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Response of the soil microbial community to different fertilizer inputs in a wheat-maize rotation on a calcareous soil

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ABSTRACT

The combined use of inorganic fertilizers with organic manures has recently attracted increasing interest in China in attempts to mitigate the deleterious environmental impacts of excessive rates of chemical fertilizers in agroecosystems. However, questions remain concerning temporal change and how the soil microbiome responds to different fertilizer inputs in intensively managed crop rotations. Here, we collected soil samples from a wheat–maize system to investigate the response of the soil microbiome to four years of application of inorganic fertilizer only (NPK), NPK plus either cattle manure or straw, NPK plus both manure and straw, or a zero fertilizer control. The soil bacterial and fungal populations and community composition, nitrogen functional genes (*amoA*) and carbon utilization patterns were assessed. Sampling time had a much greater influence on the soil microbiome than did fertilizer regime, and the effect of fertilization was mostly significant at the wheat harvest. Fertilization increased *amoA* gene copy numbers but only AOB abundance showed differences among fertilizer treatments. In June the community composition of both bacteria and fungi was clearly separated between the organic matter additions and the zero organic matter treatments. Microbial carbon source utilization was significantly affected by fertilization regime and sampling time. By contrast, at the maize harvest neither microbial populations nor microbial community composition were altered. Our results suggest that the entire soil microbiome is more responsive to organic inputs than to chemical fertilizers in the short term. Temporal shifts in microbial community composition in the crop rotation imply that crop species and environmental conditions need to be carefully integrated into nutrient management strategies.

1. Introduction

Soil organic carbon (SOC) is a key contributor to crop productivity and soil quality (Bauer and Black, 1994). Large amounts of chemical fertilizers, in particular of N, have been applied to arable fields over the past few decades in China. Excessive fertilization does not always translate into a continuous increase in crop yield (Vitousek et al., 2009). Instead, it leads to low nutrient use efficiency and deterioration of the environment through soil acidification (Guo et al., 2010), eutrophication (Zhang et al., 2011), and greenhouse gas emissions and N deposition (Liu et al., 2013; Ju et al., 2009). For instance, the minimum soil nitrate-N content achieving maximum grain yields and N uptake was about 180 kg ha^{-1} in a winter wheat–summer maize rotation, while the annual application rate of synthetic N ranged from 550 to 600 kg N ha^{-1} (Cui et al., 2008b; Zhao et al., 2006). Consequently, the apparent N recovery of cereal grain production was < 20% in the 2000 s (Cui et al., 2008a). Recently there has been increasing interest in incorporating organic manures or crop straw together with synthetic inorganic fertilizers to offset the negative impacts of the mineral fertilizers and deliver multiple benefits such as increased SOC content and crop yields (Cai and Qin, 2006; Demelash et al., 2014; Seufert et al., 2012). For example, the application of organic manure along with N fertilizers for 33 years in a fluvo-aquic soil substantially increased SOC and yields of wheat and maize (Yang et al., 2015). A global meta-analysis of 141 studies found that substituting livestock manures for synthetic N fertilizers (at equivalent N rates) significantly increased crop yields and N use efficiency, and annual SOC sequestration increased significantly by 700 and 401 kg C $\text{ha}^{-1}\,\text{yr}^{-1}$, respectively, in upland and paddy fields (Xia et al., 2017).

Soil microbes play an important role in maintaining soil functions through their involvement in the turnover of organic matter, nutrient cycling and bioremediation. Understanding how they respond to

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organic matter inputs and chemical fertilizers can be indicative for the assessment of soil quality in agriculture (Yin et al., 2010; Sharma et al., 2010). Numerous studies addressing how organic and inorganic fertilization and straw return alter microbial biomass and/or activities show contrasting results (Zhao S et al., 2016; Hartmann et al., 2015). For example, the application of inorganic fertilizer regimes over 34 years induced soil acidification which was related to lower bacterial biodiversity and abundance and changes in the bacterial community (Zhou J et al., 2015). By contrast, NPK combined with manure was shown to maintain bacterial diversity and sometimes increase bacterial populations (Sun et al., 2015). Systems receiving organic fertilizer were characterized by specific microbial guilds known to be involved in the increased relative abundance of copiotrophic taxa (Hartmann et al., 2015). In contrast, no significant differences were found in the abundance of rhizosphere bacteria or fungi between inorganic and organic amendments in a long-term fertilization experiment (Ai et al., 2012). It is generally acknowledged that the type and quantity of fertilizers are important factors affecting soil microbial communities. However, there remains only limited understanding of the temporal variability and forces driving soil microbial communities in crop rotation systems. Several studies show that both fertilizer regime and timing had significant influence on bacterial community structure in a wheat-rice cropping system (Zhao et al., 2014). The soil microbial community differs among crop species (Reinhold-Hurek et al., 2015; Ofek-Lalzar et al., 2014). Environmental factors such as soil moisture and temperature also affect the assembly of microbial communities (Lauber et al., 2013). Hence, it is necessary to understand the relative contribution of fertilization and timing on the changes in crop rotation-associated microbial communities.

Differences in the availability of N in organic amendments and inorganic fertilizers may have significant influence on nitrogen cycling. Ammonia-oxidizing microbes, the key functional microbial guilds in nitrification, are more sensitive than total bacterial counts as an indicator of nitrogen cycling in soils (Shen et al., 2008). Previous studies show that differences in fertilization regimes did not alter the abundance or composition of ammonia-oxidizing archaea (AOA) but resulted in changes in ammonia-oxidizing bacteria (AOB) in calcareous and neutral soils receiving fertilizer N (Shen et al., 2008, 2011). However, Xue et al. (2016) reported that both chemical fertilizers and the combined use of organic manures and chemical fertilizers changed the abundance and composition of AOA and AOB in a neutral soil after 23 years of fertilization. Zhou X et al. (2015) found that AOA and AOB showed different responses to organic and inorganic fertilizers, and AOA increased significantly under organic N additions, but AOB increased with the addition of inorganic N in long-term field studies. The response of nitrifying communities to environmental change under field conditions cannot therefore be readily generalized.

