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Exploring the maize rhizosphere microbiome in the field

A glimpse into a highly complex system

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Maize is one of the most economically important crops in the world. Understanding how the genetics and management of this staple crop interact with local field environments is vital to securing sustainable harvests. The interface zone between the plant root and its surrounding soil, or rhizosphere, supports essential interactions between roots and local soils. These interactions include the exchange of carbon for nutrients and are strongly influenced by the microbial constituents of the soil, or the microbiome. In a recent multi-environment study, we explored the diversity and heritability of the maize rhizosphere microbiome at flowering time. We assessed the bacterial diversity of the maize rhizosphere by pyrosequencing of 16S rRNA genes obtained from soil surrounding the roots of 27 genetically diverse maize inbreds grown in five field environments. We then partitioned variation in α- and β-diversity of the microbiome across plant inbreds in the different fields. The results of this study revealed the heritability and significance of genotype-by-environment interactions on the maize rhizosphere microbiome at a single time point. Longitudinal studies detailing the maize rhizosphere throughout an entire growing season are currently underway and should provide a more detailed view of how plant genotypes interact with the environment to shape the microbiome. Future efforts will aim to incorporate these interactions into genetic models of economically important traits such as yield.

Keywords: maize, rhizosphere, microbiome, GxE, 16S rRNA, heritability

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With nearly 850 million metric tons of global annual production,[1] maize is the most widely grown cereal[2] and provides essential carbohydrates to the diets of billions of people. Securing stable harvests requires knowledge of genetic and environmental factors influencing the crop. In developed nations, breeding and management have sought to maximize yields, exercising little constraint on the use of fertilizers, pesticides and other inputs. As economic costs and environmental consequences of these inputs grow, a comprehensive understanding of the ecological interactions influencing maize is needed to enhance cropping system sustainability while retaining competitive yields.

A promising target for crop improvement applications is the interactions between maize genotypes and microbiota colonizing their rhizospheres.[3] Plant-microbial interactions, such as those between maize and arbuscular mycorrhiza[4] or growth promoting rhizobacteria,[5] can mediate a plant's capture of soil nutrients and drought tolerance. However, with traditional microbiological methods recovering < 1% of soil-borne microbiota,[6] most plant-microbe interactions have yet to be characterized. Furthermore, many interactions are context dependent, with the direction and magnitude of effects conditional on host genetics,[7,8] the environment[9] and abundances of other microbiota.[10]

In the recent article, "Diversity and heritability of the maize rhizosphere microbiome under field conditions," we pyrosequenced 16S rRNA genes to explore the microbiome of genetically diverse inbreds grown in multiple environments.[11] We sampled roots and adherent

soil from randomly distributed plots of 27 maize inbreds grown in five field environments located in three US states (New York, Illinois and Missouri) in our study of rhizosphere microbiomes. We chose to sample the maize rhizosphere at flowering time.[11] In many plants, flowering induces a transition in carbon source-sink relations. Before flowering, roots exude carbon as organic acids and other forms, altering local soils and microbiota abundances[12] to create advantageous growth conditions. After flowering, resources are shunted to kernels to ensure reproductive fitness. We inferred sampling at flowering would increase heritability by allowing unique exudation profiles to affect rhizosphere microbiota before transition of carbon flow. We used seed from a uniform stand because seed-borne endophytes[13] differentially colonize seeds due to genetics or shared parental environment, and this effect constitutes another possible source of heritability. While our first report was a single-time point analysis of the rhizosphere microbiota, longitudinal studies of microbiome dynamics and heritability in the field are underway. The time-series analysis will allow us to assess when in plant development the influence of genotype on the microbiome is strongest and how plant-microbe interactions change as a function of plant maturation.

To survey the rhizosphere microbiome diversity, we pyrosequenced PCR amplicons of a hypervariable region in the 16S rRNA gene that provides a phylogenetically wide view of bacterial diversity. Given that several such regions exist, each with divergent discriminatory power, we compared four regions of the 16S rRNA gene for utility in describing diversity of a subset of samples. In the full experiment, we identified the region capturing substantial classifiable diversity with reduced representation of maize plastid genomes.[11] We partitioned variation in α- (within samples) and β- (between sample) diversity using bootstrapped linear models of richness estimators and principal coordinates.[14,15] To measure α-diversity, we determined genus-level operational taxonomic unit (OTUs, picked at 97% ID) richness, Chao 1[16] and phylogenetic diversity[17] metrics. These detail the number of observed OTU, estimate the number of observed and unobserved OTU, and estimate richness weighted by phylogenetic differences such that related OTU contribute less to the statistic than unrelated OTU, respectively. To measure β-diversity, we calculated weighted and unweighted UniFrac distances.[18] These detail the fraction of unique and shared branches (between any two samples) on a common phylogenic tree, considering and discounting the number of sequences mapping to those tree branches, respectively. Examining α- or β-diversity, with an analysis of variance of the model, $\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GxE} + 2\sigma_{G,E} + \sigma^2_{error}$, reveals how host genetics (G), and environment (E) affect microbiome diversity (P). It also assesses the effect of host genetics within an environment (GxE) and aids adjustment for technical artifacts like sequencing batch. Using these approaches we showed nearly 20% of variation in α-diversity could be explained by host genetics after accounting for environmental and technical factors. Similarly, broad-sense heritability estimates revealed about 5% of variation in β-diversity, as measured by UniFrac distance, was explained by host genetics. In contrast, nearly half the variation in α-diversity and 20–25% of variation in β-diversity was explained by host genetics within a given field after accounting for environmental and technical factors.

We found that greater sequencing depth is required to increase the power to discern with confidence the complement of genus-level taxa differing in abundance between inbreds. Sequencing-by-synthesis technologies (e.g., Illumina platforms) generate far greater data sets for a given effort and budget than pyrosequencing, and our use of these technologies in the time-series study will increase precision in abundance estimates. Although the technology's sequence lengths are reduced compared with pyrosequencing,[19] they sufficiently typify many microbiota in terms of sequence content and taxonomic assignments achieved by matching to reference databases that are also increasingly comprehensive. Plant genotype probably selects for function rather than microbial lineage, and even though 16S rRNA gene sequence data can be used as a proxy for function, these inferences are limited. Future studies will benefit from concentrating efforts on microbiota with functions of interest either in a targeted approach or by generating shotgun metagenomes, which can be parsed into gene functions as well as used for their phylogenetic information content. Regardless of the approach, statistic modeling applied to well-designed experiments will be key to partitioning the variance in microbiome traits between plant genotype and environmental effects.

Our results indicated diversity in the maize rhizosphere microbiota is heritable, and that environment has a strong influence on the specific host-microbiota interactions. This important finding lays a foundation for future applied work. Yet, knowing variation in microbiomes exists between inbreds has few direct applications in crop improvement. These metrics will be far more useful when conditioned on functionally-relevant microbiota. Also, microbiome diversity metrics may have more applications if treated as environmental factors influencing traits such as yield. In this model, the heritable component of the microbiome may explain a portion of the covariance of host genetics and environment ($\sigma_{G,E}$) and enhance our understanding of the true mechanics underlying heritability.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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