J. Microbiol. Biotechnol. (2015), 25(5), 569–578
http://dx.doi.org/10.4014/jmb.1407.07066

jmb

Effects of Supplementary Composts on Microbial Communities and Rice Productivity in Cold Water Paddy Fields

Kaizhi Xie[1,2,3], Peizhi Xu[1,2,3*], Shaohai Yang[1,2,3], Yusheng Lu[1,2,3], Ruiping Jiang[1,2,3], Wenjie Gu[1,2,3], Wenying Li[1,2,3], and Lili Sun[1,2,3]

[1] *Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P.R. China*
[2] *Key Laboratory of Plant Nutrition and Fertilizer in South Region, Ministry of Agriculture, Guangzhou 510640, P.R. China*
[3] *Guangdong Key Laboratory of Nutrient Cycling and Farmland Conservation, Guangzhou 510640, P.R. China*

Received: July 24, 2014
Revised: November 3, 2014
Accepted: November 18, 2014

**Corresponding author*
Phone: +86-20-85161405;
Fax: +86-20-85161437;
E-mail: xupz66@163.com

pISSN 1017-7825, eISSN 1738-8872

Cold water paddy field soils are relatively unproductive, but can be ameliorated by supplementing with inorganic fertilizer from animal waste-based composts. The yield of two rice cultivars was significantly raised by providing either chicken manure or cow dung-based compost. The application of these composts raised the soil pH as well as both the total nitrogen and ammonium nitrogen content, which improved the soil's fertility and raised its nitrification potential. The composts had a measurable effect on the abundance of nitrogen-cycling-related soil microbes, as measured by estimating the copy number of various bacterial and archaeal genes using quantitative real-time PCR. The abundance of ammonia oxidizing archaea and bacteria was markedly encouraged by the application of chicken manure-based compost. Supplementation with the composts helped promote the availability of soil nitrogen in the cold water paddy field, thereby improving the soil's productivity and increasing the yield of the rice crop.

Keywords: Compost, cold water paddy field, productivity, nitrification, denitrification

Introduction

Cold waterlogged paddy fields are formed when the groundwater level rises as a result of the impact of local microclimate and topographical features, leading to perpetual water accumulation on the soil surface. These paddy fields are mainly distributed in mountain valleys, lake marshes, mountain ponds, and land downstream of dams. Cold waterlogged paddy fields typically have low to average yield because of the following characteristics: (i) in the mountains, the annual average sunshine hours and total amount of astronomical solar radiation are very low, and the temperature of soil is 3-5 degrees lower compared with the average sunshine hours, total amount of astronomical solar radiation, and soil temperature on flat ground, thus negatively influencing tillering of rice; (ii) soil drying is inhibited because of long-time waterlogging, resulting in less soil aggregates, low water stability, and a poorly developed plough layer; (iii) the growth of rice is influenced negatively because of the increasing number of soil anaerobic microorganism and accumulation of soil reducing substances that result from the perpetual waterlogging; and (iv) soil mineralization ability is weakened by the decreasing number of aerobic microorganisms, resulting in lack of available soil nutrients, especially soil available P content. Such fields occupy about 3.5 Mha in China, representing 15% of the area on which patty rice grows; about half of this area is found in the mountainous and hilly regions of southern China. The achievable yield in these fields is less than half of the national average, mainly resulting from nutrient stress, which weakens the root system and slows crop growth and development.

Long-term fertilizer application can increase the soil's nitrate content but also depresses the population of nitrifying bacteria. Organic fertilizers derived from livestock and poultry manure contain large amounts of organic matter and the macronutrients essential for crop growth, but need to be well composted to provide the maximum benefit [9, 32]. The use of such composts helps both to maintain the soil's nitrogen status as well as to encourage microbial diversity and activity [6, 22, 26]. Generally, there is a so-called "win–win" status in manure-amended rice systems for its N use efficiency improvement and animal waste recycling. Consequently, the interactions between the composition of these communities and nitrogen cycling processes need to be better understood in order to optimize trade-offs between high organic matter inputs management practices and microbial-mediated N dynamics in agroecosystems.

Today, with the rapid development and increased use of molecular biology techniques, there has been some progress in the study of nitrogen functional genes in farmland ecosystems amended with organic fertilizer [14, 15, 29]. Data suggest that composts do not leave direct microbial imprints in soils after long-term amendment, but an indirect effect on the ammonia-oxidizing bacteria (AOB) community is evident. Ai *et al*. [1] suggests that N fertilization greatly enhances potential nitrification activity and AOB abundance, while manure application increases ammonia-oxidizing archaea (AOA) abundance. Kong *et al*. [19] hypothesized that (i) differences in potential gross N mineralization and nitrification rates across the systems would correspond with AOB and denitrifier abundances, and (ii) ammonia monooxygenase (*amoA*), nitrous oxide reductase (*nosZ*), and 16S rRNA gene abundances would be higher in the microaggregates than in the coarse particulate organic matter (>250 μm) and silt-and-clay microenvironments. However, even though soil management, C content, and N content differed across the organic, conventional, and low-input systems, the total bacterial communities within the whole soil were similar in size across the three systems (~5.15 × 10^8 copies/g soil). Whereas most studies to date have focused on a single functional gene, analysis of a more complete suite of genes would enable us to better address the role of community structure in controlling nitrogen cycling.

Here, quantitative real-time PCR assays, targeting part of the *amoA*, nitrite reductase (*nirK* and *nirS*), *nosZ*, and 16S rRNA genes, were used to investigate the effects of three different composts on the growth of a rice crop raised in a cold water paddy field, focusing particularly on the effect of the fertilizer regime on the soil's microbes.

