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Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis

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ABSTRACT

Significant differences in the microbial biomass and diversity in soil have been observed following long-term fertilization, but little research has explored the co-occurrence patterns among microbial taxa or functions. Soil samples from four long-term experiments in China were collected, and an Illumina amplicon sequencing of 16S rDNA amplicon was performed to decipher the differences in interactions and network organization arising from fertilization with organic amendment (OA) or chemical fertilizer (CF) across the sampling sites. We thus aimed to extend the analysis beyond the basic inventory descriptions of the composition and diversity of the microbiome and toward an assessment of relationships and effects. Nonmetric multidimensional scaling analysis revealed a clear difference between the soil microbiomes associated with the OA and CF treatments. Fertilization can interact with the soil chemical properties and sampling site, thereby significantly impacting the associated soil microbiome. Distinct network structures indicated that the OA network was characterized by more functionally interrelated operational taxonomic units (OTUs) than the CF network. The topological roles of individual OTUs and key microbial populations were distinctly different, although the generalists mainly belonging to *Actinobacteria* and *Proteobacteria* were observed in both networks. The different fertilization treatments had distinct effects on the soil pH, which represented a key variable to impact the associated microbial community modularity. Most functional groups involving carbon-, nitrogen- and phosphorus-related metabolism and recycling and antibiotic biosynthesis were present at significantly high abundance in the organic-amended soils. All of the functions with statistical significance could be traced to corresponding responsible major modules in the CF network, but it was not the case in OA soil. These results suggested that the soil functions were decentralized and assigned to small groups in OA soil. Overall, we demonstrated that long-term organic amendment supports stronger functional potentials and more interactions within soil community relative to long-term chemical-only fertilization, which may be related to OA benefits to soil stability and buffering capacity. These results explore the correlations, inter-taxa and between major groups and functional traits, to gain a more integrated understanding of microbiome and ecological rules guiding soil community fostered by fertilization regimes.

1. Introduction

Soil microbes play an important role in maintaining soil productivity through biochemical processes such as residue decomposition and nutrient recycling. It is well known that fertilization is an important agricultural practice for improving plant nutrition and achieving high yields, but it may also result in microbial community variations, which in turn can affect plant growth by altering nutrient turnover and impacting the severity of soil-borne disease. Several recent studies have focused on the biomass, activity, and community composition of microbes in rice field soils subjected to long-term fertilization. For example, chemical fertilizer applied to rice field soil did not lead to changes in the microbial

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biomass but did alter the composition of the bacterial community of the soil (Wu et al., 2011). Ai et al. (2013) demonstrated that bacterial and archaeal ammonia-oxidizing microbes were impacted by both the effect of the plant rhizosphere and fertilization regimes. Although significant differences in microbial biomass and variations in microbial diversity have been observed following fertilization in farming soils (Ling et al., 2014b; Murase et al., 2015; Wang et al., 2015), the impact of fertilization on the relationships between soil microbial community composition and the associated functions still require extensive study.

With rising costs of chemical fertilizer and growing concerns over the environmental impact of excessive fertilizer application, there has been an increasing scrutiny on how nutrients are managed on farms (Chen et al., 2014). Organic amendments, such as cover crop residues, manures and composts, are well known to supply needed nutrients for the maintenance and enhancement of plant growth (Cavagnaro, 2014). Organic amendments yield additional benefits such as an increase in soil carbon stocks (Xie et al., 2014) and improvement in soil structure and water retention (Yu et al., 2012). A key benefit of these strategies is that they can be used to manipulate soil enzyme activities and microbial community composition (Bowles et al., 2014; Kotroczó et al., 2014), and that they simultaneously provides maintenance of soil health (Chaparro et al., 2012) and suppression of soil-borne diseases (Qiu et al., 2012). However, little is currently known concerning the detailed changes in soil bacterial community composition and function that vary with organic amendment type and their downstream influence on soil ecosystem function.

Many long-term trials have been established in order to study the impact of fertilizer amendment on crop production. These well-documented experiments have been used to further study soil microbial communities under different fertilization regimes. Since soil microorganisms may be slow to respond to changes in the soil environment (Eisenhauer et al., 2010) these long-term experiments are best suited to study the specific effects of different fertilizers with microbial communities often assessed using high-throughput sequencing (Zhao et al., 2014; Hartmann et al., 2015). This methodology enables the detection of microbial populations in a high-throughput and low-cost manner (Logares et al., 2014). A better understanding of the response of microbial communities to different forms of long-term fertilizations will, in addition to expanding our knowledge on diversity and abundances of species, provide useful information on the complex interactions among microbial groups.

