Title: 3D Cartography of the Sorghum Rhizosphere

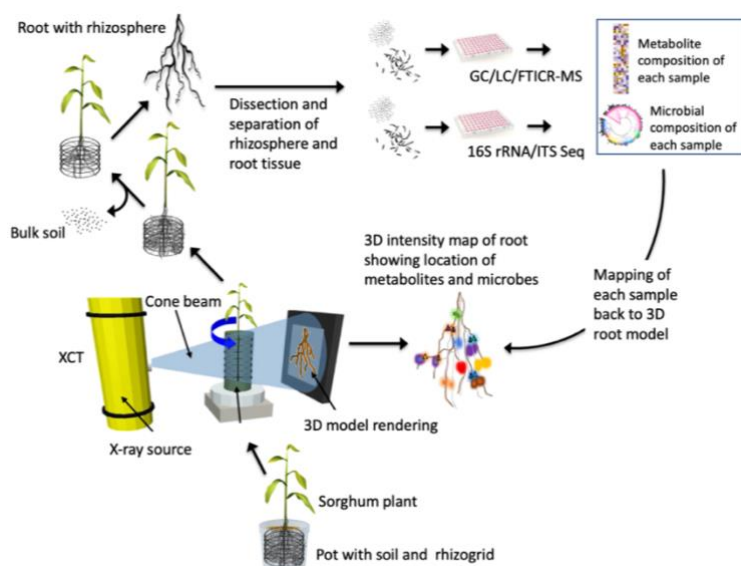**Authors:** Pubudu Handakumbura[*], Albert Rivas-Ubach, Tamas Varga, Anil Battu, Thomas Wietsma, Janet Jansson, Christer Jansson, **Robert Egbert**

Institution: Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory (PNNL), Richland, WA 99354 USA

Website: https://genomicscience.energy.gov/research/sfas/pnnlbiosystemsdesign.shtml

Project Goals: The Pacific Northwest National Laboratory Persistence Control Scientific Focus Area is focused on developing fundamental understanding of factors governing the persistence of engineered microbial functions in rhizosphere environments. From this understanding, we will establish design principles to control the environmental niche of native rhizosphere microbes for the model bioenergy crop sorghum through data-driven genome reduction and engineered metabolic addiction to plant root exudates. These principles will lead to secure plant–microbe biosystems that promote secure, stress-tolerant, and highly productive biomass crops.

Abstract Text: The rhizosphere, the zone of soil influenced by plant roots, is closely associated with a complex microbial community of bacteria, archaea, viruses, and fungi[1]. Using a correlative surface imaging approach with *Brachypodium distachyon*, a genomics model for grasses[2], we recently demonstrated that the root surface is metabolically heterogeneous with hot spots for bacterial attachment[3]. To follow up on these findings and, more broadly, to elucidate details underlying metabolic hotspots for plant-microbe interactions in the rhizosphere we developed a 3D root cartography platform (Fig. 1). The platform maps root exudate metabolites and microbes onto a 3D image of the root, generated by X-ray computed tomography (XCT). Following 3D rendering of the root structure, the root and adhering soil is pulled up from the pot. Loosely adhered soil is removed from the root by gentle shaking while the rhizosphere remains attached to the root surface. The root with its rhizosphere is segmented into short fragments, assigned with a barcode and coordinate position, and separated into root and rhizosphere fractions for subsequent metabolite and microbial profiling using LC-MS and 16S/ITS amplicon sequencing, respectively. To preserve the integrity of the root system architecture once it is removed from the pot, we developed 3D-

Figure 1. Schematics of the 3D root cartography platform.

printed polymer grids (rhizogrids) that are inserted into the pots prior to filling them with soil (Fig. 1). The rhizogrid also serves to provide coordinates for reconstructing the 3D root image from the excised root segments. Spatially resolved metabolite and microbial data on the root surface and in the rhizosphere are visualized using open-source software[4,5].

In the present study, XCT was used to obtain a 3D image of sorghum (*Sorghum bicolor* (L.) Moench). The imaged root was freed from bulk soil and dissected into 1-cm long segments. The rhizosphere was washed off from each root segment and the rhizosphere solution and corresponding root tissue were collected in micro-vials and kept at -80 °C for future analyses. The aims of this ongoing experiment are to 1) map the distribution of exuded metabolites and the soil microbiome in the 3D space of the sorghum root and rhizosphere, 2) examine if there are hotspots for microbial colonization, and 3) determine if these hotspots correlate with exudation of specific metabolites.

References/Publications

1   Ahkami, A., White, I., R. A., Handakumbura, P. P. & Jansson, C. Rhizosphere Engineering: Enhancing Sustainable Plant Ecosystem Productivity in a Challenging Climate; *Rhizosphere* **3**, 233-343 (2017).
2   Jansson, C., Vogel, J., Hazen, S., Brutnell, T. & Mockler, T. Climate-smart crops with enhanced photosynthesis. *Journal of Experimental Botany* **69**, 3801-3809 (2018).
3   Liu, W. *et al.* Correlative surface imaging reveals chemical signatures for bacterial hotspots on plant roots. *Analyst* **145**, 393-401 (2020).
4   Protsyuk, I. *et al.* 3D molecular cartography using LC-MS facilitated by Optimus and 'ili software. *Nature Protocols* **13**, 134-154 (2018).
5   Floros, D. J. *et al.* Mass Spectrometry Based Molecular 3D-Cartography of Plant Metabolites. *Front Plant Sci* **8** (2017).