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Research paper

Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties

Danmei Chen[a], Ling Yuan[a,*], Yiren Liu[b,*], Jianhua Ji[b], Hongqian Hou[b]

[a] College of Resources and Environment, Southwest University, Chongqing 400716, China
[b] Institute of Soil Fertilizer and Resources Environment, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China

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ABSTRACT

Compared to the short-term experiment, we have a lack of understanding about the long-term effect of fertilizers on rice yield and paddy soil properties under the conditions of frequent soil disturbance and intensive cropping cultivation. Thus, a 32-year (1984–2015) field experiment was established on a red clay soil (typical Ultisols) near Nanchang, Jiangxi province, China, to assess the effects of inorganic and organic fertilizers on rice yields, soil chemical properties and bacterial communities in early rice-late rice-*Astragalus sinicus* L. rotation system. Manure applications in combination with different proportions of chemical fertilizer in terms of nitrogen, particularly 70 M + 30CF (70% manure in combination with 30% chemical fertilizer), sustained high rice yields and increased soil OM, 1 N NaOH-hydrolyzed N, Olsen phosphorus, microbial biomass, and bacterial diversity but alleviated soil acidification. The soil receiving MCF had a great number of bacterial operational taxonomic units and high richness indexes. Compositions and abundances of predominant bacteria in soils varied among the fertilizer treatments and all of bacterial communities were dominated by three major phyla (Chloroflexi, Proteobacteria, and Acidobacteria), which were more than 70% of the total sequences in each of the soils examined. Among the top 15 predominant bacteria, seven were commonly found in all studied soils and only 1–2 phylotypes were unique in each soil. A large number of facultative anaerobic and aerobic bacteria, including *Thiobacillus thioparus*, *Bradyrhizobium*, and *Nitrospira*, were present in all studied soil. Therefore, bacterial community compositions can reflect soil processes such as acidification, greenhouse gas emission and nitrogen recycling in response to tillage and fertilizer managements.

1. Introduction

Crop fertilization can be supplied with organic and inorganic fertilizers. Chemical fertilizers, because of their high nutrient concentration, easy availability, and convenient transportation and application, are very attractive and commonly used to enhance crop yields. However, the long-term use of large amounts of chemical fertilizers only may contribute to degrade soil structure and deteriorate soil productivity (Blanco-Canqui and Schlegel, 2013; Guo et al., 2010). On the other hand, sole application of organic fertilizers may not be able to maintain and synchronize the required supply of nutrients to the growing crops for optimum production, because of relatively less quantity of plant-available nutrients and more time needed for mineralization to release nutrients available for effective plant uptake (Miao et al., 2011). Judicious and proper application of organic manures in combination with chemical fertilizers can reduce the sole dependence on chemical fertilizers for sustainable high crop production by minimizing nutrient losses into the environment and optimizing nutrient use efficiency. Combined use of organic and inorganic fertilizers may thus be an effective way to ensure high sustainable soil productivity and environmental quality (Ye et al., 2011; Zhao et al., 2010). However, the information on the relative comparisons of organic and inorganic fertilizers in improving rice yield and paddy soil properties through long term experiments is lacking, especially in the subtropical areas with intensive cropping (early rice, late rice and *Astragalus sinicus* L. each year) and excessive use of heavy tillage (Ma et al., 2011; Subehia et al., 2013).

Bacteria are the most abundant and diverse group of soil organisms (Stroobants et al., 2012), which play key role in soil quality and function due to their involvement in organic matter dynamics, nutrient

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Abbreviations: CK, without fertilizers; CF, chemical fertilizers; 30M + 70CF, 30% manure + 70% chemical fertilizers; 50M + 50CF, 50% manure + 50% chemical fertilizers; 70M + 30CF, 70% manure + 30% chemical fertilizers; OM, organic matter; C, carbon; N, nitrogen; OUTs, operational taxonomic units; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen

* Corresponding authors.
E-mail addresses: dorischan0808@163.com (D. Chen), lingyuanh@aliyun.com (L. Yuan), jxnclyr@163.com (Y. Liu), jron_jijianhua@126.com (J. Ji), hugh_hhq@yeah.net (H. Hou).

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Table 1
Annual application rates of net nutrients in experimental treatments (kg ha^{-1}).

Treatments	Early rice				Late rice			
	A. sinicus L. (N-P_2O_5-K_2O)	Urea (N)	Superphosphate (P_2O_5)	KCl (K_2O)	Swine (N-P_2O_5-K_2O)	Urea (N)	Superphosphate (P_2O_5)	KCl (K_2O)
CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CF	0.00	150.0	60.00	150.0	0.00	180.0	60.00	150.0
30M + 70CF	44.34-11.71-33.66	105.8	48.00	116.4	54.18-21.42-63.00	125.8	38.58	87.00
50M + 50CF	74.92-19.78-56.87	74.84	40.20	93.60	89.87-35.53-104.5	90.13	24.47	45.50
70M + 30CF	103.9-27.44-78.89	46.00	33.00	71.40	126.0-49.81-146.5	54.00	10.19	3.50

CK = control treatment; CF = chemical fertilizer treatment; 30 M + 70CF = 30% manure + 70% chemical fertilizers treatment; 50 M + 50CF = 50% manure + 50% chemical fertilizers treatment; 70 M + 30CF = 70% manure + 30% chemical fertilizers treatment.

