Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China

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A B S T R A C T

Repeated fertilizer applications to cultivated soils may alter the composition and activities of microbial communities in terrestrial agro-ecosystems. In this study, we investigated the effects of different long term fertilization practices (control (CK), three levels of mineral fertilizer ($N_1P_1K_1$, $N_2P_2K_2$, and $N_3P_3K_3$), and organic manure (OM)) on soil environmental variables and microbial communities by using phospholipid fatty acid (PLFA) biomarkers analysis in subtropical China. Study showed that OM treatment led to increases in soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP) contents, while the mineral fertilizer treatment led to increases in dissolved organic carbon (DOC) content. Changes in soil microbial communities (eg. bacteria, actinomycetes) were more noticeable in soils subjected to organic manure applications than in the control soils or those treated with mineral fertilizer applications. Fungal PLFA biomarkers responded differently from the other PLFA groups, the numerical values of fungal PLFA biomarkers were similar for all the OM and mineral fertilizer treatments. PCA analysis showed that the relative abundance of most PLFA biomarkers increased in response to OM treatment, and that increased application rates of the mineral fertilizer changed the composition of one small fungal PLFA biomarker group (namely $18:3\omega6c$ and $16:1\omega5c$). Further, from the range of soil environmental factors that we examined, SOC, TN and TP were the key determinants affecting soil microbial community. Our results suggest that organic manure should be recommended to improve soil microbial activity in subtropical agricultural ecosystems, while increasing mineral fertilizer applications alone will not increase microbial growth in paddy soils.

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1. Introduction

Soil microorganisms play an important role in the soil environment. They are the critical factors that determine soil organic matter decomposition, nutrient cycling, soil degradation and bioremediation of soil pollution (Larkin, 2003; Li et al., 2012). Shifts in the structure and composition of the microbial community are strong indicators of soil biological activity, soil quality and crop productivity of terrestrial agro-ecosystems (Edmeades, 2003).

China is one of the major rice cultivation countries in the world, producing approximately one-third of the global rice crop (Coats, 2003). The cultivation of rice, interchanging between dry and wet field conditions, gives rise to anoxic conditions when soils are flooded during most of the rice-growing season and aerobic conditions when soils are drained during the non-cropping season (Li et al., 2010). Thus, the composition and structure of microorganisms in paddy soils are diverse and complicated (Ge et al., 2008).

Research has clearly demonstrated that environmental conditions and soil management practices largely determine the structure of soil microbial communities (Steenwerth et al., 2002; Wu et al., 2011). Paddy soils are routinely fertilized to improve soil nutrition and maximize the rice yield. Just as different groups of microorganisms vary in their ability to adapt to the various soil nutrient conditions, fertilization will also certainly influence soil microbial growth and activity (Broeckling et al., 2008; Wei et al., 2008). Repeated fertilizer applications to soil can change the soil microbial community directly or indirectly since they change the soil physical, chemical and biological properties (Beauregard et al., 2010).

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Some studies have documented that fertilization has had significant impacts on the population, composition and function of soil microorganisms, and that organic and inorganic fertilizer amendments have increased the soil microorganisms' activity (Ge et al., 2008; Mandal et al., 2007). However, other studies have demonstrated that inorganic and organic fertilizers have had relatively little or no effect on soil microbial diversity and activities (Okano et al., 2004; Treseder, 2008). Long-term fertilization experiments can be controlled so as to modify soils in a particular manner and so can contribute significantly to existing knowledge about the evolution of soil fertility, the effects of fertilization, nutrient cycling in croplands, as well as soil biogeochemical cycles. Thus, research on soil microorganism communities under long-term fertilizer management has been one of the foci of soil ecological research in recent years (He et al., 2008; Yamaguchi et al., 2009).

Phospholipid fatty acids (PLFAs) are a vital component of the membrane (essentially the skin) of all microbes, and their polar head groups and ester-linked side chains (i.e. FAs) vary in composition between eukaryotes and prokaryotes, as well as among many prokaryotic groups (Drenovsky et al., 2004; Hao et al., 2008). These compounds rapidly degrade as cells die, making them good indicators of living organisms (White et al., 1979). Thus, analysis of microbial populations using PLFA analysis provides direct information for the identification, classification and quantification of microbial community composition which overcomes the selectivity problems associated with culture techniques (Wu et al., 2009a).

