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Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil

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ABSTRACT

The rhizosphere harbors complex microbial communities, whose dynamic associations are considered critical for plant growth and health but remain poorly understood. We constructed co-occurrence networks for archaeal, bacterial and fungal communities associated with the rhizosphere and bulk soil of wheat fields on the North China Plain. Rhizosphere co-occurrence networks had fewer nodes, edges, modules and lower density, but maintained more robust structure compared with bulk soil, suggesting that a less complex topology and more stable co-occurrence pattern is a feature for wheat rhizosphere. Bacterial and fungal communities followed a power-law distribution, while the archaeal community did not. Soil pH and microbial diversity were significantly correlated with network size and connectivity in both rhizosphere and bulk soils. Keystone species that played essential roles in network structure were predicted to maintain a flexible generalist metabolism, and had fewer significant correlations with environmental variables, especially in the rhizosphere. These results indicate that distinct microbial co-occurrence patterns exist in wheat rhizosphere, which could be associated with variable agricultural ecosystem properties.

1. Introduction

Agricultural ecosystems have lower plant diversity and greater spatial homogeneity when compared to natural environments, as a result of directed and persistent human intervention (Kennedy and Smith, 1995). The rhizosphere is a complex ecological and biological zone where root exudation can alter biogeochemistry and sustain microbial activity (Turner et al., 2013). Edwards et al. (2015) proposed a multi-step model for root microbiome assembly from soil, with each root-associated compartment harboring a distinct microbiome during pure cultivation or in greenhouse. In real agricultural systems, bacterial (Fan et al., 2017) and fungal (Zhang et al., 2017) community composition have been found to differ significantly between root-associated soils and bulk soil, with a decrease in microbial diversity closer to the root (Donn et al., 2015; Fan et al., 2017). However, most studies have focused on the bacterial or fungal community in isolation, so that the interaction between archaeal, bacterial, and fungal populations in the rhizosphere and bulk soil of agricultural crops remains unclear.

Microbial communities consist of species which compete for space and resources (Hibbing et al., 2010) or engage in symbiotic interactions (Faust and Raes, 2012). Keystone species are defined as those which other species rely on such an extent that if they were removed the ecology of an ecosystem would be dramatically altered (Ze et al., 2013). Keystone species have been identified in many environments (Zaura et al., 2009) by defining the degree of node-specific interaction for taxa within co-association networks (Fisher and Mehta, 2014). Species occupying key positions in these networks, namely as hubs or connectors, have the potential to act as keystone species, as the removal of these nodes can have outsized impact on overall network structure. Keystone species often have more defined ecological roles, such as bacteria that suppress fungal root pathogens in the rhizosphere (Mendes et al., 2011). Shi et al. (2016) identified keystone species in soils associated with wild oats and found that some of them had low relative abundance. However, few studies have focused on microbial keystone species among multiple kingdoms in agricultural ecosystems, let alone attempt to determine the environmental parameters that shape their

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distribution and co-associations.

Network analyses have been used to explore the ecological interaction patterns among microbial species in many different environments including human gut (Chow et al., 2014; Sung et al., 2017), oceans (Fuhrman and Steele, 2008) and soils (Ma et al., 2016; Jiang et al., 2017). Co-occurrence patterns can help decipher the structure and assembly of complex microbial communities (Barberán et al., 2012), and predict potential interactions (Kara et al., 2013). Because co-occurrence patterns are based solely on simultaneous changes in pairwise taxa abundance, it is not possible to differentiate between environmental filtering (species with similar niches changing in response to the same environmental gradients) and direct interspecific interactions. However, species occupying similar niches are likely to compete under many, though not all circumstances (Tilman, 1982), so differentiation between direct interactions and environmental filtering may only be necessary when specific interactions are critical to understanding community behavior. Mendes et al. (2014) used co-occurrence networks to demonstrate that the rhizosphere community was a subset of the bulk soil community, and the rhizosphere bacterial community had a less complex network compared to that of bulk soil in a short-term plantation system (Mendes et al., 2014). Ma et al. (2016) investigated the microbial community co-occurrence patterns of forest soil across five climate regions, demonstrating a random distribution of interactions within the archaeal community and a non-random pattern for bacterial and fungal communities. Jiang et al. (2017) found the alkaline phosphomonoesterase (ALP) producing *Mesorhizobium* by analyzing the network correlations between bacterivores and ALP-producing bacteria in maize rhizosphere. However, there is little information about the topological shifts of archaeal, bacterial and fungal co-occurrence interactions in rhizosphere compared with bulk soil.

