Testing predictions of plant–microbe–environment interactions to achieve climate adaptation and improve sustainability in switchgrass

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**OBJECTIVES**

Utilize switchgrass common gardens to continue studies of local adaptation and to identify beneficial candidate genes and microbial associates (bacteria/archaea, fungi, and viruses). Generate predictions of optimal plant ideotypes across adaptation zones and test these predictions with direct manipulations in the field.

Establish “sow, select, sequence” experiments to study the genetic and microbial drivers of seed dormancy, seedling recruitment, and establishment ability across broad environmental gradients.

Screen a novel *Panicum* mutant population to discover genes controlling key traits and plant–microbe interactions.

Investigate candidate genes affecting switchgrass traits (e.g., phenology, seedling recruitment, winter-survivorship, root system allocation & composition, microbial associations) using gene-editing experiments.

Expand existing switchgrass microbial isolate collections with additional collecting across field sites, host material, and years. Use microbial selection experiments to manipulate microbial communities. Study individual microbes, synthetic communities, and evolved communities and their role in stress tolerance using growth chamber, greenhouse, and field experiments.

Evaluate the impact of switchgrass traits and associated microbial diversity on ecosystem processes in stand plantings. Conduct experimental manipulations within existing stands to test predictions about effects of climate change and resource limitation on switchgrass biomass productivity and soil carbon sequestration.

**Project Description:** We needmore carbon-neutral energy sources to reduce greenhouse gas emissions and their forcing effects on climate change. There is growing interest in the potential of biofuels for meeting this need. A critical question is whether biofuel production can be ***sustainable*** over the time scales needed to mitigate our carbon debt from fossil fuel consumption. The long-term productivity of biofuels depends on the environmental factors limiting plant growth. These factors are often related to soil resources that involve complex interactions at the plant–microbe–soil interface. Our collaborative project will address sustainable switchgrass production by exploring **Plant Systems**, **Plant-Microbiome**, and **Ecosystem Processes** through the integrative lens of **Multi-Scale Modeling**.

**Progress from Earlier DOE funding:** The first cycle of the DOE Sustainability program supported our group’s studies of adaptation and plant–microbe–soil interactions in switchgrass (DE‐SC0014156). Our work centered on collecting and resequencing switchgrass natural diversity, then characterizing this diversity by establishing common gardens and stand plantings that span the species’ geographic range. A key goal of the research was to understand gene-by-environment interaction (GxE) as a driver of plant responses to environmental variation. We resequenced ~950 switchgrass genotypes, established sixteen garden sites spanning 24˚ degrees of latitude across North America, and collected five years of field data. These data detail the evolutionary history of switchgrass, provide insight into the key gradients affecting switchgrass performance, characterize patterns of GxE and tradeoffs across sites and years, and allow robust genetic mapping of adaptation-related traits. A key result is the identification of candidate genes that influence niche breath and adaptation. Our work also characterized the switchgrass microbiome and identified genomic regions that drive microbial community assembly. Finally, our stand-planting studies provide a benchmark on the impact of perennial grasses on ecosystem processes. Together, our results provide a rich framework for studying switchgrass biology in support of biofuel feedstock development. These studies generated testable predictions about the interplay between plant traits, genes, and microbes. Here, we propose new experiments to test these predictions.

**Plant Systems:** We will leverage our established diversity panel and common gardens to expand the scope of our new research. We will propagate (and share) material as before and will maintain most of our gardens under new funding. We will increase the power of our longitudinal studies of GxE by regularly phenotyping gardens for key traits, focusing effort on high-throughput phenotyping platforms (Juenger, Fritschi) and machine-learning based image analyses (Zare). In close collaboration with DOE JGI, we will expand population genomic studies of switchgrass, including an expansion of reference genome assemblies and pan-genome studies (Juenger, Lowry, Schmutz). This effort will help to build our understanding of switchgrass genome evolution, as well as hone resources for functional genomic studies.