Materials and Methods

Experimental Setup

Experiments were conducted between 2011 and 2013 in a cold water paddy field in Guangdong Province, China (22°50′9′′N, 114°36′3′′E), where the local climate is subtropical and monsoonal. The soil is clay with a pH of 5.1, and contains 30.4 g/kg organic matter, 132 mg/kg available N, 10 mg/kg available P, and 34 mg/kg available K. Two rice cultivars were grown: Huanghuazhan and Hefengzhan. A combination of inorganic and organic fertilizers was applied. The former had three components: urea (U) containing 46% N, superphosphate (SP) containing 12% P_2O_5, and potassium chloride (KCl) containing 60% K_2O. Three kinds of organic fertilizer were trialled: one processed from cattle manure (CAM), one from pig manure (PIM), and the third from chicken manure (CHM). The N:P:K ratio in all three organic fertilizers was about 1.5:1:1, and the organic matter content was about 30%. At the beginning of the experiment, the CAM contained (pH 7.2), 1.03% water-soluble organic C, 6.04 × 10^9/g bacterial copies, 1.82 × 10^6/g archaeal copies, 3.08 × 10^4/g AOA copies, and 1.25 × 10^4/g AOB copies. The PIM contained (pH 6.5), 0.37% water-soluble organic C, 1.32 × 10^{10}/g bacterial copies, 3.24 × 10^6/g archaeal copies, 1.21 × 10^5/g AOA copies, and 2.65× 10^5/g AOB copies. The CHM contained (pH 7.9), 3.58% water-soluble organic C, 2.49 × 10^{11}/g bacterial copies, 1.32 × 10^7/g archaeal copies, 8.45 × 10^5/g AOA copies, 1.18 × 10^7/g AOB copies.

Experimental Design

Four treatments were imposed (Control, PIM, CAM, and CHM), and the experiment was set out as a randomized complete block design with three replications. Each 8.7 × 7.1 m plot was isolated by a ridge covered with plastic film to prevent any plot-to-plot movement of water, while leaving a guard row for protection. The control involved the application of 325 kg/ha U, 375 kg/ha SP, and 200 kg/ha KCl, and the PIM, CAM, and CHM treatments each used 3,000 kg/ha organic fertilizer supplemented with 228 kg/ha U and 150 kg/ha KCl. The full complement of fertilizer was applied one day before transplanting the 30-day-old rice seedlings. The space within each row was 18 cm, and the space between each row was 20 cm. Field management followed standard agricultural practice.

Phenotypic Evaluation and Soil Sampling

Five plants in the center of each plot were scored for the number of panicles per plant (PPP), the number of fertile panicles per hectare (EP), the number of spikelets per panicle (SPP), the fertility of the main panicle (SR), and the thousand grain weight (GW). The grain yield of each plot (YEP) was calculated from that of individual plants. A 1 kg soil sample, constituted by mixing samples collected at between 0 and 20 cm from the soil surface, was taken from each treatment in the sixth season (November 2013), and divided into three parts. The first part was used for the determination of nitrogen and nitrification/denitrification, the second as a source of soil microbes for DNA analysis, and the third to determine various physicochemical properties.

Soil Physicochemical Characteristics

The soil solution pH was determined using a pH meter (Delta

320; Mettler-Toledo Instruments Co., China) after extracting a sample in 2.5 volumes of water. A San++ continuous flow analyzer (Skalar Analytical, Breda, The Netherlands) was used to quantify ammonium-N and nitrate-N from extracts made with five volumes of 2 M KCl. Total C and N contents were determined using a Thermo Finnigan FLASH EA 1112 CHN Analyzer (ThermoQuest, Milan, Italy). Nitrification potential was determined according to Kurola [12]. In brief, 5.0 g of fresh soil was added to 50 ml centrifuge tubes containing 20 ml of phosphate buffer solution (PBS) (g/l: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 0.2; NaH_2PO_4, 0.2; pH 7.4) with 1 mM $(NH_4)_2SO_4$. Potassium chlorate at a final concentration of 10 mM was added to the tubes to inhibit nitrite oxidation. The suspension was incubated in a dark incubator at 25°C for 24 h, and nitrite was extracted with 5 ml of 2 M KCl and determined by a spectrophotometer at a wavelength of 540 nm with *N*-(1-naphthyl) ethylenediamine dihydrochloride. Denitrification potential was measured using the acetylene (C_2H_2) inhibition technique [10]. In this study, we modified the method by omitting any extra nitrate and carbon, since the content of NO_3^-–N and organic carbon was already high (384 mg/kg and 19.9 g/kg, respectively) owing to excessive fertilization. It was reported that the optimum concentrations for NO_3^-–N should not exceed 50 mg/kg, and an excess of NO_3^-–N (>100 mg/kg dry soil) might inhibit N_2O production[20, 24].

DNA Extraction

DNA was extracted from soil samples using a NucleoSpin kit (Macherey-Nagel, Germany) following the manufacturer's protocol. DNA purity was evaluated on the basis of its OD_{260}/OD_{280} ratio.

Real-Time PCR Analysis

Amplicons of bacterial and archaeal 16S rRNA sequences along with those generated from the *amoA*, *nirK*, *nirS*, and *nosZ* genes present in ammonia oxidizing archaea and bacteria were analyzed using bacterial and archaeal 16S rRNA gene sequences, and *amoA*, *nirK*, *nirS*, and *nosZ* of ammonia oxidizing archaea and ammonia oxidizing bacteria were analyzed using a fluorescence quantitative PCR instrument (ABI PRISM7500 ABI Co., USA). The bacterial 16S rRNA sequence was analyzed according to the Taqman probe method [12]. Each PCR mixture (25 µl) contained 12.5 µl of SYBR Premix Ex Taq (TaKaRa, Japan), 1 µl of template DNA (~50 ng), and 0.5 µl of each primer (10 pmol/µl). The other genes were analyzed according to the instructions of SYBR Premix Ex Taq. Plasmids and the standard curve were prepared according to Guo *et al*. [10]. Primers, probes, and reaction procedures of the real-time PCR analysis are shown in Table 1.

