

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 N₂-fixing microorganisms, contributes substantial nitrogen inputs to peatlands. *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 initiated a project using synthetic communities, microbiome transfers, genotype-to-phenotype associations, and metabolic characterization to address the overarching hypothesis that genetic variation in *Sphagnum* host and associated microbiome play a key role in determining the environmental tipping point of beneficial symbiosis (i.e., environmental disruption).

To address this hypothesis and more specifically investigate the interaction of host genetics and microbiome thermal origin on host thermotolerance, we mechanically separated the microbiome from *Sphagnum* plants residing in a whole-ecosystem warming study, transferred the component microbes to a 180 member pedigree of germ-free plants, and exposed the new hosts to temperature stress. In some *Sphagnum* individuals the addition of a microbiome from a thermal origin that matched the experimental temperature completely restored plants to their pre-warming growth rates while other *Sphagnum* individuals did not receive a benefit from the addition of a conditioned microbiome. To confirm the results we repeated the experiment with a subset of *Sphagnum* genotypes that corresponded with the highest and lowest benefit from the addition of thermal conditioned microbiomes and found similar results. To identify candidate plant genes responsible for receiving thermal benefits from the microbiome, we performed genome scans using a single normal QTL (quantitative trait loci) model, Haley-Knott regression, and default parameters. A seven gene QTL region was identified and is being tested in heterologous expression systems. Together, our findings show that the microbiome can transmit thermotolerant phenotypes to host plants, providing a valuable strategy for rapidly responding to

environmental change. Future research efforts from this award are progressing in two areas: 1) candidate gene functional analysis for microbial provided thermal benefits for bioenergy crop improvement, and 2) field-based biogeochemical studies exploring the role of genetically predisposed *Sphagnum* genotypes to warming.

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