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ORIGINAL PAPER

Effect of long-term fertilization on bacterial composition in rice paddy soil

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Abstract We investigated the effect of long-term fertilization on bacterial abundance, composition, and diversity in paddy soil. The experiment started in 1990 in Taoyuan Agro-ecosystem Research Station in China (111°33′ E, 28° 55′ N). The molecular approaches including real-time quantitative PCR, terminal restriction fragment length polymorphism, and clone library construction were employed using 16S rRNA gene as genetic marker. Application of inorganic fertilizers did not affect bacterial abundance, and rice straw incorporation combined with inorganic fertilizers significantly ($P<0.05$) increased bacterial abundance with shifts in bacterial community composition. Among phylogenetic groups, γ-Proteobacteria was responsive to all fertilization regimes while Acidobacteria was relatively stable to fertilization practices. Inorganic fertilizer mainly affected γ-Proteobacteria and δ-Proteobacteria, while rice straw incorporation influenced β-Proteobacteria and Verrucomicrobia. Therefore, long-term fertilization can affect abundance and composition of bacterial communities in paddy soil.

Keywords 16S rRNA · Long-term fertilization · Inorganic fertilizer · Rice straw incorporation · Bacterial community

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Introduction

Soil microorganisms play an important role in maintaining soil productivity through biochemical processes such as residue decomposition and nutrient recycling. Microbial diversity and activity are sensitive indicators that reflect the sustainability and productivity of terrestrial agroecosystems (Bell et al. 2005; Cardinale et al. 2006; Costa et al. 2007). It has been reported that soil type and management practice largely determine the structure of bacterial communities (Gelsomino et al. 1999; Clegg et al. 2003; Da Silva et al. 2003; Steenwerth et al. 2003). Marschner et al. (2001, 2004) found that plant species and fertilization can affect composition of microbial communities in the rhizosphere soil. It is well known that fertilization is an important agricultural practice for improving plant nutrition and achieving high yield, but it may also result in shifts in the microbial communities, which in turn can affect plant growth by changing nutrient turnover and increasing disease incidence or disease suppression (Marschner et al. 2003; Melero et al. 2008; Saha et al. 2008). Significant differences in microbial biomass and variations in microbial diversity have been observed following fertilization in upland soils (Sarathchandra et al. 2001; Burke et al. 2006; Wasaki et al. 2005; Esperschütz et al. 2007; Parrent and Vilgalys 2007).

Paddy soil is different from upland soil for its flooding and intermittent irrigation. Kikuchi et al. (2007) found a

difference in the bacterial communities between flooded and drained/upland conditions in a Japanese paddy soil. Succession of bacterial community structure and diversity in paddy soil can also be affected by oxygen gradient (Noll et al. 2005). The bacterial communities of different habitats (including floodwater, plow layer soil, microcrustaceans, rice straw and rice straw compost incorporated in soil, and rice straw placed on the soil surface) of paddy field ecosystem differed in diversity and composition (Okabe et al. 2000; Nakayama et al. 2006; Asakawa and Kimura 2008). However, the effect of long-term fertilization on composition and abundance of bacterial communities of paddy soil are poorly known. Several studies have been focused on the responses of some functional microbes including denitrifiers, methanotrophs, and diazotrophic bacteria to fertilization in paddy soil (Qiu et al. 2008; Chen et al. 2010).

Rice straw can be returned to field to improve soil fertility. The application of rice straw stimulated N2 fixation with 40% increase compared with the urea fertilizer control (Tanaka et al. 2010). Takahashi et al. (2003) showed that the continuous application of rice straw increased crop yield and N uptake by paddy rice and upland crops. Rice straw incorporation can also increase methane emission and successions of bacterial population responsible for straw decomposition with the fast-growing bacteria favored at the beginning and slow-growing bacteria prevailing in the later stages (Liou et al. 2003; Sugano et al. 2005; Asari et al. 2007; Rui et al. 2009). However, the effects of the long-term application of rice straw on the bacterial community structure are poorly understood.

