Applied Soil Ecology 99 (2016) 1–12

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effects of organic–inorganic compound fertilizer with reduced chemical fertilizer application on crop yields, soil biological activity and bacterial community structure in a rice–wheat cropping system

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ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 6 November 2015
Accepted 11 November 2015
Available online 28 November 2015

Keywords:
Organic–inorganic compound fertilizer
Rice–wheat cropping system
Temporal dynamics
Bacterial community structure
Core microbiome

ABSTRACT

The development of more stable and sustainable agroecosystems for improving food production has caused wide public concern in recent years. In the present study, we conducted a field experiment to investigate the effect of pig manure organic–inorganic compound fertilizer with reduced chemical fertilizer on the crop yields, soil physicochemical properties, biological activities and bacterial community structure in a rice–wheat cropping system over two crop seasons (rice and wheat). The results showed that at all sampling times, this fertilizer regime enhanced the soil nutrient availability, microbial biomass, enzymatic activities, and soil nitrogen processes and, to some extent, promoted crop yields. Across all soil samples, bacterial communities were dominated by *Proteobacteria*,*Acidobacteria*, and *Chloroflexi* at the phylum level. Hierarchical cluster analysis based on the weighted UniFrac distance revealed that the bacterial community structures were strongly separated by the sampling time, and the treatments in the wheat harvest soils. A Venn diagram of shared OTUs showed a core microbiome across different treatments and sampling times, in which the relative abundance of each abundant phylum (class) was stable in the different treatments and at different sampling times. Specifically, the relative abundance of *Alphaproteobacteria*, *Gammaproteobacteria*, *Nitrospirae*, *Bacteroidetes*, and *Actinobacteria* was largely and particularly enriched under the organic–inorganic compound fertilizer regime, indicating that soil functions, such as nitrification and the turnover of organic matter, might be strengthened under this treatment. Collectively, these results indicate that the application of organic–inorganic compound fertilizer may reduce chemical fertilizer use and improve the long-term productivity and sustainability of agroecosystems.

1. Introduction

The rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) cropping system, which covers an area ranging from 9.5 to 13.5 million ha, is a long-established grain production system in China and is considered to be of utmost importance for China's food security and livelihood (Gupta et al., 2003). Since the early 1980s, the productivity of rice and wheat in China has increased dramatically. This has mainly been attributed to the introduction of high-yielding varieties and the increased input of chemical fertilizers (Ladha et al., 2004; National Bureau of Statistics of China, 2009). Recently, concerns have been raised regarding the stagnation and even decline in the productivity and sustainability of this cropping system (Ladha et al., 2003; Mandal et al., 2003). Therefore, to meet the ever-increasing food demand in China, the development of highly productive and sustainable food production practices is becoming increasingly important.

A rice–wheat cropping system is a nutrient exhaustive system that requires appropriate fertilizer management to maintain soil productivity, improve plant nutrition, and increase crop yields. Organic fertilizers are known to improve soil quality and structure

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http://dx.doi.org/10.1016/j.apsoil.2015.11.006
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(Bronick and Lal, 2005) as well as stimulate soil microbial biomass (Masto et al., 2006; Jannoura et al., 2014), enzyme activities (Ge et al., 2010; Insam et al., 2015; Plaza et al., 2004), and functional diversity and abundance (Orr et al., 2012) in soil community structure. However, the effect of organic fertilizers on crop yield is slow and variable (Khaliq et al., 2006). Thus, farmers prefer to use inorganic fertilizers rather than organic fertilizers to preserve crop yield, particularly in intensive agricultural systems under fluctuating environmental conditions (Smith et al., 2007). However, the long-term oversupply of inorganic fertilizers, especially nitrogen fertilizer, has resulted in nitrate pollution of groundwater, eutrophication of surface waters, acid rain and soil acidification, greenhouse gas emissions, and other forms of air pollution (Ju et al., 2009). In particular, Guo et al. (2010) reported that soil acidification had become a major problem in intensive Chinese agricultural systems. Moreover, the long-term application of inorganic fertilizers without organic inputs has been identified as the driving force in the loss of soil organic matter, deterioration of the soil structure, decrease in biological activities, and thus reduction in soil fertility (Bronick and Lal, 2005; Ladha et al., 2004; Zhong and Cai, 2007), which is assumed to be nonsustainable. Accordingly, the combination of organic and inorganic fertilizers is a promising approach to develop more sustainable fertilizer management strategies.

It has been recognized that the addition of organic amendments and a reduction in synthetic inorganic fertilizer can optimize the soil microbial-driven internal cycling of nutrients (Germaine et al., 2010). For instance, reduced inputs of nitrogen fertilizer can enhance the activity of nitrogen-fixing bacteria and thus promote biological nitrogen fixation, which supplies nitrogen to agroecosystems (Hsu and Buckley, 2009; Orr et al., 2012). Liu et al. (2009) reported that the application of organic amendments coupled with reduced chemical fertilizer enhanced the microbial biomass, activity, and nutrient availability compared with the use of chemical fertilizer only. Similarly, combined inorganic/organic fertilization improves N utilization efficiency and increase rice productivity through organic carbon accumulation (Pan et al., 2009). Recently, organic–inorganic compound fertilizer has become a novel and popular fertilizer in China because of slow nutrient release, high fertilizer use efficiency, and low environmental pollution (Ni et al., 2010). Thus, it is necessary to understand the influence of this type of fertilizer on crop productivity, soil biological activity and microbial assemblages.

