

Determining the genetic and environmental factors underlying mutualism within a plant-microbiome system: insights from genome sequencing, mass spectrometry imaging and exometabolite characterization

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Project Goals: To characterize the *Sphagnum*-diazotroph symbiosis by incorporating plant host *Sphagnum* and microbial genetic variation, variable climatic drivers, and complex communities that scale across biological organizations to regulate peatland carbon and nitrogen cycling.

The importance of plant-microbiome systems on terrestrial carbon and nitrogen processes is perhaps most pronounced in *Sphagnum* dominated ecosystems, which occupy 3% of the Earth's land surface yet store approximately 25% of terrestrial carbon as recalcitrant organic matter (i.e., peat). The foundation plant genus *Sphagnum* is responsible for much of the primary production in peatland ecosystems and produces recalcitrant dead organic matter. Together with associated N₂-fixing microorganisms, *Sphagnum* contributes to substantial peatland nitrogen inputs. *Sphagnum* growth and production (carbon gain) depends, in part, on a symbiotic association with N₂-fixing, diazotrophic microbes. Under changing environmental conditions, a central question about these ecosystems is whether the *Sphagnum*-diazotroph symbiosis will maintain its beneficial interaction, or will it shift to neutral or even antagonistic interactions that ultimately influence peatland carbon gain and storage. To begin to address this question, we are initiating a 5-year project using synthetic communities, genotype-to-phenotype associations, and metabolic characterization to address two overarching hypotheses, 1) *Sphagnum* host and diazotroph genetic variations play a key role in determining the environmental tipping point of beneficial symbiosis (i.e., environmental disruption), and 2) the surrounding microbiome can further adjust the tipping point through facilitation, competition, and antagonism.

The first year of this project is centered on developing the genetic and analytical resources necessary for synthetic community and transfer community experimentation. Through a JGI CSP, we now have draft genomes of 15 *Sphagnum* species and (re)sequencing for a 200-member pedigree is currently underway. On the microbial side, 72 *Sphagnum* associated heterotrophic bacteria strains, along with 12 cyanobacteria and 30 putative methanotrophs have been isolated on multiple medium types including N free. The JGI collaboration includes exometabolite characterization from a cross feeding experiment among *Sphagnum*, a cyanobacterium and a fungal partner. A pilot optimization experiment confirms that our synthetic community approach is amenable to exometabolite characterization with the identification of over 65 key metabolites. A complementary collaboration with EMSL is now adding spatial characterization of target metabolites among the tri-partite members through different phases of symbiosis using matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) along with

liquid extraction surface analysis (LESA). This approach allows for accurate metabolite structural information from LESAs and high spatial resolution from MALDI. Equipped with these resources, our team is now initiating experimentation to address the quantitative genetics of symbiosis, metabolite exchange and codependency, and ultimately how environmental perturbations interact with plant and microbial genetics to form and break symbiosis.

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