The aim of this study was to characterize the variations in abundance and composition of bacterial communities in paddy soil as affected by long-term application of inorganic fertilizer and rice straw using 16S rRNA as a genetic marker.

Materials and methods

Soil sampling and processing

The study site was located at the Taoyuan Agro-ecosystem Research Station (111°33′ E, 28°55′ N) in China, where soil was derived from quaternary red clay. Before the long-term fertilization experiment, the field had been cropping rice for many years; the soil properties were: pH 5.41, soil organic C (SOC) 15.4 g kg^{-1}, total N 1.88 g kg^{-1}, available N 164.3 mg kg^{-1}, available P 16.2 mg kg^{-1}, available K 74.3 mg kg^{-1}, and cation exchange capacity 9.1 cmol kg^{-1}. Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Available P in soil was extracted by sodium bicarbonate and determined using the molybdenum blue method (Olsen et al. 1954). Available K in soil was extracted by ammonium acetate and determined by flame photometry. The cation exchange capacity, soil organic C, total N, and available N were determined by ammonium acetate, dichromate oxidation, Kjeldahl digestion, and alkali-hydrolytic diffusion, respectively.

The experiment started in 1990 with a double cropping rice system with the following treatments: CK (no fertilizer), N (182.3 kg N ha^{-1} year^{-1}), NPK (182.3 kg N ha^{-1} year^{-1}; 39.3 kg P ha^{-1} year^{-1}; 197.2 kg K ha^{-1} year^{-1}), NPKR (NPK plus rice straw produced in the plot). Nitrogen, P, and K fertilizers were added as urea, superphosphate, and potassium chloride, respectively. Rice straw produced from NPKR plots was returned to the corresponding plots at a rate of about 8.5 t/ha which varied based on rice growth. The applying time was in July and November just after the harvests of early and late rice. Rice straw properties were: organic carbon 364 g kg^{-1}, total N 6.73 g kg^{-1}, total P 0.86 g kg^{-1}, total K 16.04 g kg^{-1}. All treatments have three replicated plots, which were randomly arranged in the field: each plot had an area of 33 m^2. Soil samples were collected in March 2007 when the field was wet and in the idle period before cropping early rice. Five soil cores (diameter, 3 cm) were taken from upper layer (0–15 cm) from each plot and were well mixed. About 200 g of soil were placed into sterile bag, immersed in liquid nitrogen immediately, and stored at −70°C. After freeze-drying (Neocool, Yamato), the samples were ground to powder (sterile mortar and pestle) for the molecular analysis and stored at −70°C. The rest of the fresh original soil was air-dried for soil property analysis (Table 1). Microbial DNA was extracted from 0.5 g soil as described by Chen et al. (2010).

Quantification of 16S rRNA genes by qPCR

The bacterial abundance was determined by quantitative PCR (qPCR; ABI 7900, Foster City, CA, USA) with 1369F/1492R primers (Dandie et al. 2007). The 25 μl of reaction mixture contained 1×SYBR Premix Ex*Taq* (Takara Bio Inc, Shiga, Japan), 10 pM of each primer, and DNA extract. Amplification conditions were as follows: one cycle of 95°C for 2 min and then 35 cycles of 95°C for 15 s, 54°C for 20 s, and 72°C for 20 s. Threshold cycle (*Ct*) values were determined with the thermocyclers internal software (7300 System SDS software, V1.2.2, Applied Biosystems, CA, USA). The determination of each sample was replicated three times, and the mean values were used for the subsequent analyses. The standard curves for 16S rRNA gene was created using tenfold dilution series (ranging from 103 to 108) of the plasmids containing the 16S fragment from the soil. The efficiency of the reaction was 103% (based on the slope of the standard curve). The R2 value for the standard curve