Data Analysis

One-way analysis of variance and the Duncan multiple range test were used was to identify means that differed significantly from one another. A Pearson exact analysis was used to obtain correlations. All statistical calculations were carried out using programs implemented in SPSS ver. 13.0 software (IBM, USA).

Results

Plant Performance

The grain yield achieved by including any of the organic

Table 1. Primers, probes, and reaction procedures of the real-time PCR analysis.

Genes	Primers and probes	Primers (5′ → 3′)[b]	PCR procedure	References
Bacterial 16S rRNA	1369F 1492R TM1389F	CGGTGAATACGTTCYCGG GGWTACCTTGTTACGACTT CTTGTACACACCGCCCGTC	An initial cycle of 10 sec at 95°C; 35 cycles of 15 sec at 95°C, 1 min at 56°C, and 40 sec at 72°C.	[12]
Archaeal 16S rRNA	A364aF Ar958R	CGGGGYGCASCAGGCGCGAA YCCGGCGTTGAVTCCAATT	An initial cycle of 30 sec at 94°C; 40 cycles of 20 sec at 94°C, 30 sec at 63°C, and 30 sec at 72°C.	[18]
AOA[a]	Arch-amoAF Arch-amoAR	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	An initial cycle of 30 sec at 95°C; 40 cycles of 10 sec at 95°C, 30 sec at 53°C, and 1 min at 72°C.	[12]
AOB[a]	amoA-1F amoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	An initial cycle of 2 min at 94°C; 40 cycles of 30 sec at 94°C, 45 sec at 55°C, and 30 sec at 68°C.	[12]
nirK	1F 5R	GGMATGGTKCCSTGGCA GCCTCGATCAGRTTRTGG	An initial cycle of 5 min at 95°C; 34 cycles of 30 sec at 95°C, 40 sec at 58°C, and 30 sec at 72°C.	[10]
nirS	1F 6R	CCTAYTGGCCGCCRCART CGTTGAACTTRCCGGT	An initial cycle of 2 min at 94°C; 40 cycles of 30 sec at 94°C, 30 sec at 58−53°C (the first 5 cycles per cycle was lowered by 1°C), and 30 sec at 72°C.	[10]
nosZ	F 1622R	CGYTGTTCMTCGACAGCCAG CGCRASGGCAASAAGGTSCG	An initial cycle of 2 min at 94°C; 40 cycles of 30 sec at 94°C, 30 sec at 58−53°C (the first 5 cycles per cycle was lowered by 1°C), and 1 min at 72°C.	[10]

[a] *amoA*-AOA: ammonia monooxygenase (*amoA*) of ammonia oxidizing archaea (AOA); *amoA*-AOB: ammonia monooxygenase (*amoA*) of ammonia oxidizing bacteria (AOB).

[b] Y = C or T; M = A or C; W = A or T; R = A or G; K = G or T; S = C or G.

fertilizer formulations was higher than that achieved in the control, but the addition of pig manure-based compost did not produce a statistically significant improvement (Table 2). The chicken manure-based product was the best-performing additive, followed by the cattle manure-based one. The performance of the crops with respect to three of the four yield-related traits (the exception was EP), when fertilized with either chicken manure- or cattle manure-based compost, was not significantly better than in the control.

Soil pH and Nitrogen Content

The three composts had varying effects on the soil's pH and nitrogen content (Table 3). The presence of the composts increased the soil pH and the total nitrogen and ammonium N content, particularly when the compost was formulated with either chicken manure- or cattle manure-based compost. The content of nitrate and nitrite was higher in the control soils than in any of the organic fertilizer-supplemented (PIM, CAM, and CHM) soils. Both soil pH and the form of nitrogen provided had a substantial effect on crop productivity, most notably in the CAM and CHM soils (Tables 2 and 3).

Soil Nitrification and Denitrification Potential

The soil nitrification and denitrification potential is given in Figs. 1 and 2. The nitrification potential increased gradually over time, but changed faster in PIM, CAM, and CHM than in control soil during the 10 days after transplanting. Differences in the nitrification potential were nonsignificant between PIM, CAM, and CHM soils at 20 and 25 days after transplanting. The denitrification potential also increased gradually over time in the presence of each of the organic fertilizers.

Abundance of Functional Soil Microbes

The abundance of bacterial and archaeal cells, as indicated

Table 2. Rice yield and its components from different treatments.

Year and season	Treatments	EP	SPP	SR (%)	GW (g)	Yield (kg hm^{-2})
2011 early	CK	2,865,000	107	87.6	22.5	5,718.0 b
	PIM	2,865,000	112	85	22.6	5,896.5 ab
	CAM	2,925,000	113	87.5	22.5	6,075.0 a
	CHM	2,970,000	111	87.3	22.7	6,123.0 a
2011 late	CK	3,060,000	149	83.5	20.1	6,637.5 c
	PIM	3,075,000	145	82.6	20.5	6,948.0 b
	CAM	3,015,000	143	82.3	20.6	7,176.0 ab
	CHM	2,925,000	150	83.5	20.5	7,374.0 a
2012 early	CK	2,850,000	133	82.3	21.0	6,283.5 c
	PIM	2,895,000	130	82.7	21.1	6,532.5 b
	CAM	2,985,000	130	83.5	21.2	6,604.5 b
	CHM	3,075,000	134	84.6	21.4	7,042.5 a
2012 late	CK	2,910,000	139	81.5	20.1	6,337.5 c
	PIM	3,000,000	135	80.6	20.5	6,798.0 b
	CAM	3,015,000	133	81.3	20.6	7,026.0 ab
	CHM	3,045,000	140	81.5	20.5	7,074.0 a
2013 early	CK	2,655,000	149	77.3	21.0	6,345.0 b
	PIM	2,580,000	162	77.9	21.0	6,525.0 b
	CAM	2,640,000	160	80.9	21.3	6,841.5 a
	CHM	2,730,000	184	79.7	21.2	6,930.0 a
2013 late	CK	2,775,000	127	88.2	20.5	6,465.0 b
	PIM	2,760,000	140	80.5	20.5	6,645.0 b
	CAM	2,865,000	146	88.9	20.4	6,952.5 a
	CHM	3,180,000	147	86.1	20.6	7,129.5 a

Means followed by the same letter are not significantly different at the 5% level by the Tukey test. CK: control; PIM: pig manure; CAM: cattle manure; CHM: chicken manure.

