In contrasting cropping systems, root-associated prokaryotic and fungal communities exhibit distinct shifts at different maize developmental stages

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Hypothesis

Due to differences in fertilization of contrasting agricultural systems, the root-associated microbial communities of maize will differ most at stages of rapid growth and high nitrogen uptake compared to stages of slower growth and low nitrogen uptake.

Background

- Root-associated microbes play an important role in liberating nutrients for plant uptake[1]
- Plant roots select for microbial communities by exuding C and altering soil abiotic properties[1]
- Agricultural management alters soil microbial communities and their functions[2, 3, 4]
- The interactive effects of root selection and agricultural management on soil microbial communities are still poorly understood

Field Site and Sampling

- Conventional Soil (**Conv.**): 2-yr (corn-soybean) rotation, inorganic fertilizer
- Diversified Soil (**Div.**): 4-yr (corn-soybean-oats/alfalfa-alfalfa) rotation, manure application
- Bulk soil, rhizosphere soil, and rhizoplane sampling (Fig. 1)
- Sampled at four time points (Fig. 2)
- Three samples per plot (9 reps total)

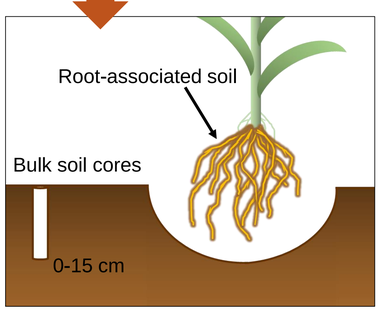


Fig. 1. Soil harvest. Bulk soil was collected by coring the first 15 cm of soil between corn rows. Rhizosphere and rhizoplane (root-associated) soil was collected by shaking/scraping and then vortexing/sonicating the root respectively.

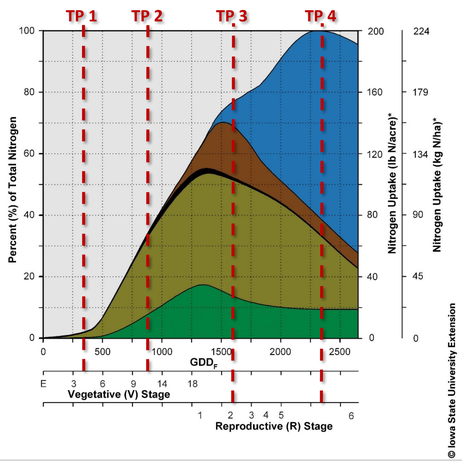
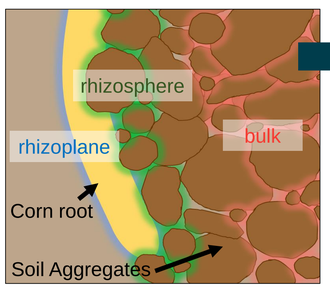


Fig. 2. N uptake by maize[6]. Samples were taken at V4, V11, R2, and R5 at 21, 41, 68, and 105 days after sprouting respectively.

Data Analysis



Illumina Miseq 16S and ITS DNA amplicon sequencing

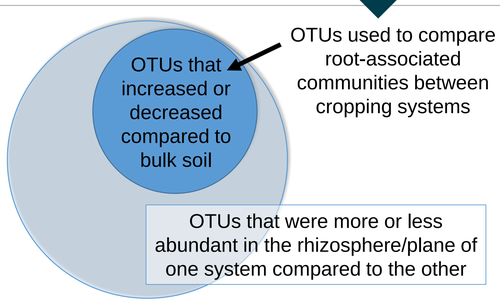
Quality Filtering, Clustering, Taxonomic Assignment

Beta Diversity

- CSS normalization[7] with singletons removed
- Phyloseq[8] in R v 3.3
- Bray-Curtis Dissimilarity

Differential Abundance

- DESeq2[9] in R v. 3.3
- Significance at $\alpha < 0.01$
- Comparisons of bulk soils, rhizosphere soils, and rhizoplanes between soil systems (see right for how root-associated comparisons were conducted)