Table 1 Chemical properties of soil samples from the fertilization practices

Parameters	Fertilization practice			
	CK	N	NPK	NPKR
pH (H_2O)	5.17±0.08a	5.17±0.11a	5.10±0.19a	5.05±0.22a
Soil organic C (SOC, g kg^{-1})	18.14±0.66a	20.14±0.86a	20.58±0.49a	27.35±0.33b
C/N	10.3±0.56	10.6±0.49a	11.0±0.59a	10.2±0.14a
Available N (mg kg^{-1})	129.29±15.73a	146.55±7.13ab	120.83±12.34a	174.91±7.43b
Available P (mg kg^{-1})	5.72±0.31a	5.56±0.75a	12.42±2.59b	22.14±0.88c
Available K (mg kg^{-1})	33.27±3.11a	30.35±0.43a	51.45±8.58a	136.70±16.54b

Different letters following means±standard error indicates significant difference ($P<0.05$) between treatments

Fertilization practices treatments were: *CK* control without fertilizer, *N* treatment fertilization with inorganic N, *NPK* treatment fertilization with combination of N, P and K, *NPKR* treatment fertilization with NPK and incorporation of rice straw

was 0.999. One sharp peak was observed in the melt curve. Appropriate negative control containing no template DNA gave null values. Standard curves, negative controls, and environmental DNA samples were amplified on a single 384-well plate.

Validation of 16S rRNA primers and T-RFLP analysis

Primers of F299/R1307 were designed with Primer Premier (version 5.0) and Oligo (version 6) to target a fragment length of about 1,000 bp allocated in the variable regions of V3 to V8 of 16S rRNA with 35 sequences representing different family and species collected from NCBI databases. The forward F299 and reverse R1307 primers were 5′-ACAYTGGDACTGAGACACGG and 5′-GATTACTAGCGATTCCRRCTTC, respectively. The primer specificity was validated by PCR through testing 45 reference strains obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), China General Microbiological Culture Collection Center (CGMCC), Agricultural Culture Collection of China (ACCC), Culture Collection Center of Guangdong Microbial Institute (GIM), Beijing City University (BCU), Institute of Applied Ecology (IAE), and Institute of Subtropical Agriculture, CAS (ISA; Table 2).

PCR was carried out with the forward primer F299 labeled with 6-FAM (6-carboxyfluorescein) at the 5′ end and the reverse primer R1307. The PCR products were purified by Gel DNA Purification Kit Ver.2.0 (TaKaRa Bio Inc., Japan) following the manufacturer's instruction. Restriction digestion was conducted in a 10-µl reaction mixture containing 10 U of endonuclease *Taq*I (TaKaRa Bio Inc., Japan) that was maintained in a water bath at 37°C overnight, and the digested sample was purified with the Agarose Gel DNA Purification Kit. Then, an ABI 3100 DNA sequencer (Applied Biosystems) determined the precise lengths of the peaks from the amplified 16S rRNA products. The analysis and standardization of the terminal restriction fragment length polymorphism (T-RFLP) profiles was conducted as described by Dunbar et al. (2001). Only terminal restriction fragments (T-RFs) with a signal above 1.0% of the sum of all peaks and larger than 35 bp were included in the analyses. Because T-RFs can vary slightly in size, the T-RFLP patterns were inspected visually, and peak size differences of ±1 base pair were confirmed by comparing the respective peaks of all patterns. The relative abundances of the T-RFs in a sample, given in percent, were calculated after normalization of peak heights in an iterative standardization procedure, as described by Dunbar et al. (2001).

