**Microbiome transfer and synthetic community approaches for determining the genetic and environmental factors underlying mutualism within a *Sphagnum* peatmoss system**

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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 30% of terrestrial carbon as recalcitrant organic matter (i.e., peat). The foundation plant *Sphagnum* is responsible for much of the primary production in peatland ecosystems and produces recalcitrant dead organic matter. *Sphagnum* together with associated $N_2$-fixing microorganisms, contributes substantial nitrogen inputs to peatlands. *Sphagnum* growth and production (carbon gain) depends, in part, on a symbiotic association with $N_2$-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 initiated a project using synthetic communities, microbiome transfers, genotype-to-phenotype associations, and metabolic characterization to address two overarching hypotheses, 1) Genetic variation in *Sphagnum* host and associated diazotrophs 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 address the first hypothesis, we developed a synthetic community approach where strains of symbiotic cyanobacteria and moss genotypes from our sequenced pedigree population of 186 genotypes can be cultured together in symbiosis or separately and then cross-fed spent medium containing characterized exometabolites. Additionally, moss or cyanobacteria metabolite, metatranscriptomic and metaproteomic data were obtained. Using this approach, we were able to identify putative metabolites involved in nutrient and carbon transfer between community members. Specifically, we discovered the exchange of nitrogen- and sulfur-rich compounds between host and symbiotic cyanobacteria.

In an effort to link our findings from synthetic communities to the broader *Sphagnum* phylogeny, we sequenced 15 additional *Sphagnum* species and applied phylogenetic analyses. We discovered three putative whole genome duplications, two large chromosomal inversions, and

identified a potential hybridization event between hummock and hollow clades. Furthermore, we used gene family analysis to identify 15 genes under positive selection in hummock forming species including a sulfur transporter that was repressed in the cross-feeding study.

To address the second hypothesis, we developed a microbiome transfer approach where field collected *Sphagnum* microbiomes, conditioned to three-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. Consistent over two consecutive years, we found that *Sphagnum* grows better at elevated temperatures when inoculated with a warming-conditioned microbiome than when inoculated with an ambient microbiome or no microbes at all. Metatranscriptome data revealed that changes in microbiome nitrogen, carbon, sulfur metabolism and heat shock response differed as a result of microbiome origin (i.e., conditioned to warming or ambient field temperature). On the plant side, expression of stress related genes, including those encoding heat shock genes, were reduced at elevated temperatures when in symbiosis with a microbiome originating from warm field conditions. Future experiments will apply these communities across sequenced moss pedigrees for identification of plant genes mediating beneficial microbial interactions.

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