

Characterizing bacterial and fungal communities associated with plant drought tolerance

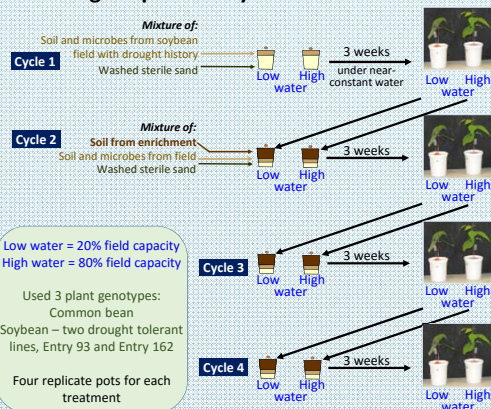
Chiliang Chen¹, Jeffrey Williams¹, Bo Xie¹, Shulin Yang² and Gwyn A. Beattie¹

¹Dept of Plant Pathology & Microbiology, Iowa State University; ²School of Life Sciences, Guizhou Normal University, China

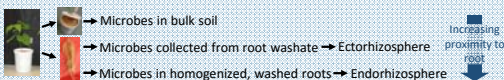
Introduction

Water limitation is a major problem for crop production worldwide. Plant microbes are known to enhance drought stress tolerance, we therefore applied a systematic approach to begin to associate microbial community members with plant tolerance to water deficits. We subjected soybean and bean plants to successive cycles of growth under water-limited and water-rich conditions with the goal of enriching for beneficial communities under each condition. By characterizing the microbial communities near, on and in roots, we were able to assess the relative impact of the root, water, plant species, and enrichment cycle on microbial community composition, and to reveal specific bacterial and fungal taxa that thrive under low or high water conditions. These studies provide insights into the role of water as a driver that shapes microbial communities.

Enriching for potentially beneficial communities



Methods and analysis



- Total DNA was extracted from each sample and used for the amplification of microbial biomarkers: 16S rRNA gene for bacteria (with 340F/806R primers) and ITS1 for fungi (with ITS1F/ITS2 primers)
- Amplicons were purified, normalized and pooled into a bacterial library and a fungal library
- Libraries were assessed for quality (Bio-analyzer) and concentration (qPCR)
- Libraries were sequenced using a MiSeq platform and 2x300 paired-end reads, with separate runs for the bacterial versus fungal communities
- A bioinformatics pipeline was applied that included Cutadapt, Trimmomatic, QIIME (1), and UPARSE (2) for processing sequencing data and generating OTU tables
- Data presented include an average of 22,500 and 19,500 high-quality reads (Q>30, avg length 264 bp) per sample for bacterial and fungal communities, respectively
- Taxonomy was assigned to individual OTUs at a 97% identity using SILVA and UNITE databases, respectively, and plant OTUs were removed
- Statistical analyses were performed using Phyloseq (3) and LEfSe (4) based on weighted and unweighted UniFrac distance or Bray-Curtis dissimilarity

Acknowledgements

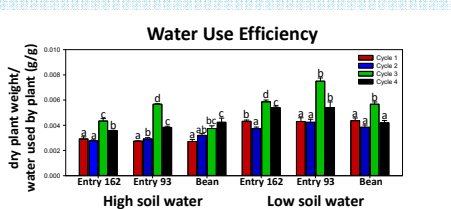
We thank Larry Purcell, Univ Arkansas, for the field soil and drought tolerant soybean lines.

This work was funded by the Iowa State University Plant Science Initiative.

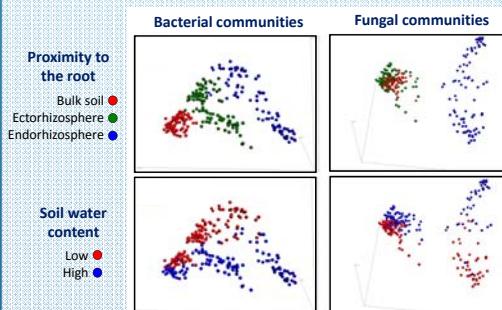
References

- Caporaso et al. (2010) *Nat Methods* 2010, 7:335-336.
- Edgar (2013) *Nat Methods* 10:996-998.
- McMurdie and Holmes (2013). *PLoS ONE* 8(4): e61217.
- Segata et al. (2011) *Genome Biol* 12:R60.

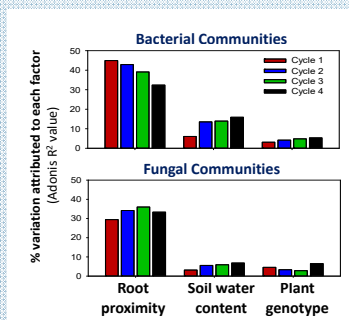
Plant water use efficiency significantly increased with successive plant growth cycles



Soil water content influenced bacterial and fungal community composition, and had a greater effect on and in roots than in the bulk soil

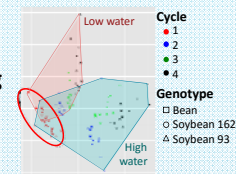


The experimental factors that influenced the microbial community composition all had significant effects (P<0.05) on both the bacterial and fungal communities

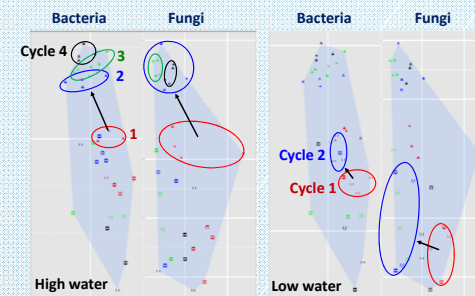


The soil water content had an increasing impact as the enrichments continued, particularly for the bacteria

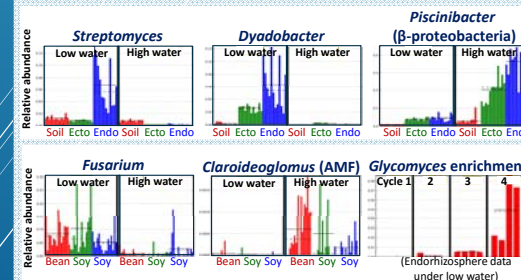
Endorhizosphere microbial communities were initially similar but diverged during subsequent enrichment



Large shift in endorhizosphere microbial communities occurred between the first and second enrichment cycle under both high and low water conditions



The drought treatment strongly favored specific bacterial and fungal taxa in the endorhizosphere



Conclusions

- Our studies support the following ranking of drivers of microbial community development under these conditions: **root proximity > water availability > serial plant growth cycle > genotype**
- The improved water use efficiency after only two successive plant growth cycles coincided with a noticeable shift in microbial community structures, supporting the possibility that plants rapidly recruit microorganisms to enhance drought tolerance
- Similar changes in taxa across three plant genotypes provided evidence for reproducible community development patterns in response to soil water content