Clone library construction

PCR products obtained with F299/R1307 primers were purified using PCR Clean-up System Kits (Promega Wizard Company, USA), ligated into the pGEM-T vector (Promega, USA) and transformed into the *Escherichia coli* strain DH5α (Wu et al. 2008). Clones were randomly selected by the blue–white method. Then, 150 clones in each library containing the correct size of inserts were screened by PCR with the M13 primer and sequenced with the ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

In total, 600 16S rRNA sequences were achieved (150 sequences for each treatment). The sequence homology analysis was performed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned using Clustal W (http://www.ebi.ac.uk/Tools/clustalw/index.html). Phylogenetic analysis was carried out using Clustal X 1.83 and Mega 4.1 software (Kumar et al. 2004). Phylogenetic trees were constructed using the neighbor-joining method and boot-

Table 2 Reference strains included in this study and results of PCR amplification of 16S rRNA with primers F299-R1307 primers

Organisms	PCR results for 16S rRNA gene (about 1,000 bp)	Origins
α-Proteobacteria		
Ochrobactrum anthropi	+	GIM 1.002
Bradyrhizobium japonicum	+	GIM 1.227
Azospirillum brasilense	+	ACCC 10100
Acetobacter pasteurianus	+	ACCC 10181
Sphingobium xenophagum	+	ACCC 10187
Beijerinckia indica	+	ACCC 10483
Paracoccus denitrificans	+	ACCC 10489
Rhodobacter capsulatus	+	CGMCC 1.2359
Methylobacterium extorquens	+	CGMCC 4.1476
β-Proteobacteria		
Burkholderia cepacia	+	GIM 1.139
Burkholderia soli	+	GIM 1.290
Alcaligenes faecalis	+	GIM 1.64
Achromobacter cycloclastes	+	ACCC 03051
Comamonas testosteroni	+	ACCC 10192
Acidovorax facilis	+	ACCC 10287
Achromobacter xylosoxdans subsp. denitrificans	+	CGMCC 1.1800
γ-Proteobacteria		
Azotobacter chroococcum	+	IAE-B1
Azotobacter vinelandii	+	IAE-B10
Pseudomonas aeruginosa	+	GIM 1.200
Shigella flexneri	+	GIM 1.231
Shigella dysenteriae	+	GIM 1.236
Rhizobium japonicum	+	IAE-B17
Rhizobium meliloti	+	IAE-B25
Xanthomonas campestris	+	ACCC 10491
Stenotrophomonas maltophilia	+	ACCC 10525
Firmicutes		
Bacillus cereus	+	IAE-B270
Clostridium pasteurianus	+	ACCC 11114
Bacillus subtilis	+	ACCC 10634
Lactobacillus rhamnosus	+	CGMCC 1.0120
Bacillus licheniformis	+	DSM 13
Actinobacteria		
Mycobacterium sp.	+	ACCC 41095
Corynebacterium glutamicum	+	DSM 20300
Micromonospora echinospora	+	IAE-A191
Actinomadura sp.	+	IAE-A265
Pseudonocardia sp.	+	IAE-A266
Rhodococcus sp.	+	IAE-A270
Streptomyces griseus	+	BCU-A1
Streptomyces lavendulae	+	BCU-A2
Nocardia mediterranea	+	BCU-A3
Deinococcus-Thermus		
Deinococcus radiodurans	+	GIM 1.276
Filamentous fungi		
Chaetoceratostoma lonyirostre	–	IAE-F356
Rhizoctonia solani	–	ISA
Yeast		
Saccharomyces cerevisiae	–	IAE-Y14
Saccharomyces cerevisiae	–	IAE-Y57
Candida pseudotropicalis	–	IAE-Y83

+ PCR product of the right size obtained, − no PCR product obtained

strap analysis with 1,000 replicates. Nucleotide sequences with >98% similarity were treated as identical and represented individual operational taxonomic units (OTUs).

Statistical analysis

T-RFs of different lengths were considered to be indicative of different 16S rRNA OTUs present in a sample, and the relative peak heights were used as a measure for the relative abundance of the 16S rRNA OTUs. The diversity and evenness of the T-RFLP profiles were estimated by PC-ORD version 5.0 (MJM software, Oregon). Correspondence analysis (CA) and canonical CA (CCA) were performed with CANOCO version 4.53 (Microcomputer Power, Inc., Ithaca, NY). Correspondence analysis was performed with the T-RFLP profiles to view the quality of replication. The effects of the environmental variables, such as pH, SOC, C/N, available N, P, and K on the T-RFLP profile were estimated by the ordination techniques of CCA. Monte Carlo permutation tests were used to test whether the environmental variables affected the relative abundance of bacterial OTUs. One-way analysis of variance was used to determine the significance of differences between soil properties, bacterial diversity, and evenness through SPSS 13.0 (SPSS Inc., USA).

