

## Stability of Switchgrass Leaf Microbiome in the Face of Natural Aerial Colonizers

Lukas Bell-Dereske<sup>1,2\*</sup> (belldere@msu.edu), and Sarah Evans<sup>1,2,3,4</sup>

<sup>1</sup>DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI, <sup>2</sup>W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, <sup>3</sup>Department of Integrative Biology, Michigan State University, East Lansing, MI, <sup>4</sup>Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI

<https://www.glbrc.org/research/sustainable-cropping-systems>

<https://rhizosphere.msu.edu>

**Project Goals: We are interested in exploring the role microbial communities play in sustainable growth of dedicated bioenergy crops and investigating the role of aerial dispersal on the assembly and stability of the switchgrass (*Panicum virgatum*) leaf microbiome. We will use this information to inform efforts to develop fungal inocula that can colonize and persist in the endosphere of switchgrass, with the potential to improve switchgrass disease resistance and drought tolerance.**

Plant-microbial interactions are critically important for the sustainable production of cellulosic energy crops. While the majority of previous research into plant-microbial interactions has focused on the root and rhizosphere community, plants also host a diverse leaf microbial community. This leaf microbiome has the potential to be utilized to improve host disease resistance, drought tolerance, and other benefits improving the yield of cellulosic energy crops. However, past efforts to manipulate and inoculate leaves with beneficial microbial communities have had little success, perhaps because communities establish from seed microbes and are difficult to invade. For this reason, it is important to understand what are the sources of the microbes that colonize the leaf community and the stability of this community in the face of outside, natural, colonists. We established two experiments to test the effects of aerial microbial colonists on leaf microbiome development of switchgrass (*Panicum virgatum*). To determine prevalence and magnitude of natural aerial dispersal into leaf microbiomes, we germinated and grew switchgrass seedlings in pots with sterile soil and placed them near mature switchgrass field monocultures. Seedlings were left in the field for 51 days, and we collected rain and dry deposited microbes throughout the period to characterize the abundance and composition of the aerial fungal colonists. We found that the fungal community inhabiting the endosphere of seedling leaves (endophytes) showed rapid and significant shifts that were driven primarily through enrichment of species found in the aerial community. Interestingly, the fungal endophyte community of the mature switchgrass leaves did not significantly differ from the seed microbiome but did host many aerial dispersed species. In our second experiment we tested the direct effects of single rain events on the development of the leaf microbiome of germinating seedlings. We germinated and grew seedlings for ~24 days in sterilized sealed petri dishes inoculated with either live rain, autoclave sterilized rain, or nanopure water. Seedlings that germinated in live rain showed significant shifts in the fungal endophyte community compared

to the starting seed microbiome but seedlings grown in both sterile treatments showed little to no shift in the leaf endophyte community, suggesting an active interchange between the leaf microbiome and the aerial community. In further support of this, we found that the temporal turnover of the aerial fungal community strongly tracked the seasonal development of switchgrass across the year and a half of rain events under analysis. Our results suggest that the leaf microbiome of seedlings has greater sensitivity to these aerial colonists while the mature endophyte community is more difficult to invade. The invadability of seedling leaf microbiome makes it a great target for inoculation of beneficial microbes, but this flexibility also suggests that it may be necessary to reinforce inoculations with repeated applications.

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