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Responses of microbial activity, abundance, and community in wheat soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen

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ABSTRACT

A three-year field experiment in a winter wheat–rice cropping system was performed to compare the influence on soil microbial parameters including microbial activity, abundance and community by heavy application of manure-based compost (CP) and inorganic nitrogen (CF, inorganic nitrogen applied as urea). The same amount of total nitrogen that was calculated from the local farming habits was applied in each season for the two treatments. Samples were analyzed after the third season of wheat harvested. In contrast to the untreated control (CK), CP and CF treatments both significantly increased the soil nutrient content ($p < 0.05$) with converse results obtained for improvements in soil water-holding capacity and acidification. Heavy application of organic compost markedly increased the soil microbial activity and gene copies of bacteria, archaea, and ammonia-oxidizing bacteria (AOB). However, pyrosequencing results demonstrated that heavy application of inorganic nitrogen had no influence on soil bacterial diversity. Diversity was significantly decreased by organic compost. Changes occurred in the bacterial community composition in compost treated soils, and most abundant taxa correlated with the moisture content, pH, organic C, total N, available P, and available P. The findings of this study indicated that compost application was not always beneficial to soil quality, and might decrease soil diversity. Therefore, studies to maintain the relationship between compost application rate and microbial diversity should be further conducted to protect soil ecosystems against disturbances

1. Introduction

Soil microorganisms are of great importance to agroecosystem functioning and sustainability through their contributions to nutrient cycling and soil structure maintenance (Helgason et al., 2010; Nacke et al., 2011). Numerous studies have suggested that microbial parameters such as soil enzyme activity, microbial biomass content, and microbial diversity are potentially valuable indicators of soil quality (Garbeva et al., 2004; Kong et al., 2011; Romaniuk et al., 2011). Microorganisms also can rapidly respond to changing environmental conditions by modifying microbial biomass and community composition (Schloter et al., 2003). Therefore, investigating the shifts in soil microbial activity, abundance, and community driven by different agricultural practices would be conducive to maintaining and enhancing the fertility and productivity of soils and protecting soil ecosystems against disturbances.

Fertilization is an indispensable agricultural practice for improving plant nutrition and increasing soil organic matter. This practice produces changes in soil microbial activity, abundance, and community either directly or indirectly (Chu et al., 2007; Saha et al., 2008). Various studies have investigated the disturbance effects of different fertilization regimes on these soil biological properties. The incorporation of organic residues from different sources (Ros et al., 2006; Crecchio et al., 2007; Cytryn et al., 2011) and different rates of chemical fertilizers (Yu et al., 2014) are the most commonly addressed issues. However, comparative studies between the application of equal amounts of chemical and organic nitrogen are scarce, if available, for paddy soils (Zhong and Cai, 2007; Zhang et al., 2012). Moreover, the available information is sometimes conflicting with regard to the influence of different

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fertilization regimes on soil microbial biomass and microbial diversity (Dong et al., 2014).

Denaturing gradient gel electrophoresis (DGGE) analysis and phospholipid fatty acid (PLFA) analysis are popular methods to investigate the changes in microbial communities that result from different fertilization regimes. For instance, the abundance and composition of native soil bacterial communities under different fertilization practices using DGGE were examined by Shen et al. (2010), while PLFA analysis was employed by Zhang et al. (2012). They found opposite patterns of microbial community structure after chemical fertilizer and organic manure inputs. However, their results may introduce an incomplete estimate of phylogenetic diversity, particularly in environmental samples containing diverse microorganisms, because only the predominant and specific microbial types can be identified by DGGE and PLFA, respectively (Zelles, 1997; Crecchio et al., 2007). Conversely, pyrosequencing can provide a large number of sequences and even reveal small changes in microbial populations for obtaining massive sequences in parallel (Poulsen et al., 2013).