Nucleotide sequence accession numbers

The partial 16S rRNA gene sequences were deposited in the GenBank database and assigned with the following accession FJ265066-FJ265112, FJ265134-FJ265136, FJ265138-FJ265166, FJ265177-FJ265179, FJ265185-FJ265198, FJ265202-FJ265203, FJ265209-FJ265266, FJ265276-FJ265279, FJ265286-FJ265356, FJ265380-FJ265382, FJ265386-FJ265411, FJ265420-FJ265422, FJ265424-FJ265448, FJ265450-FJ265466, FJ265473,

FJ265476-FJ265492, FJ265496-FJ265497, FJ265499-FJ265513, and FJ265520-FJ265528.

Results

Primer validation

The primers F299/R1307 for the 16S rRNA gene were validated by testing 40 bacterial strains including nine Actinobacteria strains, and five fungal strains (Table 2). All bacteria and Actinobacteria strains showed positive PCR results, and the five fungal strains showed negative results. Therefore, the F299/R1307 primers were used in the bacterial community analysis.

Effect of fertilization on bacterial abundance

Based on the real-time PCR results, the copy numbers of 16S rRNA gene representing soil bacteria were $(5.39 \pm 0.98) \times 10^9$, $(5.90 \pm 0.79) \times 10^9$, $(6.29 \pm 0.77) \times 10^9$, and $(1.23 \pm 0.15) \times 10^{10} g^{-1}$ dry soil in the CK, N, NPK, and NPKR treatments, respectively. Only the use of NPK with rice straw incorporation significantly ($P<0.05$) increased the bacterial abundance compared with the value of the CK treatment (Fig. 1).

Bacterial community profiles

The similar T-RFLP profiles among the three replicated plots of each treatment (data not shown) indicated that they had relatively uniform bacterial compositions. By comparing T-RFLP profiles, the diversity and evenness among the four fertilization treatments were not obviously different (Table 3).

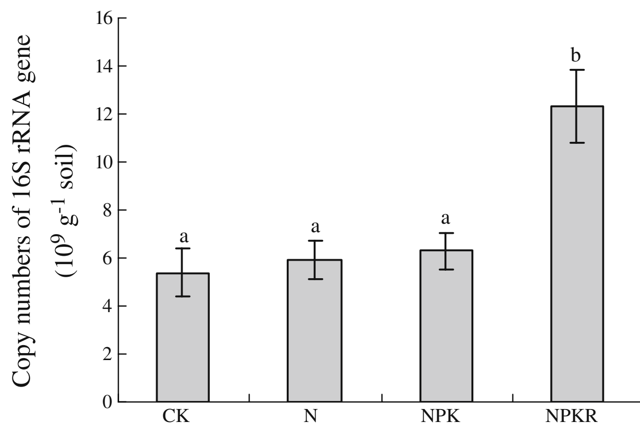

Fig. 1 Copy numbers of 16S rRNA gene measured by real-time PCR. **Different letters* indicate significant differences by Fisher's LSD ($P<0.05$). *CK*, *N*, *NPK*, and *NPKR* treatments represent control without fertilizer and fertilization; with N; with N, P, and K, and with NPK and rice straw incorporation, respectively

Table 3 Diversity indices of 16S rRNA from soils treated with different fertilizers

Treatment	Mean (SEM)	
	Evenness	Shannon index
CK	0.77 ± 0.03^a	2.33 ± 0.08^a
N	0.78 ± 0.05^a	2.34 ± 0.16^a
NPK	0.76 ± 0.01^a	2.33 ± 0.04^a
NPKR	0.83 ± 0.01^a	2.51 ± 0.03^a