The North China Plain has a long agricultural history with a wheat-maize rotation system (Zhao et al., 2006; Liu et al., 2010). Wheat (*Triticumaestivum* L.) is one of the most important crops globally, however, the increase of wheat productivity has slowed down to 0.9% per year (Fischer and Edmeades, 2010). One potential way to increase wheat productivity is by manipulating microbial community interactions that support plant health, especially those in the rhizosphere. In this study, we investigated archaeal, bacterial, and fungal communities in wheat rhizosphere and bulk soil on the North China Plain. We proposed two hypotheses: *1) Microbial co-occurrence patterns in wheat rhizosphere are distinct from those in bulk soil, which is affected by both abiotic and biotic factors; 2) The keystone microbial species are usually metabolic generalists that demonstrate fewer correlations with environmental variables.*

2. Materials and methods

2.1. Sample collection and soil physiochemical analysis

Samples were collected from nine sampling sites across the typical wheat planting fields (32° Ñ38° N; 110° E ~ 118° E) on the North China Plain during the wheat filling stage (22nd −27th of the May, 2015). The soil type in most sampling sites were Fluvic Calcaric Eutric Cambisols, Haplic Luvisols, Cambic Calcisols, Calcaric Eutric Cambisols and Endocalcaric Luvisols according to the soil taxonomy of FAO. At each sampling site (~100 km^2 plot), five replicate locations were sampled. In each location, ten to twelve wheat plants were extracted. After shaking off the loosely bound soil, we brushed off the tightly adhered soil, which serve as rhizosphere soil (RS). Beside each wheat group, the topsoil (0–15 cm) without plants were collected by soil auger, which serve as bulk soil (BS). Soil pH was determined by pH meter (Thermo Orion-868) with a 1:5 fresh soil to water ratio. Soil texture was determined by using Laser Particle Sizer (LS13320). Soil moisture was determined gravimetrically by drying 5 g fresh soil to the constant weight under 105 °C for 12 h. Total carbon (TC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were determined by $K_2Cr_2O_7$-H_2SO_4 oxidation method, semi-micro Kjeldahl method, Mo-Sb colorimetry method and flame spectrophotometry method, respectively.

2.2. High throughput sequencing

DNA was extracted from 0.5 g fresh soil using the Power Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The archaeal and bacterial 16S rRNA genes were amplified by primer pairs 524F-10-ext (5′-TGYCAGCCGCCGCGGTAA-3′)/Arch958-modR (5′-YCCGGCGTTGAVTCCAATT-3′) (Baker et al., 2003) and 515F (5′-GTGCCAGCMGCCGCGGTAA-3′)/907R (5′-CCGTCAATTCCTTTGAGTTT-3′) (Biddle et al., 2008), respectively; the fungal ITS2 region was amplified by primer pair ITS3 (5′-GCATCGATGAAGAACGCAGC-3′)/ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Gade et al., 2013). The sequences have been submitted to the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra/SRP117302) with accession number SRP 117302.

2.3. Sequence analysis

The Quantitative Insight into Microbial Ecology (QIIME) pipeline (http://qiime.sourceforge.net/) was used to analyze the sequence data (Caporaso et al., 2010). 1,545,509 high quality sequences of archaea; 3,595,706 high quality sequences of bacteria; 2,383,721 high quality sequences of fungi were acquired after removing < 200 bp long and average quality score < 25 reads. OTUs were generated based on a 97% similarity level through UCLUST (Edgar, 2010). The greengenes database (http://greengenes.lbl.gov/) was used to assign the taxonomic identity of each phylotype of archaea and bacteria; fungal taxonomic identity was determined using the UNITE database (Köljalg et al., 2005).

2.4. Statistical analysis

NMDS (based on Bray-Curtis distance), Mantel test, Envfit, ANOSIM, MRPP and ADONIS analyses were conducted using the 'vegan' R package (Oksanen et al., 2013) in R × 32 (3.2.2) (https://CRAN.R-project.org/package=vegan). And the physicochemical parameters were fitted on the NMDS map based on non-permutation regression. The rank abundance distribution, which was calculated by the frequency of sequences (OTU Table), was used to test whether stochastic or deterministic processes best explain the community assembly of archaeal, bacterial, and fungal communities. TeTame (Jabot et al., 2008) was used to test whether a rank abundance was consistent with zero-sum multinomial (ZSM) distribution, predicting the dominance of stochastic processes (Hubbell et al., 2001). Tests for dominance of deterministic processes, i.e. rank abundance distributions fitting the Broken stick model, Pre-emption model, Log-normal model, or Zipf-Mandlebrot model were performed using 'radfit' in the 'vegan' R package (Oksanen, 2010).

The co-occurrence network was constructed with the 'WGCNA' R package based on the Spearman correlation matrix (Langfelder and Horvath, 2012). We removed OTUs occurring in less than 30% of all samples, kept OTUs with relative abundances greater than 0.01% for archaeal, bacterial, and fungal communities (Ma et al., 2016). The nodes and the edges in the network represent OTUs and the correlations between pairs of OTUs, respectively. P-values were adjusted by Benjamini and Hochberg false discovery rate (FDR) test (Benjamini et al., 2006), and the adjusted P-values had a 0.001 cutoff. We calculated the network properties with the 'igraph' R package (http://igraph.org), and generated network images with Gephi (https://gephi.org/). The natural connectivity provides sensitive discrimination of network structural robustness, we estimated network stability by removing nodes in the static network to assess how quickly robustness degraded and we assessed network robustness by natural connectivity (Peng and Wu,