Table 3. Soil pH and nitrogen contents of four treatments.

Treatments	pH	Total N (g/kg)	Ammonium N (mg/kg)	Nitrate N (mg/kg)	Nitrite N (mg/kg)
CK	5.55 b	1.63 c	183.41 c	6.10 a	0.320 b
PIM	5.58 b	1.87 b	200.65 b	6.10 a	0.212 c
CAM	5.78 a	1.90 ab	238.23 a	5.88 a	0.505 a
CHM	5.83 a	2.08 a	243.13 a	3.98 b	0.249 c

Means followed by the same letter are not significantly different at the 5% level by the Tukey test.

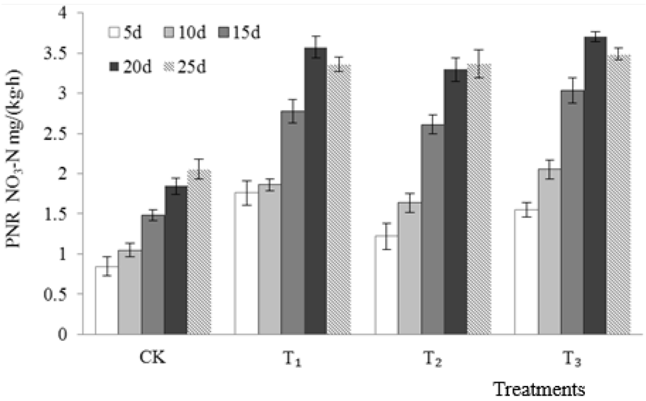

Fig. 1. Soil nitrification potential.

PNR: potential nitrification rate.

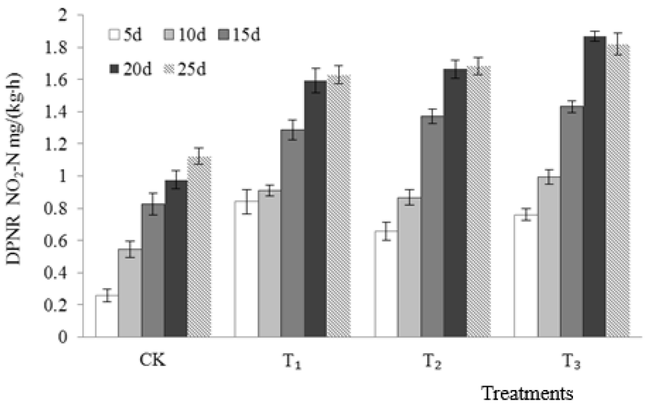

Fig. 2. Soil denitrification potential.

DPNR: potential denitrification rate.

by the quantities of 16S rRNA, *nirK*, *nirS,* and *nosZ* amplicons produced, is shown in Fig. 3. The 16S rRNA and *amoA* profiles showed that the population of bacteria and archaea was the highest in CHM soil, followed by the populations in CAM, PIM, and control. On the basis of the *nirK* and the *nosZ* amplicons, the highest predicted population size was in the CAM soil, whereas on the basis of the *nirS* amplicon, it was in the CHM soil. The abundance of *nirK*, *nirS*, and *nosZ* among the three refined organic fertilizer treatments was different from each other, indicating that the three refined organic fertilizers participated in different soil nitrogen metabolic pathways. It is clear therefore that the application of organic fertilizers can encourage the growth of nitrogen-related functional soil microbes, improving the availability of soil nitrogen to the plant, which in turn promotes crop growth and yield.

Correlations Between the Copy Numbers of Nitrogen Related Functional Soil Microbe Genes and the Soil's Physicochemical Characteristics

The correlation analysis between the abundance of the nitrogen-related functional soil microbes and the soil's physicochemical characteristics is given in Table 4. Archaea number was very significantly positively correlated between AOA copies, AOB copies, and soil denitrification potential, whereas archaea number was very significantly negative with AOA/AOB. AOA and AOB copies were very significantly positively correlated with soil pH, total-N and ammonium-N, and denitrification potential, but very significantly negative with C/N and nitrate-N. It was indicated that the ammonia oxidizing archaea and ammonia oxidizing bacteria were the main strains driving the nitrification process. The copies of *nirK*, *nirS*, and *nosZ* were very significantly positively correlated with total-N and ammonium-N, and were positively correlated with the denitrification potential, whereas they were significantly negatively correlated with C/N. *nirK*/*nirS* was significantly positively correlated with soil pH, ammonium-N, and nitrite-N. (*nirK* + *nirS*)/*nosZ* was significantly positively correlated with the nitrification potential, denitrification potential, and total-N, but significantly negative with C/N. This indicated that different composts could improve the soil pH, total nitrogen, and C/N by material property itself, causing a change of abundance of nitrogen-related functional soil microbes and the soil nitrogen form and content. This activated the soil nitrogen nutrient in the cold

