Fungal Nutrient Acquisition and Transport in Soil Micromodels is Regulated by Organic Acid Chelation and Specific Membrane Transporters

Arunima Bhattacharjee*[1](arunimab@pnnl.gov), Christopher R. Anderton[1], Dušan Veličković[1], Jocelyn Richardson[2], Odeta Qafoku[1], Lindsey Anderson[1], Sneha Couvillion[1], Zihua Zhu[1], **Kirsten S. Hofmockel[1], Janet K. Jansson[1]**

[1]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA; [2]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA

Website: https://www.pnnl.gov/projects/soil-microbiome-science-focus-area

Project Goals: PNNL's Phenotypic Response of Soil Microbiomes SFA aims to achieve a systems-level understanding of the soil microbiome's phenotypic response to changing moisture. We perform multi-scale examinations of molecular and ecological interactions occurring within and between members of microbial consortia during organic carbon decomposition, using chitin as a model compound. Integrated experiments are designed to confront spatial challenges and inter-kingdom interactions among bacteria, fungi viruses and plants that regulate community functions. These data are used to parametrize individual- and population-based models for predicting interspecies and inter-kingdom interactions. Predictions are tested in lab and field experiments to reveal individual and community microbial phenotypes. Data is captured and shared through an optimized data management pipeline. Knowledge gained will provide fundamental understanding of how soil microbes interact to decompose organic carbon and enable prediction of how biochemical reaction networks shift in response to changing moisture regimes.

Abstract: Soil fungi are integral for soil nutrient cycling and transport due to their widespread interactions with plants and bacteria. Nonetheless, the precise mechanisms by which soil fungi uptake and transport specific mineral nutrients remains unknown. We previously illuminated the mechanisms underpinning directional growth into soil micropore like spaces by the soil fungus *Fusarium sp. DS 682*[1] using a custom designed mineral doped soil micromodel. Here, we expand on this work and reveal the mechanisms underlying fungal mineral derived nutrient transport, where mineral K translocation through fungal hyphae occurs via specific fungal proteins that facilitate passage of organic acid-chelated K.

To investigate fungal nutrient transport proteins, we analyzed *Fusarium sp. DS 682* grown on agar with and without contact with specific soil minerals, such as K - Feldspar and mica. From these samples, several families of cell membrane and mitochondrial transporters were detected in both the presence of minerals (+M) and the absence of minerals (-M). Transporters common under both + M and - M included the major facilitator superfamily transporters, solute carrier transporters, ATP-binding cassette type transporters, and aquaporins. In addition, nine specific transmembrane transporter proteins, such as a drug transporter (K03446), electrochemical potential driven transporters (K16261, K07300), a sugar transporter (K08145), and a xenobiotic transporter (K05658) were detected in our *Fusarium sp.* in

the presence of minerals. Moreover, several fungal proteins associated with organic acid transport (K23630, K03448) were enriched under +M conditions, perhaps for transport of mineral nutrients.

The underlying biology regulating micronutrient acquisition by soil fungi has not yet been identified. Our finding of enriched expression of organic acid transporters in the +M condition suggests that uptake of mineral nutrients was facilitated by fungal secreted organic acids, as has been previously suggested[2] through correlative evidence. To confirm our findings, we grew *Fusarium sp. DS 682* in our previously developed soil micromodels that are compatible with matrix-assisted laser/desorption ionization-mass spectrometry imaging (MALDI-MSI). These devices permitted us to map the location of small molecular weight molecules (e.g., organic acids) in an untargeted fashion using MALDI-MSI. Here, we detected several organic acids, such as citric acid, tartaric acid, malic acid and fumaric acid, on the micromodel surface after fungal growth, and found that the spatial distribution of citric acid and tartaric acid were notably different after fungal growth. Moreover, we detected K-citrate in fungal biomass grown in mineral doped micromodels using micro-X-ray fluorescence (μ-XRF) and X-ray absorption near edge structure (XANES), through our collaboration with the Stanford Synchrotron Radiation Lightsource (SSRL). K-citrate was not detected in fungal hyphae in micromodels without minerals. These results provide direct evidence of a previously uncharacterized mechanism of inorganic K uptake and transport, where fungal hyphae produce organic acids (i.e., citric acid) for uptake of mineral nutrients through organic acid transporter proteins expressed for translocation of mineral derived K+. Moreover, this study provides a pathway for discovery of specific fungal transport mechanisms that contribute toward nutrient translocation under different moisture conditions in soil.

References

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Funding statement: *This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (OBER), as part of BER's Genomic Science Program (GSP), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Soil Microbiome Scientific Focus Area "Phenotypic Response of the Soil Microbiome to Environmental Perturbations." A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by OBER and located at PNNL. PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RLO 1830.*