

Determining the genetic and environmental factors underlying mutualism within a *Sphagnum* peatmoss microbiome system

David J. Weston^{1*} (westondj@ornl.gov), Travis J. Lawrence¹, Alyssa Carrell¹, Megan Gable¹, Sara Jawdy¹, Adam Healey², John T. Lovell², Jeremy Schmutz²

¹Oak Ridge National Laboratory, Oak Ridge, TN; ²Joint Genome Institute, Walnut Creek, CA

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.

To facilitate the testing of hypothesis one, we collaborated with the DOE JGI to develop a *Sphagnum fallax* genetic map for quantitative trait analysis (QTL). A 220 member pedigree was established in tissue culture conditions with 167 individual genotypes resequenced to 7X depth. The initial map is 5395.77 cM in length with an average spacing of 1.8 cM between markers. The largest gap is 9.3 cM between markers. Overall there are 19 well-defined linkage groups with an additional linkage group possibly representing a sex chromosome or micro-chromosome. Furthermore, 15 additional *Sphagnum* species have been sequenced to enable comparative genomic analysis. The end goal of this approach is to link gene and gene family evolution to QTLs playing a key role in symbiosis and tolerance to environmental perturbations. This combined approach has the potential to reveal the history of selective pressure underlying symbiosis QTLs.

To address the second hypothesis, we developed a community transfer approach where native *Sphagnum* microbiomes conditioned to two-years of elevated temperature (ambient + 9 °C) or ambient temperature were isolated and applied to germ-free tissue culture *Sphagnum* and exposed to temperature manipulations. Our initial results indicate that the warming conditioned microbiome reduced *Sphagnum* mortality by 50% at warming temperatures relative to *Sphagnum* cultured with an ambient derived microbiome or no microbiome at all. Ongoing metagenome

analyses have assembled 38 microbial genome bins with greater than 90% completeness and less than 10% contamination. Relative abundance of binned genomes shows putative patterns of temperature specific taxa suggesting changes in metabolic potential. These data are now being augmented with metatranscriptomics.

The second year of this project will continue to link phylogenetic and quantitative genetic analyses to investigate the evolution of adaptive symbiosis traits. Furthermore, we are developing an additional pedigree and genetic map for *Sphagnum fuscum*, and will screen the temperature conditioned microbiomes across all *Sphagnum* pedigree members.

Funding: This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Early Career Research Program; and the DOE JGI Community Science Program 504399; and FICUS 504306.