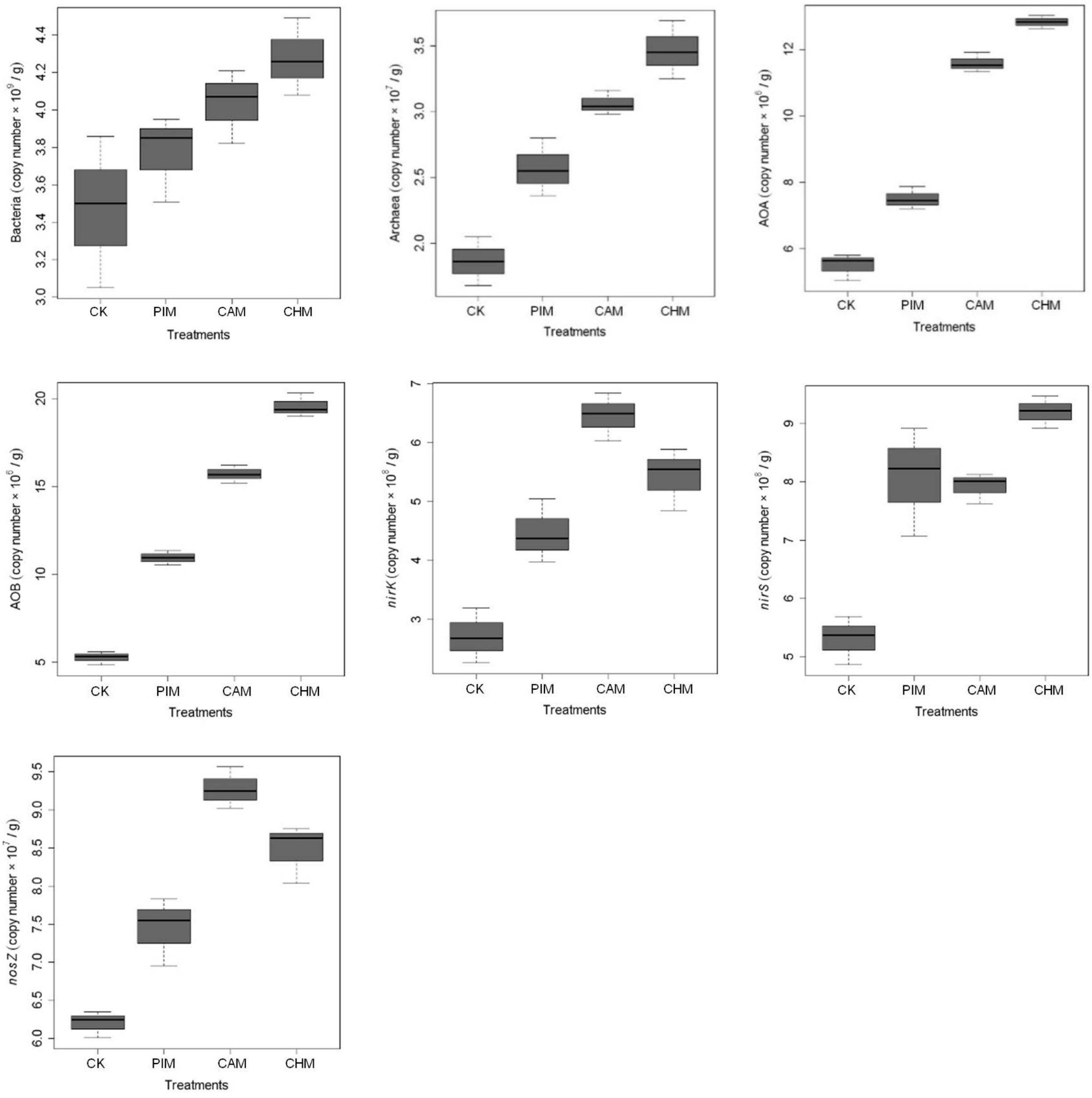

Fig. 3. Copy number of 16S RNA, *amoA*, *nirK*, *nirS*, and *nosZ* amplicons in soils sampled from the various fertilizer treatments.
The rectangular box is the main part of the diagram. The three lines represent (from top to bottom) the 75, 50, and 25 percentiles of the variable and 50% of the observed value drops in this area. The vertical line in the center is a tentacle line, whereas the horizontal lines at the top and bottom represent, respectively, the maximum and minimum values of the variable.

water paddy field and enhanced the soil productivity.

Discussion

Use of Organic Fertilizers to Improve the Productivity of Cold Water Paddy Field Soils

Long-term use of organic fertilizer has been suggested as a means of soil improvement [3, 7, 11, 15, 28]. It has been claimed that the application of composts can help release nutrients from the O-horizon to the mineral soil [2], and been shown to raise the nitrogen content over the top 20 cm of the soil profile [25]. Hartz *et al.* [11] proposed that composts are able to significantly increase short-term soil N supply. Chalhoub *et al.* [3] suggested that the application

Table 4. Correlation analysis among abundance of nitrogen-related functional soil microbes and physicochemical characteristics.