Soil microorganisms are considered to play a vital role in maintaining soil health, productivity, and sustainability (Zhao et al., 2014a). Therefore, understanding the soil microbial community and its response to various agricultural management practices will guide us to select a suitable management strategy for the establishment of more stable and sustainable agroecosystems (Li et al., 2012; Zhao et al., 2014b). It is well known that nitrogen availability often limits plant productivity in terrestrial ecosystems (LeBauer and Treseder, 2008), and soil nitrogen transformations are driven directly by a diverse microbial community (Robertson and Groffman, 2007). For example, biological N_2-fixation is the conversion of dinitrogen (N_2) to biologically available ammonium (NH_4^+) by free-living, associated and symbiotic diazotrophs from a wide range of bacterial phyla (Zhang et al., 2006). Therefore, it is also necessary to understand the dynamics of microbial functional genes associated with nitrogen cycling in soil applied with different fertilizer regimes.

We hypothesized that the organic–inorganic compound fertilizer with reduced chemical fertilizer would help to establish a distinct microbial community, facilitate soil microbial-driven nutrient cycling, enhance enzymatic activities, and thus improve crop yield. To test this, a field experiment was conducted in a rice–wheat cropping system to evaluate the effects of this fertilizer regime on crop yields, soil physiochemical properties, soil enzyme activities, microbial nitrogen functional genes, and microbial community structure.

2. Materials and methods

2.1. Field site and experiment description

The experiment was performed in a paddy field under rice–wheat rotation in Changshu, Jiangsu, China (31°18′N, 120°37′E, 6 m a.s.l.). This region has a northern subtropical monsoon climate with an average annual temperature of 15.4 °C and a mean annual precipitation of 1054 mm. The soil type is classified as clay loamy endogleyic-Fe-leachic-stagnic anthrosol, and the soil properties before experiment are described by Zhao et al. (2014a).

The fertilization experiments were established in 2010 and were designed in a randomized complete block with four replicates, each measuring 40 m^2. The treatments were the control without fertilizer (CK), chemical fertilizers (NPK) (conventional dosage for this region), 50% chemical fertilizers plus 6000 kg/ha manure (NPKM) and 30% chemical fertilizers plus 3600 kg/ha pig manure organic–inorganic compound fertilizer (NPKMOI). The pig manure organic–inorganic compound fertilizer granules were comprised of pig manure compost and a suitable amount of multi-elements with a special coating material. Each plot received the same levels (except control) of nitrogen, phosphorus, and potassium from fertilizers applied in the wheat (N: 240 kg/ha, P_2O_5: 120 kg/ha and K_2O: 100 kg/ha) and rice (N: 300 kg/ha, P_2O_5: 120 kg/ha and K_2O: 100 kg/ha) seasons. The N fertilizer (urea) was applied as a basal fertilizer and a supplementary fertilizer, while the P, K, and manure fertilizers were only used as basal fertilizers. Rice was irrigated routinely to maintain shallow standing water from the seedling stage to the maturity stage, whereas wheat was irrigated when necessary for agriculture. The crop grains from the entire plot were weighed and recorded after air drying.

2.2. Soil sampling and analysis

Soil samples were collected from the plough layer (0–20 cm) of each replicate plot for each treatment using a 2.5-cm diameter auger after the harvest of wheat and rice in June and October 2012, respectively. Each soil sample was a composite of ten soil cores that were randomly collected, pooled in a sterile plastic bag, and transported to the laboratory on ice. The soil samples were sieved (2-mm-mesh sieve) and divided into three subsamples before being thoroughly homogenized. One portion was stored at −80 °C for DNA extraction, another portion was stored at −4 °C for microbial biomass measurement, and the remainder was air-dried for soil characteristic and enzyme activity analyses. The soil edaphic properties of each sample were evaluated in the soil testing laboratory at the Qiyang Red Soil Experimental Station of the Chinese Academy of Agricultural Sciences.

2.3. Enzyme activities and microbial biomass analysis

The activities of the soil enzymes were assayed in triplicate with two non-substrate controls using air-dried soil samples according to Guan et al. (1986). Alkaline phosphatase (EC 3.1.3.1) activity was determined using disodium phenyl phosphate as a substrate and phenol as a product after incubation at 37 °C for 24 h. Urease (EC 3.5.1.5) and invertase (EC 3.2.1.26) activities were analyzed by the released ammonium and glucose equivalent, respectively. Catalase (EC 1.11.1.6) activity was measured by shaking for 20 min with H_2O_2 (3.5%) as a substrate, and the suspension was titrated with 0.1 mol L^{-1} $KMnO_4$ solution. Catalase activity was expressed as 0.1 mol L^{-1} $KMnO_4$ ml g^{-1} soil 20 min^{-1}, whereas the other enzyme