

Comparison of Actively Growing (RNA) and Potentially Active (DNA) Soil Microbial Communities in *Miscanthus x giganteus*

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Project Goals: This study provides insights into plant-soil-microbe interactions that are associated with fertilization management and stand age in miscanthus. Actively growing microbial communities (RNA) are used to identify key taxa and functions that contribute to nitrogen cycling. These results are directly compared to observations from DNA-based characterizations, where varying patterns have been observed. This effort will provide clearer insights into how to sustainably manage nitrogen needs in CABBI feedstocks based on microbial community characterization.

<https://cabbi.bio/research/sustainability-theme/>

Introduction

Miscanthus x giganteus is a promising bioenergy crop because of its ability to produce a large amount of biomass. Plant, soil, and microbe interactions have been shown to influence its productivity under varying fertilization rates and stand ages. Previously, the characterization of microbial communities has been limited to DNA-based analyses which provide information on community membership but not activity. In this study, we use RNA-based approaches to expand our understanding of the impacts of the actively growing soil microbial community on the sustainable production of *M. giganteus*.

Research Approach

Soil samples (n=403) from plots receiving 0, 200, and 400 kg N ha⁻¹ were obtained from fields containing two-, three-, and four-year-old *M. giganteus*. Paired DNA and RNA extractions were performed using the MagAttract PowerSoil DNA EP kit (Qiagen) and Rneasy PowerSoil Total RNA kit (Qiagen), respectively. Amplicon sequencing was performed with 150 bp paired-end libraries for the 16S rRNA gene of both extracted DNA and RNA (Illumina Miseq platform, Argonne National Laboratory). Taxa associated with DNA and RNA communities were identified using the DADA2 package and annotated with the closest representative in the RDP classifier. Statistical comparisons of enriched taxa under fertilization and differences between RNA and DNA communities were made with PERMANOVA and PCoA using the Bray-Curtis dissimilarity matrix.

Results

RNA and DNA membership were observed to be significantly different in our miscanthus soils (Figure 1A), highlighting the need to understand and differentiate actively growing communities in response to management strategies. The difference between RNA- and DNA-microbial communities was largest in the more mature miscanthus stands, with significant differences observed in three- and four-year-old miscanthus (Figure 1B). The effect of nitrogen fertilization on actively growing communities was most pronounced in the four-year-old miscanthus stands (Figure 1C). In these communities, nitrogen fertilization generally reduced the proportion of taxa

associated with nitrogen fixation and nitrification. Additionally, taxa identified as unique to actively growing microbiomes in three-year and four-year-old miscanthus include the nitrifying bacteria including *Nitrosomonas*, *Nitrospira*, and *Sporosarcina* (Figure 1D).

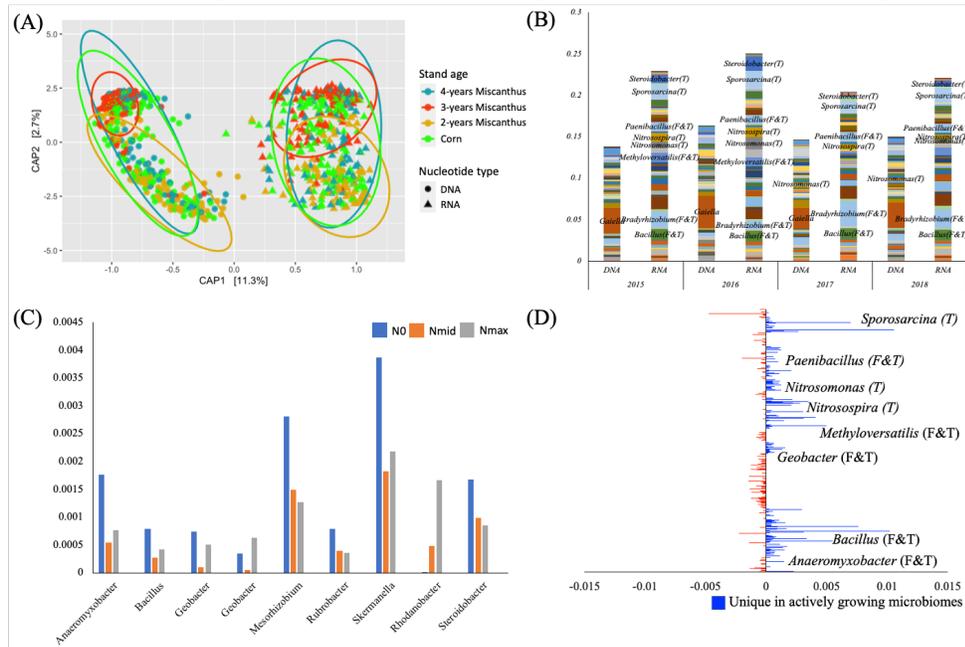


Figure 1. (A) microbial membership difference between actively growing and potentially active microbiomes, (B) abundance of nitrogen cycling bacteria with different stand ages of miscanthus, (C) differences in the nitrogen-fixing and nitrifying bacteria with different concentration of fertilizer, and (D) unique bacteria in the actively growing microbiomes.

Summary

The potentially active (DNA) and actively growing (RNA) microbiomes in miscanthus are different. Nitrogen-fixing and nitrifying bacteria were 1.5 times more abundant in the actively growing soil microbial communities compared to DNA-based estimates, suggesting that these communities can provide contrasting insights into responses to N-cycling. The actively growing microbiome of mature miscanthus was associated with less turnover, in contrast to younger miscanthus where the actively growing community had a more variable impact of fertilization.

Future Work

Seasonal patterns of microbial response to fertilizer between RNA and DNA approaches will be examined, with the hypothesis that more mature miscanthus will have a more predictable response in the actively growing membership. The role of actively growing microbiomes will be further explored through the measurement of N-cycling genes in RNA extracts.

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