	Archaea	AOA	AOB	AOA/AOB	*nirK*	*nirS*	*nirK*/*nirS*	*nosZ*	(*nirK*+*nirS*)/*nosZ*	NP	DP	pH	Total-N	Total-C	C/N	Ammonium-N	Nitrate-N
Archaea	1.000																
AOA	0.951**	1.000															
AOB	0.968**	0.979**	1.000														
AOA/AOB	-0.812**	-0.724**	-0.831**	1.000													
nirK	0.819**	0.863**	0.829**	-0.715**	1.000												
nirS	0.871**	0.782**	0.869**	-0.933**	0.671*	1.000											
nirK/*nirS*	0.417	0.532	0.425	-0.237	0.813**	0.125	1.000										
nosZ	0.824**	0.888**	0.849**	-0.704*	0.957**	0.662*	0.751**	1.000									
(*nirK*+*nirS*)/*nosZ*	0.702*	0.565	0.678*	-0.851**	0.533	0.916**	0.027	0.421	1.000								
NP	0.521	0.377	0.522	-0.825**	0.454	0.682*	0.128	0.409	0.704*	1.000							
DP	0.712**	0.586*	0.698*	-0.946**	0.645*	0.826**	0.239	0.614*	0.784**	0.896**	1.000						
pH	0.800**	0.851**	0.789**	-0.481	0.780**	0.552	0.608*	0.697*	0.471	0.216	0.393	1.000					
Total-N	0.934**	0.918**	0.940**	-0.736**	0.712**	0.859**	0.262	0.715**	0.707*	0.391	0.572	0.741**	1.000				
Total-C	0.237	0.478	0.329	0.130	0.381	-0.042	0.502	0.425	-0.233	-0.361	-0.275	0.515	0.344	1.000			
C/N	-0.906**	-0.800**	-0.880**	0.868**	-0.650*	-0.956**	-0.117	-0.643*	-0.858**	-0.579*	-0.747**	-0.572	-0.924**	0.026	1.000		
Ammonium-N	0.813**	0.878**	0.840**	-0.606*	0.854**	0.624*	0.659*	0.773**	0.541	0.335	0.495	0.965**	0.739**	0.459	-0.607*	1.000	
Nitrate-N	-0.691*	-0.712**	-0.757**	0.485	-0.364	-0.604*	-0.010	-0.391	-0.470	-0.340	-0.345	-0.576	-0.816**	-0.348	0.687*	-0.550	1.000
Nitrite-N	0.117	0.269	0.096	0.178	0.430	-0.156	0.656*	0.490	-0.346	-0.494	-0.251	0.267	0.053	0.591*	0.129	0.247	0.301

AOA: ammonia oxidizing archaea; AOB: ammonia oxidizing bacteria; NP: nitrification potential; DP: denitrification potential; *positive ($p < 0.05$); **significantly positive ($p < 0.01$).

of composts was able to reduce soil evaporation during the spring. Tejada *et al*. [28] suggested that compost may be responsible for the increase of soil biochemical and chemical properties. Here, the application over three years of formulations of specifically cattle manure- and chicken manure-based composts was effective in increasing the pH, total and ammonium N contents, and nitrification potential of a cold water paddy field soil. The CAM and CHM treatments had the highest pH, around 5.8. The PIM and control treatments had the second and third highest pH, 5.58 and 5.55, respectively. Significant differences ($p < 0.05$) in soil pH (Table 3) were observed between the control and CAM, and control and CHM treatments. The research of Naramabuye and Haynes [23] showed that the presence of organic matter, such as poultry, pig, and cattle manure, can cause an increase in soil pH. The organic matter's high pH and significant $CaCO_3$ content are significant factors to this increase. Other factors may have also contributed to the increased pH. For example, partly decomposed organic materials contain many phenolic, carboxylic, and enolic groups associated with humified material that can consume protons at their natural pH. Their capacity to consume protons, therefore, partly controls their buffering characteristics and their ability to neutralize acidity when added to acid soils. High levels of total and ammonium N contents often lead to a rapid increase in soil potential nitrification activity, which is correlated with soil pH [5]. These changes are significant in terms of the potential yielding capacity of rice in such poor soils.

Use of Composts Affects the Abundance of Bacterial and Archaeal Soil Microbes

Soil microbes participate in oxidation, nitration and ammonification reactions in the soil, which all serve to speed the decomposition and transformation of soil organic matter. The use of composts is a widespread agricultural practice and its beneficial effect on soil microfauna is well documented [17, 25]. Kayikcioglua [17] suggested that composted aromatic plant wastes can be used to enhance soil microbial activity, thereby promoting plant growth. Here, the long-term application of animal waste-based compost materially increased the abundance of bacteria and archaea, as indicated by the quantities of 16S rRNA: in particular that of ammonia oxidizing archaea and bacteria. In the current research, the application of organic manures resulted in larger overall bacterial populations and greater microbial activity than their application in control or chemical fertilizer treatments [35]. Several studies have shown that the type and amount of organic matter had an impact on the AOB and AOA abundance [15, 30, 31]. This was primarily due to organic amendments that are not only nutrient-rich but are also longer lasting than chemical fertilizers. They provide required the carbon, nitrogen, and energy for microbial growth and reproduction [33].

Recent findings have extended the known ammonia-oxidizing prokaryotes from the domain bacteria to archaea. However, in the complex rice ecosystem, it remains unclear whether AOA or AOB are exclusively or predominantly linked to prevailing plant and soil conditions over a rice crop season [13, 34]. In our study, there were no significant effects in the abundance of AOB and AOA. Therefore, soil pH might have played a more important role in affecting AOB and AOA populations in the soil [12]. Contrary to our findings, Chen *et al.* [4] reported that the rice cultivation under microcosm experiment led to a greater abundance of AOA relative to AOB *amoA* gene copies and to differences in AOA and AOB community composition.

Use of Composts Affects the Abundance of Nitrogen-Related Functional Soil Microbes

Nitrogen cycling processes require a diverse bacterial community that possesses several functional genes, including ammonia oxidation (*amoA*), nitrate reduction (*nirS* and *nirK*), and nitrous oxide reduction (*nosZ*), which are responsible for nitrogen transformation. Our quantitative results from samples showed that the lowest AOB and AOA population sizes were in the CK treatment. AOA was significantly correlated with the abundance of denitrification potential ($r = 0.586$, $n = 12$, $p < 0.05$), pH ($r = 0.851$, $n = 12$, $p < 0.01$), total-N ($r = 0.918$, $n = 12$, $p < 0.01$), and ammonium-N ($r = 0.878$, $n = 12$, $p < 0.01$), but was negatively correlated with nitrate-N ($r = 0.712$, $n = 12$, $p < 0.01$). AOB showed the same correlations as AOA. In line with our findings, Wessén *et al.* [31] reported that the AOA community size was negatively correlated to the soil organic carbon content and the C/N ratio. This negative correlation could be due to the competition of ammonia oxidizers with N-demanding heterotrophs for available ammonium and oxygen, since the latter would be favored under high C/N ratios. He *et al.* [12] reported that soil pH was significantly correlated with the abundance of AOB ($r = 0.719$, $n = 8$, $p < 0.05$) and AOA ($r = 0.775$, $n = 8$, $p < 0.05$). Statistical analyses showed that *nirK* was positively correlated with the abundance of AOA ($r = 0.863$, $n = 12$, $p < 0.01$) and AOB ($r = 0.829$, $n = 1$, $p < 0.05$), and with soil denitrification, pH, total-N, and ammonium-N. *nirS* and *nosZ* had the same correlations as *nirK* with one exception: the pH of *nirS* showed no significant correlation.

