

## **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<sub>2</sub>-fixing microorganisms, contributes substantial nitrogen inputs to peatlands. *Sphagnum* growth and production (carbon gain) depends, in part, on a symbiotic association with N<sub>2</sub>-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 diazotrophs 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 importance of 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 germ-free plants, and exposed the new hosts to temperature stress. Although warming decreased plant photosynthesis and growth in germ-free plants, the addition of a microbiome from a thermal origin that matched the experimental temperature completely restored plants to their pre-warming growth rates. Metagenome and metatranscriptome analyses revealed that warming altered microbial community structure, including the composition of key cyanobacteria symbionts, in a manner that induced the plant heat shock response, especially the Hsp70 family and jasmonic acid production. The plant heat shock response could be induced even without warming, suggesting that the warming-origin microbiome provided the host plant with thermal preconditioning. Together, our findings show that the microbiome can transmit thermotolerant phenotypes to host plants, providing a valuable strategy for rapidly responding to environmental change.

In an effort to understand how broad these findings extend to other warming sites, we performed a microbiome transfer study using 5 donor *Sphagnum* species across global peatland warming sites spanning Sweden, France, Iceland and USA (SPRUCE sites). The microbiomes were transferred onto three germ-free *Sphagnum* recipients in the laboratory and exposed to a range of experimental temperatures. Growth rate of *Sphagnum* recipients was recorded over 4 weeks. Enhanced growth rates of recipient *Sphagnum* were measured when cultured with microbiome from a matching donor *Sphagnum* species and was 48% and 252% greater than the maximum growth rate of the non-matched and germ-free *Sphagnum* host-microbiome pairs, respectively. Normalized growth rates were maximized for matched host-donor pairs when the treatment temperature was similar to the origin temperature. Ongoing metagenome and metatranscriptome analyses are being conducted to determine if the microbiome influence on host HSP70s and jasmonic acid is conserved across these diverse conditions.

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