cycling and decomposition processes including detoxification from xenobiotics (Hayat et al., 2010; Xu et al., 2014). Understanding soil microbial community structure shifts following implementation of various agronomic managements is an important component in developing management practices to improve soil fertilities and functions. Fertilizers have huge influences on compositions in soil bacterial communities because of the direct supply of organic matter and nutrients, and indirect return of more organic materials to soils by crop growth stimulation. However, much research work has focused on either surveying bacterium in a relatively short time (Klimek, 2013; Li and Zhang, 2013) or analyzing soil bacteria with traditional techniques that provide little detail about the phylogenetic structure of the bacterial communities (Ashelford et al., 2003; Edenborn and Sexstone, 2007). There is still lack of information about the specific changes in soil bacterial diversity and community structure as affected by long-term fertilizer application. The 454 pyrosequencing after ribosomal amplification is a recent and powerful molecular technique, which can identify huge number of individual bacteria and let us to look virtually at bacterial diversity in any type of soils. By determining composition in soil bacterial community after long-term fertilization, we may be able to better improve soil function and sustain soil productivity because many soil processes may depend partly on bacterial components in the soil systems (Kaur and Reddy, 2014; Mirjam et al., 2015).

Compared to the dry lands, manures decompose slowly and might produce some negative effects on rice growth in paddy soil under flooding conditions (Li et al., 2015). However, the frequent soil aeration and disturbance occur in early rice-late rice-*Astragalus sinicus* L. rotation system and manures might behave otherwise in this case. Long-term experiments can provide a means of evaluating sustainable management practices in ecology and agriculture. The objective of this study was to evaluate the long-term effectiveness of chemical fertilizer and manure in different combinations in improving rice yields, paddy soil chemical properties, and bacterial community structures under the conditions of frequent soil disturbance and intensive rice cultivation. We hope that the results obtained from this long-term experiment might provide the farmers in South China and Southeast Asia a fertilizer management practice for continually increasing rice yield and at the same time sustaining soil fertility and quality in intensive rice cultivation.

2. Materials and methods

2.1. Site description

The experiment was established in spring 1984 on a red clay soil (typical Ultisols) near Nanchang City, Jiangxi province, China (28°57′ N, 115°94′ E, 25 m above the sea level). The natural vegetation is evergreen subtropical forest with long-term average annual air temperature of 17.5 °C (ranging from 6.5 °C in January–February to 29.8 °C in July–August), annual precipitation of 1600 mm, and annual evaporation of 1800 mm. As much as 80% of the annual precipitation is received between February and August, with a mean annual humidity of 86% (Hou et al., 2011). The soil is intensively cropped each year (early rice from the beginning of April to the middle of July, late rice from late July to the middle of October, and *Astragalus sinicus* L. from late October to the middle of March next year). Some characteristics of the experimental soil at beginning are presented in Table 3.

2.2. Experimental design

A randomized complete block design was used to layout the treatment with four replications. Each plot (10 m long and 3.33 m wide) was separated by concrete walls from others. The rice was supplied with manure in combination with different proportions of chemical fertilizer in terms of nitrogen to form 5 treatments: 1. control (without fertilizers, CK); 2. chemical fertilizers (CF); 3. 30% manure + 70% chemical fertilizers (30 M + 70CF); 4. 50% manure + 50% chemical fertilizers (50 M + 50CF); and 5. 70% manure + 30% chemical fertilizers (70 M + 30CF). In various fertilizer treatments, early rice received 150 kg N ha^{-1}, late rice 180 kg N ha^{-1}, and both of them received 60 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1}. Manure was applied as *A. sinicus* L. (early rice) and swine (late rice) at transplanting stage and chemical fertilizer as urea, simple superphosphate, and potassium chloride at transplanting (all phosphorus and potassium fertilizers, and half of urea) and tillering stage (other half of urea). *A. sinicus* L. contained 0.30–0.35% of N, 0.05–0.10% P_2O_5 and 0.20–0.25% K_2O, and swine 0.40–0.50% N, 0.15–0.20% P_2O_5 and 0.50–0.60% K_2O. The amount of manures and chemical fertilizers used in each treatment is shown in Table 1.

2.3. Soil sampling and analyses

5 soil (0–20 cm) samples were taken from each plot and mixed homogenously after late rice harvest in 2015. To sample soils at this time can avoid the interference of fertilizers and reflect the real soil properties. There were four soil samples obtained from each treatment for chemical and bacterial analysis. Each sample was divided into two parts. One part of the soil sample was air-dried for determination of chemical properties and the other part was liquid N_2-frozened immediately for microbial analysis. The chemical properties measured included organic matter by dichromate oxidation method, pH in a 1:1 soil: water slurry by a glass pH meter, Olsen P by extraction with 0.5 M $NaHCO_3$ and measurement by molybdenum blue spectrophotometry, and exchangeable K by displacement with 1 M ammonium acetate followed by flame photometry (Pansu, 2006). In addition, 2 g of air-dried soil was mixed with 0.2 g $FeSO_4 \cdot 7H_2O$-Ag_2SO_4 (2:1 in weight) and put into the outer sub-cell in a diffusion cell, added with 10 mL of 1 N NaOH, and incubated at 40 °C for 24 h. NH_3 collected in 2% H_3BO_3 solution placed in the inner sub-cell was measured by HCl titration (Pansu, 2006). The soil microbial biomass carbon and nitrogen were determined on a 15 g oven-dry equivalent field-moist soil by the fumigation-extraction method (Vance et al., 1987).

454 FLX pyrosequencing were performed in Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) as described by Fierer et al.