Therefore, our data suggested that the gene abundance was more significantly correlated with the total-N and ammonium-N than nitrate-N ($p < 0.01$). The lack of correlation between the soil nitrate content and the abundance of the 16S rRNA, *narG*, *nirK*, *nirS*, and *nosZ* genes is in agreement with Kandeler *et al.* [16]. Moreover, other studies have also found that gene abundance and microbial community composition cannot predict the corresponding ecosystem processes. For example, Ma *et al.* [21] found that, although different nitrifier and denitrifier communities were specific to landforms within cultivated versus uncultivated wetlands, these differences were not related to land-use or landform differences in N_2O emissions. In line with our findings, Guo *et al.* [10] showed a positive correlation between the potential denitrification activity to the copy numbers of denitrifying functional genes (*nirK*, *nirS*, and *nosZ*). These two quantities were both negatively correlated with pyrene concentrations.

Composts Application Can Improve Rice Productivity

The long-term application of organic fertilizer has been suggested as a means to increase the rice yield through soil health improvement [8, 26, 27]. Dadhich *et al.* [8] found that the application of using composts as supplements with chemical fertilizers could improve the soil health and plant productivity of rice. Shu and Chung [27] suggested that the chemical fertilizer was a fast-release fertilizer used to supply nutrients at the early stage of rice growth in the first crop, and the beneficial effect of the composts on rice growth and nutrient uptake was conspicuous in the second crop. In this study, the results of three consecutive years showed that the rice yield of the three kinds of compost organic fertilizer together with inorganic fertilizer was significantly higher than the treatment of only chemical fertilizer, except for pig manure, which increased 7.1%–12.1%. The order of yield was chicken manure > cattle manure > pig manure > control. Different organic manure compostings have different effects on rice yield, and the reasons for that can be summarized as follows: (i) different organic manure compostings change the soil pH value, form and validity of soil N, and activation and efficiency of soil nutrients (Table 3), (ii) the poultry feeds are very different; for example, chicken feed contains much more energy and protein compared with that of pig and cattle. Although the total contents of N, P, and K among the different organic manure compostings are similar after artificially equalizing the NPK content between the different feeds, the availability of the original N, P, and micro-elements varies among them, resulting in different effects on soil microenvironment; and (iii) in most soils, the majority of carbon is held as soil organic carbon (SOC). The term soil organic carbon is used to describe the organic constituents in the soil (tissues from dead plants and animals, the products produced as these decompose, and the soil microbial biomass). The constituents of SOC can be divided into non-humic substances (which are discrete identifiable compounds such as sugars, amino acids, and lipids) and humic substances. As organic compounds, both humic and non-humic substances contain carbon (C), oxygen (O), and hydrogen (H) and can also contain nitrogen (N), phosphorus (P), and sulfur (S). Carbon is essential to plants for photosynthesis, the chemical reaction that plants use to create energy. Plants take carbon from carbon dioxide in the atmosphere and produce sugars, proteins, enzymes, hormones, and signal transfer substances. The minimum factor law of classical principles of plant nutrition uses the image of Law of the Minimum to show the importance of the element that is in shortest supply on increased yield.

In large-scale agricultural supplementation, the carbon supplementary has long been neglected while applying large amounts of N, P, and K. Under natural conditions, the amount of carbon acquired by plants is only 1/5 of the required amount, which inhibits the increase of crop production. This problem can be solved through fertilization, which increases the carbon uptake and optimizes the nutritional balance. Our study concluded that three livestock showed significantly different absorbing and digestion on different sources of fodder, resulting in different water-soluble characteristics of organic carbon in compost. The water-soluble value of organic carbon in chicken manure was 3.58%, 3.5 and 9.7 times more than that in pig manure and cow manure, respectively. Furthermore, the fertilizer efficiency of chicken manure increased three times in terms of carbon supplementary. Our results not only provided a basis of how to improve cold spring paddy soils and raise rice production by providing the optimal fertilizer, but it also provided the practical basis for carbon balance in the nutrient balance of plants. The study also provided a theoretical and practical basis for us to select a suitable organic fertilizer to improve cold water paddy fields.

Acknowledgments

The research was financially supported in part by a grant from the China Special Fund (201003059-04) for Agro-scientific Research and in part by a grant from the Special Foundation (201416) of President of Guangdong Academy of Agricultural Sciences, China.

