Succession of Rhizosphere Biotic Communities During Switchgrass Establishment in Marginal Soils

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Project Goals: Switchgrass (SG; *Panicum virgatum* L.) is a perennial C_4 grass native to the tallgrass prairies and a most promising feedstock in the U.S. for bioenergy production. Capable of abundant biomass yield with minimal fertilizer or water, SG can survive on marginal soils, and even thrive once established. We hypothesize that successful establishment and sustainable cultivation of SG in marginal soils are, in part, enabled by beneficial plant-microbial interactions. We are investigating the succession of rhizosphere microbial communities, and ecosystem-scale effects of high- and low-performing SG plants grown in nutrient-limited soils in southern Oklahoma. The outcome of this research will provide a better genomic basis for SG cultivation in marginal soils, expand our knowledge of the interactions between soil microbes, plants, and ecosystems.

In the soils surrounding roots (rhizosphere), biotic, chemical and physical drivers enrich for specific biotic communities. To generate a better understanding of the plant-microbe interactions that facilitate switchgrass cultivation in marginal soils, we investigated the succession of rhizosphere communities at multiple trophic levels during switchgrass establishment in marginal soils. Two research farms (Red River Farm and Third Street Farm) featuring 'marginal' nutrient soils were selected from Southern Oklahoma, and genetically diverse switchgrass seedlings (lowland ecotype Alamo) were planted at both sites in the spring of 2016. Rhizosphere soils were sampled from sixty plants (thirty from each site) at five time points, corresponding to different plant developmental stages. Plant growth was monitored at the same time. We then surveyed the rhizosphere communities by amplicon sequencing of phylogenetic marker genes specific to bacteria (16S), fungi (ITS), and soil protozoa (18S).

The two marginal soils surveyed in this study have distinct background communities. Regardless of the site effect, the bacterial, fungal, and protist communities all showed a significantly lower diversity in the rhizosphere than those found in the background soils at both sites. This indicates that switchgrass plants grown in the marginal soils exert a selective effect on the soil bacterial, fungal and protist populations. The composition of rhizosphere communities also differed from that of the background soils. Betaproteobacteria and Gammaproteobacteria were enriched, and Firmicutes, Verrucomicrobia and Planctomycetes were depleted in the rhizosphere at both sites. The enrichment of beta-proteobacteria in the rhizosphere was particularly dramatic at Red River site (silt loam). Rhizosphere fungi that were enriched included Eurotiomycetes (Red River, silt loam) and Leotiomycetes (Third Street, clay loam). Specific groups of protozoa were also recruited to the rhizosphere, including those feeding on bacteria and fungi from the genera Rhogostoma, Cermononas, and Allas.

Rhizosphere bacterial, fungal, and protozoan communities all dynamically changed over the growing season. In the early growing season (T1-T3), the rhizosphere bacterial communities strongly differed from their background populations, while in the late plant developmental stages (T4 and T5), they became more similar to background communities. Moreover, specific bacterial OTUs were significantly correlated with plant biomass production at the first three time points. Analysis of ITS amplicons showed fungal communities in the background soils changed over time, and rhizosphere communities became more dissimilar from background communities at late plant vegetative growth stages (T2) and late in the growing season. The rhizosphere protist communities differed from those found in the background soils starting from two months after planting (T2); the largest difference occurred at the end of the growing season (T5).

We assessed if these differing community-level responses are due to different mechanisms of rhizosphere community assembly for bacteria, fungi, and protists. Both decreased stochasticity and increased selection were observed in the switchgrass rhizosphere for bacterial communities (Red River site) and fungal communities (Third Street site). This site-dependent rhizosphere selection might be related to the baseline of the soil communities: Red River soils exhibited generally high bacterial diversity, whereas Third Street soils have relatively high fungal diversity. Rhizosphere-enhanced selection of bacteria and fungi was most evident in later plant developmental stages (T3 and T4). Switchgrass roots also increased the importance of dispersal limitation in microbial community assembly, which was more obvious in Red River. In general, homogeneous selection mechanisms were more influential for bacteria rather than fungi or protists; heterogeneous selection was more critical for protists; and dispersal limitation showed a more noticeable impact on fungi.

In conclusion, during establishment in marginal soils, switchgrass plants recruited specific groups of bacteria, fungi, and protists into their rhizosphere, leading to less stochastic and more selected rhizosphere communities that are distinct from background soil communities in diversity, composition and structure. These rhizosphere communities were strongly influenced by plant developmental stages, and different microorganisms (bacteria, fungi and protists) had distinct successional patterns over the growing season. The specific assembly mechanisms of rhizosphere communities and their dynamic changes with the plant development may play an essential role in switchgrass establishment in marginal soils.

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