In the paddy soils of subtropical China, long-term fertilizer trials were conducted to study the effects of fertilization on soil microbial communities (Beauregard et al., 2010; Zhang et al., 2012). However, most of these long-term studies have focused on different mineral fertilizer application rates, and the effects of long-term applications of different types of fertilizers on the soil microbial community structures remain poorly understood. In this study, we collected soil samples from a 13-year fertilization experiment, during which time mineral fertilizer and organic manure amendments were applied to an agricultural soil in subtropical China. We hypothesized that (i) the effects of fertilization on soil microbial community composition would vary with the fertilizer type; (ii) the organic manure would increase the abundance of the main soil microbial communities (e.g. bacteria and fungi); while different mineral fertilizer rates may have positive or negative effects on soil microbial communities (e.g. bacteria and fungi) by altering soil properties. We investigated the effects of different long term fertilization practices (CK, organic manure, different rate of mineral fertilizer) on soil microbial communities using phospholipid fatty acid (PLFA) analysis. The aims of this study were to (i) evaluate the effects of organic and mineral fertilizer treatments on the community structure of soil microorganisms and (ii) investigate the influence of soil chemical properties on soil microbial communities.

2. Materials and methods

2.1. Study site

The experiment was conducted at the Chinese Academy of Sciences Qianyanzhou Experimental Station (115°03′29.2″E, 26°44′29.1″N), located in the hill red-earth region of South China. The region has a subtropical monsoon climate. According to the meteorological statistics, the mean annual temperature is 17.8 °C, and the annual active accumulated temperatures (above 0 and 10 °C) are 6543.8 and 5948.2 °C, respectively. The annual precipitation and evaporation are 1471.2 mm and 259.9 mm, respectively, and the mean relative humidity is 83%. The frost-free period is

290 days and global radiation is 4223 MJ m^{-2}. Our experimental site was located in the flat floodplain where the soil-forming parent material consists of red sandstone and sandy conglomerate. Investigation and analysis before our experiment showed that the main soil type was paddy soils, the bulk density of which was 1.50 g cm^{-3} (0–20 cm), pH was 5.97, SOC content was 9.71 g kg^{-1}, the total N content was 1.02 g kg^{-1}, and available P was 1.6 mg kg^{-1}.

2.2. Experimental design

A long-term fertilizer experiment was initiated in 1998 under a double rice cropping system (rice–rice–winter fallow), which is one of the most common cropping systems in the region. In this system, summer rice is sown at the end of April and harvested in July, and winter rice is sown at the end of July and harvested in November. During the growing season, we used the same field practices (field preparation, tillage, puddling, and irrigation) as the local farmer. No pesticides were applied during the growing season and weeds were controlled manually.

We analyzed samples from five different treatments: (1) CK (unfertilized control), (2) $N_1P_1K_1$ (mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 225–135–225 kg ha^{-1}, (3) $N_2P_2K_2$ (mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 450–270–450 kg ha^{-1}, (4) $N_3P_3K_3$, mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 675–405–675 kg ha^{-1}, (5) OM (organic manure) applied at a rate of 41,000 kg ha^{-1} fresh weight (N content is 0.55%). In the NPK treatments, N, P, and K were applied as urea, calcium–magnesium phosphate, and potassium chloride. The organic manure used in the OM treatment was comprised of pig feces, which was collected from a nearby pig farm in Taihe County. Prior to being applied as a manure, it was subjected to a high temperature composting-process, which resulted in a good organic fertilizer after a few months of fermentation, sterilization, and deodorization (Zhang et al., 2009). All fertilizer was applied twice a year: 44% was applied in April for early rice and 56% was applied in July for late rice.

Treatments were arranged in a randomized block design with three replications (Bi et al., 2009; Sikka and Kansal, 1995), totaling 15 plots. Each plot was 15 m^2 (3 m × 5 m) and was isolated by concrete walls (50 cm depth and 15 cm above the soil surface). These fertilization systems were chosen as they were consistent with methods used by local farmers.

2.3. Sampling collection and analysis

Soil samples were collected from the 15 plots at 10 days after the late rice harvest in 2011. In each plot, soils were sampled in the plow layer (0–20 cm) using an auger with a 5 cm internal diameter at five randomly selected locations and then mixed as one sample. All the fresh soil samples were air-dried and sieved twice using 2.0 mm and 0.25 mm meshes and stored for nutrient analysis. Soil chemical properties were measured using the methods described by Bao (2005). Soil pH was measured with a glass electrode in a 1:2.5 soil/water suspension. The dissolved DOC content of filtered 0.5 M K_2SO_4 extracts from fresh soil was measured with a TOC analyser (Liqui TOC Elementar, Vario Max, Germany). Concentrations of NH_4^+-N and NO_3^--N of filtered 2 M KCl extracts from fresh soil were measured with a flow injection autoanalyser (AutoAnalyzer 3, Bran + Luebbe, Germany). Concentrations of C and N were measured with an elemental analyzer (Elementar, Vario Max, Germany) (Huang et al., 2012). Total soil P was analyzed with a flow injection autoanalyser following H_2SO_4–$HClO_4$ digestion (Benke et al., 2010).

The soil microbial community was characterized using phospholipid fatty acid (PLFA) analysis. Phospholipid fatty acid (PLFA) was extracted from the soil using the procedure of Bossio and Scow