Shannon index = $H' = \sum pi \ln pi$; where pi is T-RF abundance divided by total abundance in a profile

CK, N, NPK, and NPKR treatments represent control without fertilizer and fertilization with N, with N, P, and K, nitrogen, combination of nitrogen–phosphorus–potassium and with NPK and rice straw incorporation, respectively

Evenness = $H'/\ln S$

[a] Indicates no significant differences ($P<0.05$) between treatments based on the LSD procedure for multiple comparisons among means

As shown in Table 4, inorganic fertilizer treatments had similar patterns of the CK treatment as indicated by the relative abundance (in percent) of T-RFs; however, there were a few minor differences, such as the N fertilization

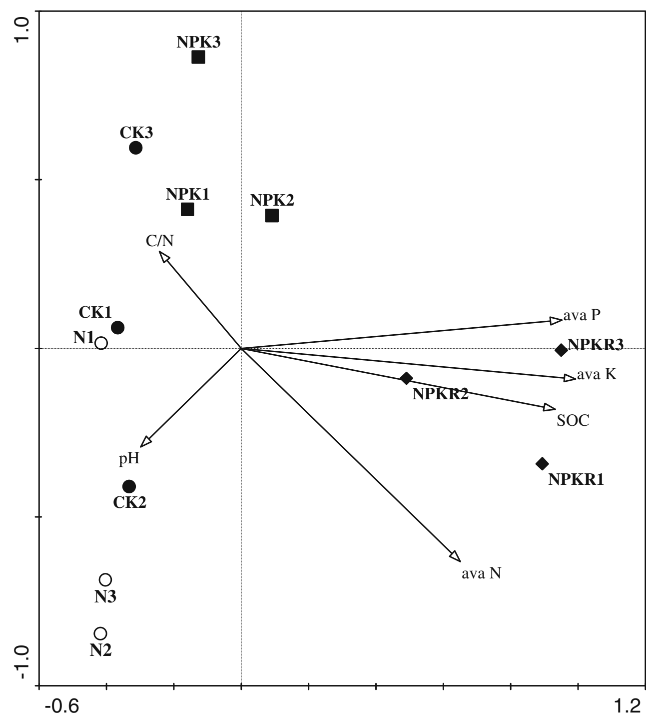

Fig. 2 CCA of the effect of fertilization on the composition of the bacterial community in the paddy soil. Soil properties are represented by *arrows*, the lengths of which indicate the correlation between the soil property and the community data. The eigenvalues of the first and second axes in the ordination diagram are as: λ_1=0.174 and λ_2=0.036, respectively. CK, N, NPK, and NPKR treatments represent control without fertilizer; fertilization with N, with N, P, and K, and with NPK and rice straw incorporation, respectively. *Ava N* is available N, *ava P* is available P, and *ava K* is available K

Table 4 Bacterial T-RFLP patterns with relative abundance

T-RFs (bp)	Fertilization			
	CK (%)	N (%)	NPK (%)	NPKR (%)
44[a]	3.3	2.5	1.7	3.5
123	25.8	24.1	24.6	18.7
145	0	0	0	1.0
148	1.0	0	0	0
153	1.1	0	1.1	0
161	1.2	1.8	1.4	0
176	0	1.8	0	0
214	1.5	1.6	1.4	1.9
260	1.1	0	1.1	1.1
296	13.4	14.3	14.3	12.3
298	2.4	0	1.7	1.7
312	3.2	3.4	3.8	4.3
322	4.1	6.6	5.6	1.4
324	0	0	0	4.5
338	1.7	2.0	1.5	1.3
424	3.5	0	7.3	7.1
429	1.4	1.6	1.5	1.4
439	0	0	0	1.2
503	1.4	0	1.1	2.2
509	1.00	1.2	1.2	1.1
529	0	1.2	1.3	0
548	0	1.0	1.4	6.3
631	3.7	3.9	5.1	0
658	4.2	6.5	4.2	2.1

