

Vertical niche analysis of rhizosphere and soil fungal and bacterial communities: A deep look into sustainable biofuel cropping systems

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Project Goals: The main goal of the Great Lakes Bioenergy Research Center is to develop sustainable biofuels and bioproducts from all usable portions of dedicated energy crops grown on marginal or non-agricultural lands. One tool we are exploring to sustainably improve the productivity of switchgrass (*Panicum virgatum*) biomass is the plant microbiome. Here we report on amplicon sequence analysis of fungi and bacteria from soils and roots sampled across different depths at the GLBRC Biofuel Cropping System Experiment. The aim of this research is to assess how the switchgrass rhizobiome relates to other plant species, how it correlates to plant biomass, and how it changes according to soil depth. Ultimately, we seek to uncover key microbes that can be used and manipulated to improve plant productivity, health and resilience in marginal lands.

Plant-microbe interactions can be extensively exploited for sustainable biofuel cropping. It is well established that fungi and bacteria living in and around plant tissues directly affect plant physiology and development, and that understanding the details in the dynamics of microbial communities is critical for 21st century agriculture. Advances obtained through Next Generation Sequencing (NGS) technologies have shown, however, that decrypting the plant microbiome is a long-term endeavor. This is due to the inherent complexity of microbial communities and plant-microbial interactions. Further, soils deeper than 20cm compose a poorly understood and characterized environment, but are likely to have important implications on switchgrass development, as *Panicum virgatum* develops long and deep root systems. Here, we utilize integrated research resources from the GLBRC Biofuel Cropping System Experiment to explore the microbial community composition over a soil-depth profile, with the ultimate goal of modeling how soil microbial networks affect switchgrass sustainable production. The hypotheses we are testing are that: (1) soil depth will create a non-linear frequency gradient of specific microbial taxa; (2) higher connectivity between soil and root compartments across the depth gradient will be positively correlated to plant health and microbial biodiversity; (3) significant correlations between microbe OTU frequency and crop yield will be higher in native species than in exotic species, especially in deeper soil layers less exposed to perturbations; (4) communities with multiple plant species will have more complex co-occurrence OTU networks compared to monocultures, with an inverse relation between soil depth and network complexity; (5) occurrence and relevance of indicator genera across soil depth will be correlated to higher

network connectivity of the same genera. To test these hypotheses, soil cores with depths of 0 to 10cm, 10 to 25 cm, 25 to 50cm, and 50 to 100cm were collected from plots with native switchgrass (*Panicum virgatum*, var. Cave-in-rock) monocultures, exotic hybrid poplar (“NM-6”, *Populus nigra* x *Populus maximowiczii*) monocultures, and restored prairie 17-species mix. Three representative samples were obtained from each of the 5 plot replicates, totaling 180 samples that were divided between soils and root fragments. Total DNA was extracted from these samples and amplified with the primer set 515F/806R targeting the V4 region of prokaryotic 16S rDNA and ITS1f/ITS4 targeting fungal internal transcribed spacer (ITS) rDNA region. Amplicon libraries were sequenced on the Illumina MiSeq platform. A total of 20,515,462 raw reads were obtained with the ITS1f/ITS4 primer in 390 samples (including controls), while 25,205,138 raw reads were obtained with the 515F/806R primer set in 387 samples (including controls). Data filtering, standardization, and preliminary analysis are currently underway.

References

1. Zhang B, Penton CR, Xue C, Quensen JF, Roley SS, Guo J, Garoutte A, Zheng T, Tiedje JM (2017) Soil Biology & Biochemistry Soil depth and crop determinants of bacterial communities under ten biofuel cropping systems. *Soil Biol Biochem* 112:140–152. doi: 10.1016/j.soilbio.2017.04.019.

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