A key component of our new project is the development of a genetic manipulation pipeline. This pipeline will leverage advances in gene editing to test new predictions about gene function. We have completed extensive genetic mapping which has helped to identify candidate genes for traits and their interactions with environmental variation. We will make new crosses to recombine key traits associated with desirable ideotypes and will plant these new lines in gardens to test performance predictions. We will initiate transgenic studies using both diploid *Panicum hallii* and tetraploid *P. virgatum*, with a focus on candidate genes related to flowering time, abiotic stress tolerance, and belowground root and microbiome related traits. We plan to incorporate a novel reverse genetics approach to validate candidate genes using our newly developed *P. hallii* fast neutron mutant population. Swaminathan (HudsonAlpha) will spearhead our genetic manipulation pipeline, with support from team members leading biological studies of specific candidate genes (Juenger, Lowry, Bartley, Fritschi).

We now have a growing understanding of traits influencing adaptation for adult spaced plants (grown as specimens in low-density plantings). However, we know much less about the traits or genes that affect establishment at the seedling stage and performance in denser competitive stands. We will establish new experiments using a “sow, select, and sequence” strategy at the existing garden sites to discover traits, genes, and microbes affecting establishment. These studies will rely on highly replicated plot plantings of diverse seed pools exposed to natural conditions. We will use high-throughput sequencing to identify genes under natural selection by determining changes in allele frequencies over the course of replicated establishment. In addition, we will identify a set of “top lines” from our existing diversity panel studies to evaluate in seeded stand plantings to confirm performance in competitive environments and to initiate studies of their impact on ecosystem processes.

**Plant Microbiome:** There is growing appreciation that plants are intimately associated with diverse microbes. We have characterized the switchgrass microbiome using culture and sequence-based assays. These methods have identified thousands of microbes and quantified their abundance across the species range. In parallel, we have used our genetic mapping population to identify genome regions influencing associations with individual microbes, as well as genomic hotspots that appear to drive abundances of microbial consortia. Together these studies have generated exciting hypotheses about plant–microbe interactions and their role in adaptation and stress responses.

Our new projects will study individual microbes, and synthetic and evolved communities as well as their role in stress tolerance using growth chamber, greenhouse, and field experiments. We will target microbes with clear evidence of host interaction and differentiation and likely functional significance based on existing annotation. We will use natural microbial inoculates and lab cultures to generate diverse microbial communities. We will use a novel microbial selection approach to evolve these communities to increase plant performance, targeting seedling establishment and performance under stress. We will test these communities against diverse switchgrass material to examine mechanisms driving plant–microbe interactions and to evaluate their impacts on plant performance and sustainability attributes.

**Ecosystem Services:** Switchgrass is a promising feedstock because of its widespread adaptability and productivity. However, the degree to which biomass can replace fossil fuels depends on the long-term sustainability of biomass production. We have begun to use our field plantings to evaluate sustainability based on yield stability and impacts on ecosystem processes, including carbon sequestration, gas exchange, and nutrient and water cycling. Our initial data have identified strong Site x Year and Cultivar x Site x Year interactions for many response variables. Overall, lowland cultivars had higher biomass production in the south and variable productivity in the north, and upland cultivars showed the opposite north-south pattern.

To assess sustainability, it is critical to conduct longitudinal studies over sufficient time because ecosystem properties can exhibit response inertia. For example, soil carbon and nutrient stocks respond on a timescale of years to shifts from annual to perennial plantings. As our established stands offer an invaluable opportunity to detect and characterize such shifts over time, we will continue monitoring these stands over the course of new funding. We will also add new treatments to our existing stands to expand our scope to explore the impact of abiotic stress and climate variability on ecosystem drivers. For example, we are likely to include new subplots that initiate soil-warming treatments and manipulations of limiting nutrients. Coupling natural variation in site–soil–climate relationships with experimental manipulations will improve our ability to characterize mechanisms underlying beneficial plant–soil–microbe interactions.

**Multi-Scale Modeling:** Multi-scale integration of information and understanding is an important goal of this project. We will link information from several levels of analysis and fields of study to improve prediction across spatial and temporal heterogeneity, including predictions across future climate scenarios. Behrman and colleagues have used process-based plant growth models scaled across space and time to predict biomass yields based on parameters for switchgrass obtained from our field trials. We have also explored the use of tolerance curves obtained from common garden plantings to make trait-based predictions of switchgrass range distributions and performance. We will strengthen and test the sensitivity of predictions with expanded data, then utilize forecasts from these initial models to design specific field plantings that test plant performance across soil–climate gradients. Ultimately, these studies will help to identify critical plant–microbe–soil traits that can be harnessed, through breeding or agronomic management, to improve the sustainability of biofuel feedstocks.