CK, N, NPK, and NPKR treatments represent control without fertilizer and fertilization with N, with N, P, and K, and with NPK and rice straw incorporation, respectively

[a] The numbers indicated the lengths of T-RFs (bp)

which induced the appearance of T-RFs 176 bp, increased the T-RFs 658 bp, and induced the disappearance of T-RFs 298 bp. The NPK treatment increased the T-RFs 631 bp and decreased the T-RFs 44 bp. The major difference between the NPKR and the other treatments was due to the fact that the percentage of the dominant T-RFs of 123 and 296 bp length decreased, whereas two new T-RFs (324 and 548 bp) appeared with the former treatment.

The influence of soil properties on the bacterial community composition was analyzed by CCA using the T-RFLP data. The effects by the contents of SOC, available P, and available K were significant (P=0.004, 0.002, 0.004 by Monte Carlo permutation test within CCA, respectively) in affecting the bacterial community composition (Fig. 2), whereas the available N content, the C/N ratio, and the pH value did not (P=0.083, 0.672, 0.518, respectively) affect the composition of bacterial community.

Taxonomic and phylogenetic analysis

In this study, 600 16S rRNA clones were classified into 427 OTUs by using a nucleotide sequence similarity value >98%. Among them, 104, 119, 134, and 111 OTUs were from CK, N, NPK, and NPKR libraries, respectively. All fertilization treatments increased bacterial genotypes compared with CK, and the NPK treatment showed the highest OTUs number. Phylogenetic analysis revealed that 427 16S rRNA OTUs were mainly grouped into: Proteobacteria (α, β, γ, δ-Proteobacteria), Acidobacteria, Verrucomicrobia, Firmicutes, Chloroflexi, Bacteroidetes, Gemmatimonadetes, *Nitrospira*, Actinobacteria, Nitrospirae, Planctomycetes, and Chlorobi; nine OTUs were clustered into an unclassified group (Table 5).

Long-term fertilization regimes caused variations of phylogenetic groups expressed as clone percentage of each phylum in the corresponding clone library (Table 5). Acidobacteria showed similar percentages among the various fertilization treatments; γ-Proteobacteria was sensitive to fertilization since the highest percentage was shown by the CK treatment. The use of NPK reduced γ-Proteobacteria and increased δ-Proteobacteria clones. Rice straw incorporation decreased β-Proteobacteria and Verrucomicrobia clones.

Table 5 The phylum level distribution of clone sequences in different fertilization treatments

Phyla	Fertilization			
	CK (%)	N (%)	NPK (%)	NPKR (%)
α-Proteobacteria	16.0*	22.0	11.3	21.3
β-Proteobacteria	9.3	10.0	9.3	4.0
γ-Proteobacteria	19.3	10.0	3.3	4.0
δ-Proteobacteria	11.3	12.7	18.0	17.3
Acidobacteria	18.0	19.3	19.3	20.7
Verrucomicrobia	12.0	10.7	11.3	5.3
Firmicutes	4.0	2.0	9.3	12.7
Chloroflexi	4.0	5.3	4.7	1.3
Gemmatimonadetes	1.3	2.0	2.7	1.3
Actinobacteria	0.7	2.7	0.7	0.7
Bacteroidetes	1.3	1.3	2.0	3.3
Nitrospirae	1.3	0	1.3	2.7
Planctomycetes	0	0	2.0	2.7
Chlorobi	0	1.3	1.3	0
Unclassified	1.3	0.7	3.3	2.7

CK, N, NPK, and NPKR treatments represent control without fertilizer and fertilization with N, with N, P, and K, and with NPK and rice straw incorporation, respectively. CK, N, NPK, and NPKR represent control without fertilizers; nitrogen, combination of nitrogen–phosphorus–potassium, and NPK with rice straw incorporation, respectively