Beta Diversity

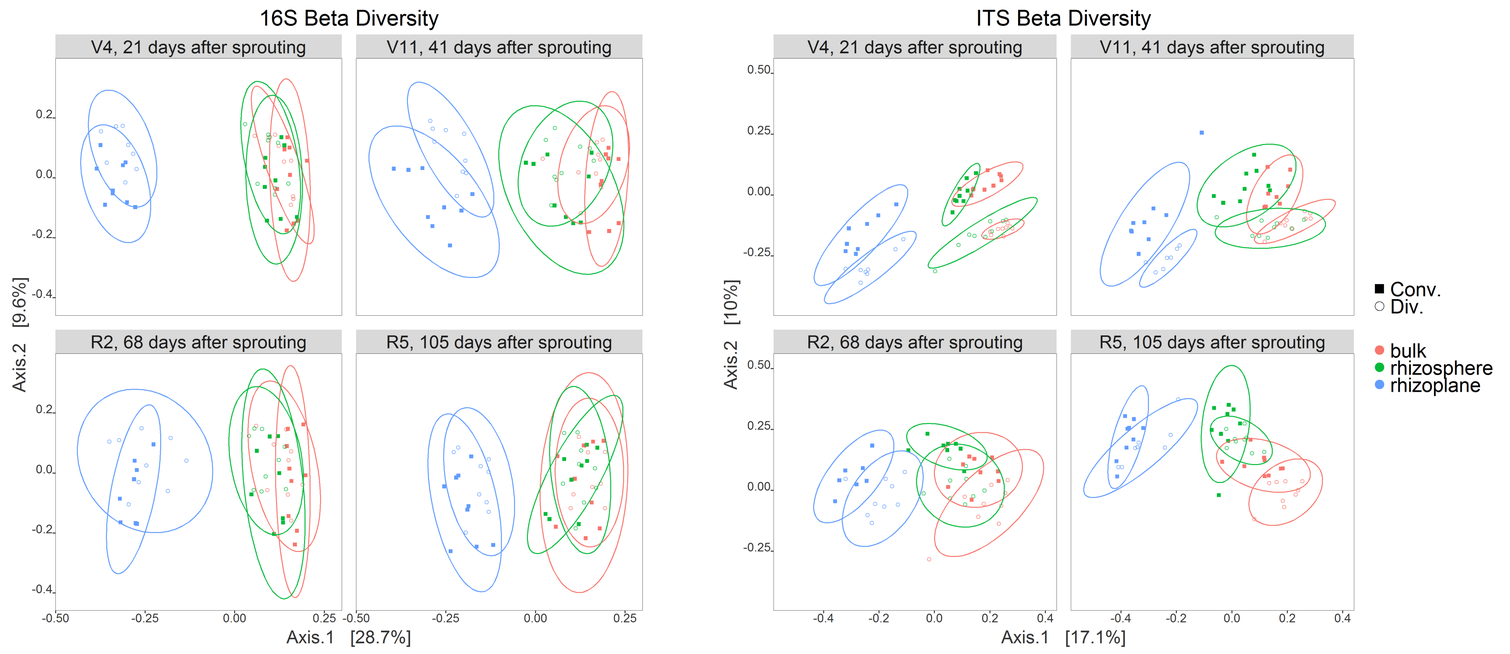


Fig 3. PCoA plots of Bray-Curtis Dissimilarity of 16S and ITS sequence data with T-normal ordination ellipses. For both 16S and ITS data, cropping system, proximity to root, and sampling time were all determined to be significant main effects ($P < 0.05$, ADONIS).

- Cropping system, root proximity, and sampling time significantly influenced both prokaryotic and fungal community composition, as evident by separation of the treatment groups
- Rhizoplane communities appear to be much more dissimilar from bulk soil communities than are rhizosphere communities

Differential Abundance

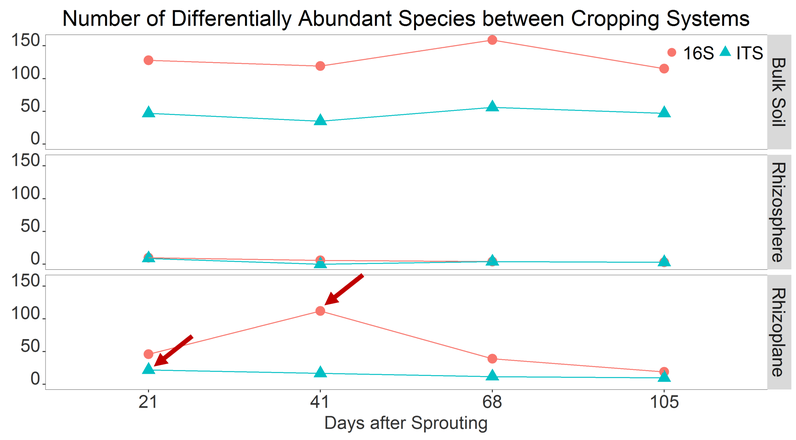


Fig. 4. Number of differentially abundant prokaryotic and fungal species between soil systems as determined by DESeq2 at $\alpha < 0.01$. Red arrows indicate comparisons highlighted in Fig. 5.

- Prokaryotic rhizoplane communities differed most at 41 days after sprouting, while fungal rhizoplane communities did so at 21 days (Fig. 4)
- Rhizosphere soils were very similar to bulk soils, resulting in fewer differentially abundant taxa (Fig. 4)
- Div. rhizoplane communities were typified by having a lower abundance of most taxa compared to the Conv. rhizoplane communities (Fig. 5)

Fig. 5. Differentially abundant 16S families (left) and ITS species (right) at 41 and 21 days after sprouting respectively. Taxonomic levels were chosen for best presentation. Negative log2Fold Change indicates a decrease in abundance in the Div. rhizoplane compared to the Conv. rhizoplane.

Conclusions

1. Our hypothesis was partially supported, prokaryotic rhizoplane communities were most distinct at V11, a period of high-N uptake, but fungal communities were most distinct earlier in plant development
2. Prokaryotic communities did not follow the same trends in the rhizosphere as in the rhizoplane but fungal communities did, indicating that the rhizosphere effect may not extend as far for prokaryotes as for fungi
3. Due to larger differences between fungal rhizoplane communities earlier in maize development rather than during rapid plant growth, fungal communities may not respond to plant N demand as do prokaryotic communities

References: **1)** Berendsen, R. L., et al. (2012). Trends in Plant Science, 17(8), 478–486.; **2)** Jangid, K., et al. (2008). Soil Biology and Biochemistry, 40, 2843–2853.; **3)** Bucher, A. E., & Lanyon, L. E. (2005). Applied Soil Ecology, *29*(1), 59–71.; **4)** Wessén, E. et al. (2010). Soil Biology and Biochemistry, 42(10), 1759–1765.; **5)** Davis, A. S., et al. (2012). PLoS ONE, 7(10).; **6)** Used with permission from Iowa State University Extension and Outreach from the publication, Corn Growth and Development, PMR 1009. Original publication date: 03/2011. **7)** Paulson, J. N., et al. (2013). Nature Methods, 10(12); **8)** McMurdie, P. J., & Holmes, S. (2013). PLoS ONE, 8(4); **9)** Paulson, J. N., et al. (2013). Nature Methods, 10(12).
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