to develop new technologies to image changes in chemistry occurring in the rhizosphere in living biosystems. A major hurdle in existing bioimaging technologies is the lack of broad chemical coverage and specificity in enormously complex chemical environments like the rhizosphere. To tackle this challenge, we developed a non-destructive liquid extraction-based mass spectrometric method that enables *in situ* measure of exogeneous chemical components in a rhizosphere-on-a-chip habitat populated with a *Populus* cutting. We demonstrate empirical measure of the distribution and heterogeneity of several amino acids along the whole of the *Populus* root structure within this environment.

The rhizosphere is an incredibly complex environment, containing thousands of unique exogenous chemical species oriented in a complex spatial network. Such compounds are known to affect plant-microbe organization, interactions and, ultimately, growth and survivability. Due to its importance, the role of exogenous compounds in the rhizosphere is under much investigation, specifically the relation between plant physiology and the spatiotemporal distribution of molecular components. However, measure of the spatial distribution of exogenous compounds in the rhizosphere is challenging given the complex and dynamic nature of the environment. Compounds include, among others, organic acids, polysaccharides, proteins, and amino acids (AAs) which can exhibit a variety of roles in the rhizosphere including acting as a nutrient source for microbial colonization or a deterrent against pathogenic species. The exudation of AAs is one of the biggest components of plant carbon loss, which when released into carbon-deficient soil can lead to significantly enhanced hot-spots of microbial growth. Therefore, methods capable of directly measuring exudates, including AAs, are necessitated to understand rhizospheric phenomena.

Direct measurement of AA distribution within the rhizosphere is challenging due to the complex and dynamic nature of the environment and the limited accessibility of the rhizosphere for analysis. Nearly every AA can be found within the rhizosphere in a complex chemical cocktail of salts, proteins, and other compounds which present difficulties for chemical differentiation though existing imaging modalities. In addition, most biological pathways are spatiotemporally regulated, which suggests the distribution of exudates and AAs will vary in space and time. However, even for abundant molecules, like AAs, little is known of how they are spatially distributed along plant roots, how their distribution changes over time and how their distribution affects microbial composition and, ultimately, plant health.

Here we show the application of liquid microjuction-surface sampling probe mass spectrometry (LMJ-SSP-MS) for the direct measure of AA distribution within a rhizosphere-on-a-chip habitat populated with a *Populus trichocarpa* cutting. Uniquely, this newly developed imaging technology enables mass

spectrometric imaging of over a dozen AAs across the root structure *in situ*, without destructively impacting *Populus* root growth and development. Our data shows the spatial relationship of AA relative to root structure and finds that AAs exhibit significant heterogenous spatial and compositional structure across the root system. Additionally, differences in AA composition across the root imply heterogenous release of AAs based on their identity, whether by direct exudation or other processes (e.g., cell lysis and sloughing).

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