Discussion

Fierer and Jackson (2006) indicated that the diversity of soil bacterial communities depended on ecosystem type. The use of pyrosequencing technique has shown that forest and agricultural soils have different bacterial diversity (Roesch et al. 2007). Sandaa et al. (2006) observed a decrease in soil bacterial diversity due to heavy metal contamination. Other agricultural managements such as tillage and fertilization can also affect composition of soil bacterial communities (Ceja-Navarro et al. 2010; Shen et al. 2010). However, we have found no statistical significant differences in bacterial diversity among the CK, N, and NPK fertilization; these fertilization treatments were carried out for 17 years. Only inorganic fertilizers combined with rice straw incorporation increased bacterial diversity. Similarly, bacterial communities were relatively non-responsive to long-term fertilization in upland soil (Freitag et al. 2005; He et al. 2008; Ogilvie et al. 2008).

Wessén et al. (2010) observed that the abundance of bacteria significantly increased with long-term calcium nitrate and ammonium sulfate fertilization. On the contrary, it was reported that N fertilization induced a significant small bacterial size with respect to no fertilizer or manure amendments (Shen et al. 2010). In this study, both abundance and composition of bacterial communities were not sensitive to inorganic fertilization whereas they were significantly affected by applying inorganic fertilizers with rice straw. It has been suggested that soil pH was the driver for affecting composition of bacterial communities (Enwall et al. 2007; Lehtovirta et al. 2009; Rousk et al. 2010). However, we did not detect any significant difference in soil pH between the fertilization regimes, and thus our observed differences in bacterial diversity were not due to changes in soil pH. Despite the NPK treatment, significantly increased rice yield compared with the N treatment (Gu et al. 2009; Yadav 2003), the two treated soils have similar bacterial abundance. Rice straw incorporation into soil probably significantly stimulated bacterial growth (Bünemann et al. 2004; Bittman et al. 2005; Gong et al. 2009; Liu et al. 2009) and successions of bacterial populations involved in straw decomposition (Sugano et al. 2005; Tanahashi et al. 2005).

By using cloning and sequencing techniques, we have found that among the dominant taxonomic groups, only Acidobacteria was not responsive to the fertilization while γ-Proteobacteria was sensitive to all the fertilization regimes. NPK treatment induced variations in percentages of γ-Proteobacteria clones, and rice straw incorporation decreased percentage of β-Proteobacteria and Verrucomicrobia. Since each phylum contains a large number of bacterial species with various functions, it is unknown which specific bacterial functions were altered by these changes. Nemergut et al. (2008) found a lower relative abundance of Verrucomicrobia in chronic N amendment, but the relative changes in soil functions were not clear. Some changes of functional groups may contribute to the taxonomic variations in relation to fertilization. Long-term fertilization significantly affected the population of free-living diazotrophs and methanotrophs in paddy fields (Zheng et al. 2008; Islam et al. 2010). Chen et al. (2010) suggested that nitrite-reducing bacteria were remarkably influenced by fertilization regimes. However, the functional bacterial groups normally consist of the species from a range of phyla. Further studies on the correlations between phylogenetic change and their functions are needed.

Soil bacterial composition can be affected by the changes of soil conditions and management (Bronick and Lai 2005). The CCA analysis in this paper suggested that content of SOC, available P, and available K were the predominant factors affecting bacterial community structure. Marschner et al. (2003) found that bacterial and eukaryotic community structures were significantly affected by soil organic C content in a field receiving long-term addition of organic and inorganic fertilizer amendments.

In conclusion, long-term fertilization with inorganic fertilizer and rice straw incorporation affected bacterial population size and community composition, whereas the only addition of inorganic fertilizer was less effective. All the fertilization regimes reduced the percentage of γ-Proteobacteria but not that of Acidobacteria. The NPK fertilization mainly affected γ-Proteobacteria whereas the combination with rice straw incorporation influenced β-Proteobacteria and Verrucomicrobia.

Further study should pay more attention on the impact of the changes of bacterial community induced by fertilization on soil functionality.

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