Previous studies have demonstrated that high mineral nitrogen inputs and low crop recoveries may lead to the reduction of bacterial diversity and increase fungal biomass (Geisseler and Kate, 2014). However, the overuse of manure-based compost in China is also popular, especially during the transition period from conventional to organic farming systems. Farmers consider consecutive and heavy application of compost to always be conducive to improved soil fertility and crop yield (Ju et al., 2007), but the changes resulting from its administration are not yet fully understood. We hypothesized that high input level of manure-based compost over a long period of time might change soil microbial activity, abundance and community more significantly than using the same amount of inorganic nitrogen. For this reason, a three-year field experiment was conducted to accurately: (1) analyze the shifts in soil physicochemical and biological properties under heavy application of organic compost and inorganic nitrogen; (2) compare the degrees of influence on soil microbial activity, abundance, and community by different fertilization regimes; and (3) examine the relationship between soil physico-chemical characteristics and microbial community composition.

2. Materials and methods

2.1. Experiment design and soil sampling

The field experiment was conducted from May 2012 to June 2014 at XueYan town in JiangSu Province of China located at longitude 120°05′05″W and latitude 331°29′24″N. This region has a typical subtropical monsoon climate with an average annual precipitation of 1048 mm and an average temperature between 12.0 °C and 20.1 °C. Winter wheat and rice are the mainly crops in this area. The properties of the loamy soil (sand 40.17%, silt 33.24%, clay 26.59 %) used in this study are listed as follows: moisture 18.34, pH 5.72, soil organic C 11.67 g kg^{-1}, total N 0.39 g kg^{-1}, total P 4.03 g kg^{-1}.

The treatments were: (1) CK (unfertilized control); (2) CF (inorganic nitrogen applied at a rate of 300 kg ha^{-1} and 180 kg ha^{-1} respectively in each rice and wheat season); and (3) CP (manure-based compost applied at the same total nitrogen amount as CF in each rice and wheat season). In the CF treatments, inorganic nitrogen was applied as urea and the application rates were calculated from local farming habits. The manure-based composts used in CP treatments were produced from pig or dairy manure mainly, and their total nitrogen contents were 1.52–2.04%. Treatments were arranged in a randomized block design with three replicates. Nine plots were built, and each plot was 30 m^2 (5.0 m × 6.0 m) with a neutral zone of 0.5 m for each neighboring plot.

Rice and winter wheat were cultivated successively in every rotation-cropping season (approximately June and November every year). Inorganic nitrogen and manure-based compost were both evenly spread on the soil surface and incorporated to a depth of 20 cm by manual hoeing before sowing. The weeds were removed by hand every month to avoid any interaction between the soil and the plants. The three treatments only differ in fertilization practice. Soil samples were collected after the third season of wheat (May 2014). They were collected at five different undisturbed spots using a hand auger (5-cm diameter) at a depth of 0–25 cm for each plot. The samples were mixed thoroughly to obtain a homogeneous composite sample. After subsamples were mixed, the entire sample was immediately divided into three parts. One was stored at −80 °C, and one at 4 °C and one immediately air-dried.

2.2. Soil characteristics and total soil microbial activity analysis

The soil moisture content was determined by oven-drying to a constant weight at 105 °C. Soil organic carbon (SOC) and total nitrogen (TN) were measured with an auto elemental analyzer (Vario EL III, Elementar, Germany). Water extracts of the fresh soil samples were prepared by shaking the fresh sample with distilled water at 1:5 (w/v) using a horizontal shaker for 24 h at room temperature. The extracts were centrifuged at 12,000 rpm for 10 min and filtered using 0.45 micrometer polytetrafluoroethylene (PTFE) filters for further analysis. The pH of fresh extracts were measured with a pH electrode (PB-10, Sartorius, Germany), and the concentrations of NH_4^+-N and NO_3^--N were determined with an AutoAnalyzer (AA3, Bran and Luebbe, Germany). The available P was extracted with sodium bicarbonate and measured via the

Table 1
Description of primers and thermal conditions used in real-time PCR.

Target microbe	Primer name	Primer set (5′-3′)	Annealing temperature (°C)	Extension time (s)	Product size (bp)
Bacteria	Eub338F Eub518R	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	55 °C	10 s	181
Archaea	ARC787 ARC1059	ATTAGATACCCSBGTAGTCC GCCATGCACCWCCTCGT3	60 °C	15 s	273
AOA	Arch-*amoA*-F Arch-*amoA*-R	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	53 °C	45 s	635
AOB	AmoA-1F AmoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	55 °C	30 s	491