Responses of Total (DNA) and Metabolically Active (RNA) Microbial Communities in *Miscanthus* x *giganteus* Cultivated Soil

Authors: Jihoon Yang[1]* (jhyang@iastate.edu), Jaejin Lee,[1] Emily Heaton,[2] and **Adina Howe**[1]

Institutions: [1]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa, [2]Center for Advanced Bioenergy and Bioproducts Innovation, Urbana, Illinois

Project Goals: *Miscanthus* x *giganteus* is one of the most efficient bioenergy crops and is supported by an active soil microbiome. Previous studies of the role of this microbiome in supplying nitrogen to miscanthus have produced inconsistent results. In this study, we hypothesize that this may be due to methods used to identify the active membership of the soil microbial community. We compared RNA-based to DNA-based soil microbial community structure analysis to improve the identification of metabolically active microbes. We also identified the impacts of management, such as stand age and nitrogen fertilization, on microbial community membership. These efforts provide insight into the best methods to study plant-soil-microbial interactions and the role of microbes in miscanthus production.

Introduction

Miscanthus x giganteus is a promising high-yielding bioenergy crop to meet growing bioenergy demands with little fertilizer compared to other bioenergy crops. Although plant-soil-microbe interactions were known to affect the productivity of *M.* x *giganteus* at various fertilization rates, previously performed characterization of the microbial community has been limited to DNA-based analysis of potentially active microbial membership. Therefore, there were limitations to identifying metabolically active microbial membership that plays a role in the nitrogen cycle. In this study, we compare DNA and RNA approaches to expand our understanding of how soil microbiome can impact the sustainable production of *M.* x *giganteus*.

Research approach

Two-, three-, and four-year-old *M.* x *giganteus* soil samples (n=271) from replicated blocks receiving 0, 224, and 448 kg ha^{-1} N were collected in 2018. Paired DNA and RNA extractions were performed using MagAttract PowerMicrobiome DNA/RNA EP kit (Qiagen, USA). The 16S rRNA gene amplicon sequencing of both extracted DNA and RNA was performed on an Illumina Miseq platform (Argonne National Laboratory). The DADA2 package in R and the RDP classifier were used for taxonomic identification of observed ASV (amplicon sequence variants). Permutational multivariate analysis of variance (PERMANOVA) was used for statistical comparison based on Bray-Curtis dissimilarity matrix.

Results

DNA and RNA microbial communities were significantly different, although similar numbers of microbial membership were detected. The profiles of the dominant microbial membership within

DNA and RNA showed significant differences in the proportions of *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. There was a seasonal response to nitrogen fertilization only in the RNA microbial communities, and this difference was associated with nitrogen-cycling bacteria with a relative abundance 7-fold higher in RNA than in DNA. Among them, genes associated with denitrifying bacteria are significantly abundant in RNA, suggesting they can be underestimated with DNA-only approaches.

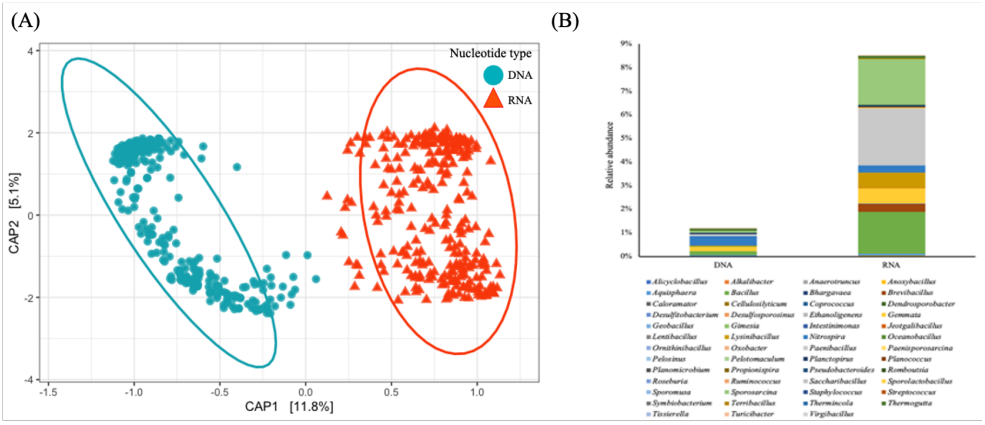

Figure 1. Difference between DNA and RNA microbial communities in *M.* x *giganteus* cultivated soil for (A) microbial membership and (B) abundance of nitrogen cycle associated bacteria.

Summary

We found that DNA and RNA-based methods for characterizing the microbial response to management changes showed different results. The RNA-based method appears to capture better the response of microbial membership known to be associated with nitrogen cycling. Increasing numbers of microbial ecology studies are identifying the environment or gradient for which the microbial community is changing. Future work needs to highlight which taxa or function is changing, and our results indicate that RNA-based SSU characterization can be a resource.

References/Publications

1. Heaton et al. Meeting US biofuel goals with less land: the potential of Miscanthus. Glob Chang Biol. 14, 2000-2014 (2008).
2. Ma et al. The impact of stand age and fertilization on the soil microbiome of Miscanthus x giganteus. Phytobiomes J. 689 (2020).
3. Yang et al. Response of total (DNA) and metabolically active (RNA) microbial communities in Miscanthus x giganteus cultivated soil to different nitrogen fertilization rates. Microbiol. Spectr. In revision

Funding Statement: *This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Energy.*