As it is predictable that long-term fertilization on agricultural crops with organic and chemical fertilizers results in changes in soil microbial community composition, function and interactions among different taxa and functional groups, we hypothesized a network analytical approach would be powerful in delineating these changes and will move beyond the basic inventory descriptions to the variation in composition and diversity of the microbial communities. In this study, soil samples were collected from 4 long-term field experiments, which were all performed over 23 years and located in different provinces of China. A random matrix theory (RMT)-based bioinformatic approach was employed to define and characterize ecological networks in microbial communities based on high-throughput sequencing data (Zhou et al., 2011). The functional profiling of microbial communities were also predicted using 16S rRNA marker gene sequences in phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) (Langille et al., 2013) to investigate variations in microbial function. Moreover, whether major modules in the respective networks showed distinct relationships with soil functions, which was not observed when people analyzed the soil microbial community as a whole, was also evaluated.

2. Materials and methods

2.1. Field sites and soil sample collection

Four long-term fixed fertilization experiments were selected throughout the major grain-producing areas of China (Fig. 1). The first long-term experiment, initiated in 1990, was situated in Jilin Province (JL) and the climate, cropping system and physical and chemical characteristics of the initial field soil have been previously reported (Ling et al., 2014a). The second long-term experiment initiated in 1978 was located in Shandong Province (SD), and detailed information about the climate, cropping system and physical and chemical characteristics of the initial field soil were described by Song et al. (2014). The third long-term experiment was established in Anhui Province (AH) in 1981, located in the Yangliu experimental base of Anhui agricultural academy. Established in 1990, the fourth long-term experiment was situated in Hunan Province (HN) with the major soil and location data were published previously (Zhang et al., 2009; Ling et al., 2014b). Basic soil properties from these sites at the start of the field experiment were shown in Table S1. The exact location and cropping regimes were shown in Fig. 1, which also showed the major soil groups based on the China Soil Classification System (Zhang et al., 2014). Application rates, fertilizer types and plot sizes were summarized in Table S2. All plots were arranged in a randomized block design.

Two treatment soils were collected from the long-term experimental sites; soils applied with only chemical fertilizers (CF) and soils treated with organic amendments (OA). Three replicates, collected from three separate plots, were sampled for each treatment at each experimental site. Ten soil cores (5 cm diameter) per plot were taken at a depth of 0–20 cm and combined into a composite sample representative for the plot. All soil samples from the four long-term experiments (2 treatments $\times$ 3 replicates $\times$ 4 experiments $=$ 24 samples in total) were collected post-harvest in 2013–2014. Samples from SD, AH and HN were collected at the fallow stage after wheat crops were harvested and before the next maize planting and soils from JL were sampled at the fallow stage after maize crops were harvested and before the next maize planting. Gravimetric water content from the fresh soils ranged from 15% to 45%. We proposed that the primary driver of microbial community structure was fertilizer application and that the range of water content played a lesser role. As such, in order to exclude possible biases due to varying water contents on the evaluation of the soil microbiomes, soil samples were air dried in an artificial, UV light sterilized ventilated chamber, for approximately 7 days to unify soil water contents to 15% because the soil samples from one of four locations had a water content of 15%. Subsequently, each sample was passed through a 5.0-mm sieve, mixed thoroughly, and then divided into two parts: one was stored at −80 °C for analysis of basic soil properties and the other part was treated according to the following description for DNA extraction.

2.2. DNA extraction from soil samples

To primarily evaluate the long-term effect of fertilization on soils, and to exclude transient effects at the sampling time most probably attributed from water content difference of soils from different locations, we utilized the mildly air-dried soils with 15% moisture as pre-treated above for further analysis. Based on the fact that alternation of wetting and drying frequently occurred in fields, before DNA isolation, all the soil samples were adjusted to approximately 30% with sterile water and incubated at 25 °C in sterilized culture vessels (250 mL) in the dark for 7 days to recover the soil activity. After the incubation, soil DNA extraction was performed with the PowerSoil DNA Isolation Kit (MoBio