

**Connecting nitrogen transformations mediated by the rhizosphere microbiome to perennial cropping system productivity in marginal lands**

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**Project Goals: We will perform a robust assessment of components of the rhizosphere microbiome-plant system by linking microbial and plant genomics and transcriptomics to N-cycling and C allocation and physiology across sites and over time (Fig. 1). We aim to understand how the rhizosphere microbiome associated with perennial biofuel crop systems (PBCS) in marginal lands:**

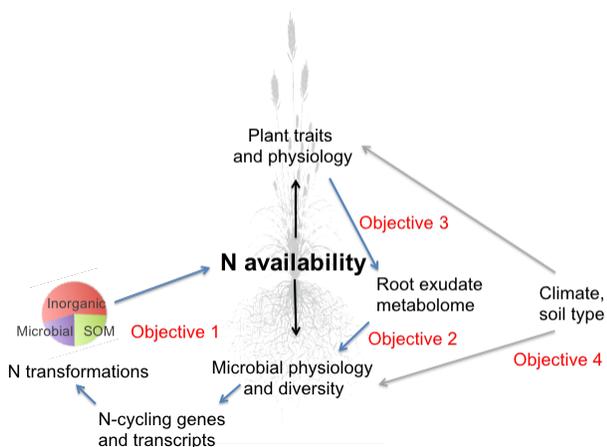
- 1) Mediates N transformations important to plant N availability;**
- 2) Acquires C resources from plants versus SOM to fuel N-transformations;**
- 3) Interacts with plant root exudation patterns and physiological pathways;**
- 4) Varies by cropping system and environment.**

The demand for energy from biofuel production is increasing, prompting concerns about the environmental impact and long-term sustainability of bioenergy cropping systems. While many recent life cycle analyses of bioenergy sustainability focus on soil organic matter (SOM) accrual and overall carbon (C) budgets, there has been less attention paid to nitrogen (N) dynamics in these systems. N is the most commonly limiting nutrient for plants, especially in marginal lands that are unsuitable for food crops because of low productivity and vulnerability to environmental stress. The introduction of perennial bioenergy cropping systems (PBCS) in marginal lands can improve whole system N use efficiency and N retention. However, **little is known overall about N-cycling and associated microbial function in marginal land biofuel cropping systems.** It is particularly important to understand the mechanisms regulating nutrient acquisition by microbes and plants, as well as SOM accrual, if we are to maximize the productivity and C benefits of these systems. **We aim to understand how the rhizosphere microbiome in PBCS on marginal lands:** 1) mediates N transformations and availability; 2) gains C resources to fuel N-transformations; 3) affects PBCS productivity and SOM accrual through trade-offs between plant C allocation to support N-transformations versus biomass accrual and; 4) changes by cropping system and environment (Fig. 1).

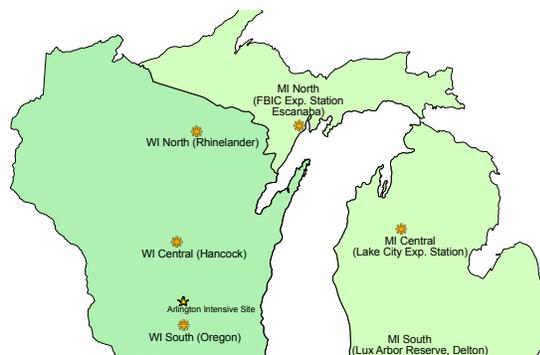
We will investigate experimental questions related to this goal using sites recently established as part of the DOE Great Lakes Bioenergy Research Center (GLBRC) in the upper Midwest (Fig. 2). On each site, six perennial crops have been established in 20 x 20 m plots replicated in four blocks together with an unplanted reference plot. We will take measurements on the reference plots, switchgrass, miscanthus, a 5-species native grass mix and a restored prairie consisting of an 18-species assemblage of native forbs, grasses, and legumes. In addition, we will sample fertilized sub-plots that will allow us to more fully explore N dynamics.

To address our objectives, we will use a systems approach that links microbial N cycling genes to N fluxes. We will first assess functional gene diversity via metatranscriptomes at each site over 3 years, followed by seasonal assessment via HTqPCR of the diversity of functional genes and their transcripts. To do this we will employ a new HTqPCR system (SmartChip Real-Time PCR Cycler, WaferGen Biosystems Inc., USA) followed by sequencing of the barcoded PCR products directly on the Illumina MiSeq platform in the MSU Core Genomics Facility. We will also measure gross N flux rates using standard biogeochemical assays and <sup>15</sup>N tracers to evaluate N transformations and plant N sources in conjunction with measures of gene diversity and expression. We will also perform two experiments alongside our field monitoring efforts: 1) <sup>13</sup>CO<sub>2</sub> pulse-chase experiments in subplots of the field experiment to measure total root exudate production. 2) We will also determine the chemical composition of exudates by isolating and analyzing the root exudates of different bioenergy crops under sterile lab

conditions using LCMS and GCMS. Exudate chemicals we identify will be used to make synthetic exudate solutions that can be isotopically labeled and applied to soils in both the greenhouse and the field. Synthetic exudates will allow us to test the effects of different chemical compositions on microbial community composition, physiology, and N-cycling processes.



**Figure 1. Conceptual illustration of project objectives**



**Figure 2. Marginal Land Experiment Sites, part of the DOE Great Lakes Bioenergy Research Center in the upper Midwest, USA**

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