In the present study we explore how microbial abundance and community shift in response to different organic and inorganic fertilization treatments in a winter wheat-summer maize cropping system in a calcareous soil on the North China Plain, one of the major grain producing regions in China. The application of manure was shown to increase bacterial diversity compared to mineral fertilizer (Li et al., 2015), and microbial community composition (PLFA) was shown to differ between manure and chemical fertilizer treatments as a result of differences in soil carbon and nutrient availability after 35 years of a long-term fertilization experiment (Wei et al., 2017). Bacterial and fungal populations and *amoA* genes were assessed in the present study using quantitative PCR, and the community composition of soil bacteria and fungi was analyzed by Miseq sequencing. The community level physiological profiles (CLPP) were analyzed using Biolog EcoPlates. Changes in the soil C pool and nutrient status with inputs of organic and inorganic fertilization treatments may lead to shifts in the ability of the microbial community to metabolize different C substrates (Liu et al., 2016; Peng et al., 2016). We hypothesized that different fertilization regimes would change microbial abundance, community composition, and functional diversity, and that application of manures and straw may increase the diversity of soil microbial communities. The effects of fertilization were expected to be stronger at the wheat harvest than the maize harvest due to the low soil temperatures and moisture contents throughout the wheat growing season.

2. Materials and methods

2.1. Field description and experimental design

The study was conducted at Quzhou Experimental Station (36°42′ N, 114°54′ E; 40 m a.s.l.) in Quzhou County, Hebei province, north China. The soil is a Cambisol with a silt loam texture. The climate is sub-humid continental monsoon with an average annual temperature of 13.2 °C and mean precipitation of 494 mm (approximately 70% of which occurs from July to September). The typical cropping system is a summer maize and winter wheat rotation. The rotation began with maize planting in June and harvesting in October followed by immediate planting of wheat. The soil had the following properties prior to the start of the experiment: pH 7.24 (H_2O), 0.90 g total N (TN) kg^{-1}, 13.7 g soil organic matter (SOM) kg^{-1}, 12.01 mg Olsen-P (AP) kg^{-1}, and 176.2 mg available K (AK) kg^{-1} (Zhang et al., 2016).

Field plots, each 50 m^2 (5 m × 10 m), were established in 2010. The experiment had five fertilization treatments with three replicates of each treatment: (1) CK control, no fertilizer application; (2) mineral NPK fertilizer only (NPK); (3) NPK + manure treatment (NPKM): 70% NPK fertilizer plus 3000 kg commercial compost ha^{-1}; (4) NPK + straw (NPKS): 100% NPK fertilizer plus wheat (maize season, 6.0 Mg ha^{-1}) or maize (wheat season, 6.8 Mg ha^{-1}) straw; (5) NPKMS: a combination of 70% NPK fertilizer + 3000 kg commercial compost ha^{-1} + wheat (7.3 Mg ha^{-1}) or maize (6.9 Mg ha^{-1}) straw. In the NPK treatment the N application rates were based on conventional farming practice with 300 kg N ha^{-1} for wheat (120 kg N ha^{-1} before planting and 90 kg N ha^{-1} at re-greening and jointing stages, respectively), and P and K rates of 50 kg P ha^{-1} and 80 kg K ha^{-1} as basal fertilizers. The N application rates for maize were 250 kg N ha^{-1} (100 kg N ha^{-1} before planting and 150 kg N ha^{-1} at the 12 leaf growth stage), and P and K rates of 20 kg P ha^{-1} and 37 kg K ha^{-1}. The fertilizers were broadcast and the chemical nitrogen, phosphorus and potassium fertilizers were urea, calcium superphosphate, and potassium sulphate. In treatments NPKM and NPMS the compost was applied before planting and the 70% NPK fertilizer was applied as in the NPK treatment. The commercial compost was derived mainly from cattle manure and contained 33.2% C, 2.0% N, 0.8% P and 0.7% K. The straw was chopped to about 2–4 cm when the crops were harvested. In the NPKS treatment the N, P and K contents of the wheat straw were 196, 15, and 154 kg ha^{-1}, and those of the maize straw were 127, 18, and 141 kg ha^{-1}, respectively. In the NPKMS treatment the N, P and K contents of wheat and maize straw were 203, 18, and 161 kg ha^{-1} and 140, 22, and 229 kg ha^{-1}, respectively.

Winter wheat (cv. ‘Good star 99′) was planted in mid-October after the maize harvest at a sowing density of 225 kg seeds ha^{-1} and harvested in early June of the following year. Summer maize (cv. ‘Zhengdan 958′) was sown with a row spacing of 60 cm and a density of ca. 63000 seeds ha^{-1} in mid-June and was harvested in October. All of the basal fertilizers were broadcast and incorporated into the upper 20 cm of the soil profile by rotary tillage prior to sowing. The use of herbicides, insecticides and irrigation was based on local conventional farming practice.

2.2. Soil sampling and analysis

In 2014 soil samples were taken twice, at the wheat and maize harvests. Eight soil cores were collected from each plot to a depth of 20 cm with a 2-cm-diameter auger following a serpentine line and mixed together to give a composite sample. The composite samples