References

1. Ai C, Liang G, Sun J, Wang X, He P, Zhou W. 2013. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biol. Biochem.* **57:** 30-42.
2. Borken W, Muhs A, Beese F. 2002. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. *Soil Biol. Biochem.* **34:** 403-412.
3. Chalhoub M, Coquet Y, Vachier P. 2013. Water and bromide dynamics in a soil amended with different urban composts. *Vadose Zone J.* **12:** 1-11.
4. Chen XP, Zhu YG, Xia Y, Shen JP, He JZ. 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environ. Microbiol.* **10:** 1978-1987.
5. Chu H, Fujii T, Morimoto S, Lin X, Yagi K, Hu J, Zhang J. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. *Appl. Environ. Microbiol.* **73:** 485-491.
6. Chung RS, Wang FN. 2000. Effect of different composts on growth and nitrogen composition of Chinese mustard in an acid red soil. *Commun. Soil Sci. Plant Anal.* **31:** 1209-1224.
7. Cooperband L, Bollero G, Coale F. 2002. Effect of poultry litter and composts on soil nitrogen and phosphorus availability and corn production. *Nutr. Cycl. Agroecosyst.* **62:** 185-194.
8. Dadhich S, Pandey A, Prasanna R, Nain L, Kaushik B. 2012. Optimizing crop residue-based composts for enhancing soil fertility and crop yield of rice. *Ind. J. Agric. Sci.* **82:** 85-88.
9. Dahlin S, Kirchmann H, Kätterer T, Gunnarsson S, Bergström L. 2005. Possibilities for improving nitrogen use from organic materials in agricultural cropping systems. *AMBIO J. Human Environ.* **34:** 288-295.
10. Guo GX, Deng H, Qiao M, Mu YJ, Zhu YG. 2011. Effect of pyrene on denitrification activity and abundance and composition of denitrifying community in an agricultural soil. *Environ. Pollut.* **159:** 1886-1895.
11. Hartz T, Mitchell J, Giannini C. 2000. Nitrogen and carbon mineralization dynamics of manures and composts. *HortScience* **35:** 209-212.
12. He JZ, Shen JP, Zhang LM, Zhu YG, Zheng YM, Xu MG, Di H. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environ. Microbiol.* **9:** 2364-2374.
13. Hussain Q, Liu Y, Jin Z, Zhang A, Pan G, Li L, *et al.* 2011. Temporal dynamics of ammonia oxidizer (*amoA*) and denitrifier (*nirK*) communities in the rhizosphere of a rice ecosystem from Tai Lake region, China. *Appl. Soil Ecol.* **48:** 210-218.

14. Hwa HS, Jung BJ. 2012. Analysis and quantification of ammonia-oxidizing bacteria community with *amoA* gene in sewage treatment plants. *J. Microbiol. Biotechnol.* **22:** 1193-1201.
15. Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H. 2006. Traceability of ammonia-oxidizing bacteria in compost-treated soils. *Soil Biol. Biochem.* **38:** 1092-1100.
16. Kandeler E, Deiglmayr K, Tscherko D, Bru D, Philippot L. 2006. Abundance of *narG*, *nirS*, *nirK*, and *nosZ* genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl. Environ. Microbiol.* **72:** 5957-5962.
17. Kayikcioglu HH. 2013. Effects of composts from agroindustrial wastes on microbial activity of a typic xerofluvent soil under Mediterranean conditions, SE Turkey. *Geomicrobiol. J.* **30:** 228-236.
18. Kemnitz D, Kolb S, Conrad R. 2007. High abundance of Crenarchaeota in a temperate acidic forest soil. *FEMS Microbiol. Ecol.* **60:** 442-448.
19. Kong AY, Hristova K, Scow KM, Six J. 2010. Impacts of different N management regimes on nitrifier and denitrifier communities and N cycling in soil microenvironments. *Soil Biol. Biochem.* **42:** 1523-1533.
20. Lalisse-Grundmann G, Brunel B, Chalamet A. 1988. Denitrification in a cultivated soil: optimal glucose and nitrate concentrations. *Soil Biol. Biochem.* **20:** 839-844.
21. Ma W, Bedard-Haughn A, Siciliano S, Farrell R. 2008. Relationship between nitrifier and denitrifier community composition and abundance in predicting nitrous oxide emissions from ephemeral wetland soils. *Soil Biol. Biochem.* **40:** 1114-1123.
22. Nair A, Ngouajio M. 2012. Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Appl. Soil Ecol.* **58:** 45-55.
23. Naramabuye FX, Haynes RJ. 2007. The liming effect of five organic manures when incubated with an acid soil. *J. Plant Nutr. Soil Sci.* **170:** 615-622.
24. Renner E, Becker GE. 1970. Production of nitric oxide and nitrous oxide during denitrification by *Corynebacterium nephridii*. *J. Bacteriol.* **101:** 821-826.
25. Ros M, Pascual J, Garcia C, Hernandez M, Insam H. 2006. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. *Soil Biol. Biochem.* **38:** 3443-3452.
26. Shu YY. 2005. Effect of application of different types of organic composts on rice growth under laboratory conditions. *Soil Sci. Plant Nutr.* **51:** 443-449.
27. Shu YY, Chung RS. 2006. Rice growth and nutrient accumulation as affected by different composts. *Commun. Soil Sci. Plant Anal.* **37:** 1139-1156.
28. Tejada M, Gómez I, Fernández-Boy E, Díaz M-J. 2014. Effects of sewage sludge and *Acacia dealbata* composts on soil biochemical and chemical properties. *Commun. Soil Sci. Plant Anal.* **45:** 570-580.
29. Wallenstein MD, Vilgalys RJ. 2005. Quantitative analyses of nitrogen cycling genes in soils. *Pedobiologia* **49:** 665-672.
30. Weng B, Xie X, Yang J, Liu J, Lu H, Yan C. 2013. Research on the nitrogen cycle in rhizosphere of *Kandelia obovata* under ammonium and nitrate addition. *Marine Pollut. Bull.* **76:** 227-240.
31. Wessén E, Nyberg K, Jansson JK, Hallin S. 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Appl. Soil Ecol.* **45:** 193-200.
32. Wong J, Li G, Wong M. 1996. The growth of *Brassica chinensis* in heavy-metal-contaminated sewage sludge compost from Hong Kong. *Bioresour. Technol.* **58:** 309-313.
33. Zeng LS, Liao M, Chen CL, Huang CY. 2007. Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil–lead–rice (*Oryza sativa* L.) system. *Ecotoxicol. Environ. Saf.* **67:** 67-74.
34. Zhang LM, Hu HW, Shen JP, He JZ. 2011. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *ISME J.* **6:** 1032-1045.
35. Zhang Q-C, Shamsi IH, Xu D-T, Wang G-H, Lin X-Y, Jilani G, *et al.* 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Appl. Soil Ecol.* **57:** 1-8.