

**Title:** Enrichment of Soil Bacterial Taxa in Switchgrass (*Panicum virgatum*) Cropping Systems Across a Latitudinal Gradient

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**Project Goals:** The primary objective of this project is to gain a better understanding of the genetic drivers and mechanisms contributing to variation in switchgrass (*Panicum virgatum*) production and fitness across a broad climatic gradient. We have established experiments at multiple sites across the United States to evaluate switchgrass as a potential source of sustainable bioenergy feedstock and study its effects on ecosystem processes. Specifically, we aim to identify beneficial traits, genes, and microbial associates that may provide locally adaptive benefits to switchgrass and improve establishment, growth, and resiliency. Here we focus on soil bacterial community dynamics in switchgrass cultivars during a 3-year period following initial plant establishment.

**Abstract text:** Plants have evolved alongside soil microorganisms over hundreds of millions of years, resulting in the establishment of many mutualistic relationships. Soil microbial associates can provide protection from pathogenic microbes and regulate nutrient accessibility through organic matter decomposition and nutrient transformation processes. Additionally, variations in root morphology and/or root metabolite exudation may facilitate the active recruitment and enrichment of beneficial microbes, leading to the development of unique consortia of microbial associates, which may vary both between- and within-species depending on the local soil environment. Understanding the relationship between switchgrass and soil microbes, and how it influences the surrounding soil environment, may contribute to establishing switchgrass as an economically viable and sustainable bioenergy crop.

To study these relationships, we collected samples from a large, multi-institutional collaborative project where thirty plots of monoculture switchgrass stands were established at each of three sites across a latitudinal gradient (Fermilab, IL; Columbia, MO; and Temple, TX). Six cultivars were selected to represent a broad range of genetic diversity within switchgrass and five replicate 6m x 6m plots were arranged in a randomized complete block design. At each site, six soil cores were collected using a stratified random design in spring 2016 to characterize the soil environment prior to switchgrass planting. In 2018 and 2019, soil microbial communities in the root zone were targeted by collecting soil cores adjacent to switchgrass crowns at multiple time points throughout the growing season. Samples were homogenized and roots removed prior to subsampling for DNA extraction. Amplicon sequencing of the 16S rRNA gene was performed at DOE's Joint Genome Institute (JGI) to determine the bacterial community structure within each sample.

Principal coordinates analyses suggest the primary drivers differentiating soil bacterial community structure in the root zone were site and collection date (differentiated by year in MO and TX, and by early vs. late season in IL). Although soil bacterial community structure in the root zone did not vary distinctly among switchgrass cultivars, we did find evidence that suggests switchgrass may recruit and enrich certain bacterial taxa. Using differential analysis (DESeq2), we identified 2,638 bacterial amplicon sequence variants (ASV's) that were significantly ( $p < 0.001$ ) more abundant in samples from 2019 (3 years after switchgrass planting) relative to samples from 2016 (before switchgrass planting). Of those, we found 96 ASV's with log<sub>2</sub> fold changes greater than 20 (or  $> 1,048,576$ -fold change). Some taxa were enriched at multiple sites, indicating that regardless of the soil environment, these microbes share a tight association with switchgrass. For instance, we identified 3 ASV's belonging to the genus *Sphingomonas* that were highly enriched ( $> 20$  log<sub>2</sub> fold change) at all three sites. *Sphingomonas* likely play a role in plant pathogen resistance and may be a valuable member of the switchgrass microbial consortia by aiding in disease suppression.<sup>[1][2]</sup> Altogether, these results reveal specific soil bacteria that were affected by the presence of switchgrass and provide a first step in understanding the relationships between soil microorganisms and switchgrass. However, many questions remain.

Future efforts will explore whether switchgrass cultivars are locally adapted to recruit soil microbes specific to their site of origin, and determine what, if any, functional benefits these microbes may provide. Using shotgun metagenomics within the context of a nitrogen fertilization experiment, we will focus on microbially mediated belowground processes, especially pathways that regulate plant available nutrient supply, and examine the potential for microbially-mediated pathogen resistance. We are also using high-throughput bacterial isolation to establish a catalog of diverse switchgrass-associated microbes. To date, we have isolated over 1,200 unique bacterial strains from three switchgrass planting sites, with whole genome sequences being assembled for each isolate. The collection is diverse, encompassing over 68 bacterial genera from 5 different phyla. We are compiling synthetic communities to test the impact on plant nutrition, root architecture, and disease resistance. This collection will be an important shared resource for the switchgrass microbiome research community and could help to improve efforts towards sustainable switchgrass production.

## References/Publications

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