Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China

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ARTICLE INFO

Article history:

Received 17 November 2009

Received in revised form 6 April 2010

Accepted 24 June 2010

Keywords:

Soil bacteria

Polymerase chain reaction

Real-time PCR

Denaturing gradient gel electrophoresis (DGGE)

Long-term fertilization

Manure

ABSTRACT

Soil bacterial communities mediate a wide range of ecosystem processes. In agricultural systems these processes determine soil health through bacterial roles in residue decomposition, nutrient cycling and their associations with other organisms. In this study, we investigated the abundance and composition of native soil bacterial communities under different long-term fertilization practices using quantitative real-time polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE). Soil samples were taken from a 20-year fertilization trial at the Plastic-film Mulching Experiment Station in Northeast China, which including four treatments of control (CK), inorganic-N fertilizer amendment (N), organic manure amendment (M), and half fertilizer-N plus half organic manure (M + N), to identify the difference of soil bacterial community under inorganic fertilizer and manure amendment. The size of the general bacterial population in the N treatment was significantly smaller than those in the CK, M and M + N treatments. Soil bacterial 16S rRNA gene copy numbers were positively correlated with soil pH and total organic carbon (TOC) content. The DGGE band patterns in CK, M and M + N treatments demonstrated more similarity to each other than to those of soil with only N amendment, while canonical correspondence analysis (CCA) revealed that the bacterial community structure in the N treatment was significantly different from the treatments of CK and M bacterial community profiles. Phylogenetic analysis of bands excised from the DGGE gels identified seven major phyla (*Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Proteobacteria*, *Actinobacteria*, *Gammaproteobacteria* and *Firmicutes*) present in all the treatments except the N treatment, in which no *Gammaproteobacteria* was observed. Results from this study suggest that agricultural fertilization practices have a great impact on soil bacterial community size and structure.

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1. Introduction

Soil microorganisms have long been recognized for their important role in residue decomposition, nutrient cycling and crop production. Understanding the shifts of microbial community structure and composition following long-term organic and inorganic fertilizer amendments may lead to development of better management practices for agroecosystems (Dolfing et al., 2004). Culture-independent molecular surveys using the 16S rDNA, encoding the RNA component of the small subunit of the ribosome, has provided major insights into species and functional diversity of bacterial populations with different soil management practice (Prosser, 2002). Results from such studies have clearly demonstrated perturbation of soil microbial community populations due to changes in environmental conditions and soil management practices

(Smit et al., 2001; Sun et al., 2004). Molecular approaches with soil DNA may answer questions on the long-term effects of fertilizer management on soil bacterial abundance and diversity.

Fertilization has been widely used as a common management practice to maintain soil fertility and crop productivity. The impacts of fertilization on the population, composition and function of soil microorganisms are significant. For example, Hallin et al. (2009) showed different fertilization treatments greatly affected N-cycling communities and ecosystem functioning in a 50-year-old fertilizer field experiment established in 1956 in Sweden. Their results showed that the size of the microbial functional groups were one to two orders of magnitude lower in the sewage sludge and ammonium sulfate treatments compared with the other treatments, which might be explained the lower pH of 4.0 in these plots. Extensive studies elsewhere have shown similar long-term community shifts (Marschner et al., 2003; Dolfing et al., 2004; Sun et al., 2004; Chu et al., 2007; Ge et al., 2008; He et al., 2008), while still other studies demonstrated that inorganic and organic fertilizers had relatively less effect on soil bacterial diversity and activities when

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investigations were conducted over the short-term (Crecchio et al., 2001; Okano et al., 2004). Additional studies focusing on the microbial community structure dynamics in response to long-term fertilizer management include a 16-year study of a Chinese red soil (He et al., 2007), a 30-year study in the Germany-reclaimed loess soil (Marschner et al., 2003), and a 70-year study of a century-old manure-treated agroecosystem in USA (Sun et al., 2004). Sun et al. (2004) observed a significantly higher number of bacterial ribotypes and more even bacterial distribution in the manure-treated soil as assessed by denaturing gradient gel electrophoresis (DGGE).

Our previous investigations have also studied the community structure of bacteria in Southern Chinese red soil and Northern Chinese alkaline soil under long-term fertilization practices using PCR-DGGE methods (Ge et al., 2008; He et al., 2008). However, little is known about the relationship between bacterial community shift and size change under long-term fertilizer application (Fierer et al., 2005; He et al., 2007; Shen et al., 2008). A 50-year-old fertilization experiment showed the relative importance of size and composition in relation to microbial process involved in nitrogen cycling (Hallin et al., 2009). However, what matters most for ecosystems functioning-composition or size of functional communities still need further studies.

In this study, we collected soil samples from a 20-year fertilization experiment which included nitrogen fertilizer and organic manure amendments to an agricultural soil in Northeast China. Earlier studies at the same site showed that long-term application of inorganic-N fertilizer and organic manure could change the availability of resources to the soil food web and the decomposition pathway (Liang et al., 2009). The aim of this study was therefore to investigate the abundance and community structure of soil bacteria and their association with soil environmental variables using real-time PCR and PCR-DGGE approaches. The relationship between bacterial community size and composition were studied in this study, which showed difference with our previous study on bacteria.

2. Material and methods

2.1. Sampling site

Soil samples were collected from a Plastic-film Mulching Experiment Station of Shenyang Agriculture University, Liaoning Province, Northeast China (41°49'N, 123°34'E). This long-term experiment site was established in 1987 with wheat continuous cropping system on loamy brown earth derived from loess-like parent materials. Four replicates of four fertilization treatments were investigated: CK (non-amended), nitrogen (application of urea as N fertilizer, 135 kg N ha⁻¹ per year), M (application of pig compost manure, 135 kg N ha⁻¹ equivalent per year), and M + N (combined application of half N fertilizer and half pig compost manure, 135 kg N ha⁻¹ equivalent per year). Five 20 cm × 5 cm diameter soil cores mixed as one sample were collected from the upper 20 cm at the seedling stage of maize in May 2007. The soil samples were sieved to 2.0 mm, and stored at -80 °C until DNA extraction. Soil chemical and physical characteristics have been described previously (Liang et al., 2009) and are presented in Table 1.

2.2. PCR amplification of bacterial 16S rRNA genes

Nucleic acids were extracted from frozen soils as described previously (He et al., 2007) using an UltraCleanTM soil DNA isolation kit (MoBio, San Diego, CA). Purified DNA was eluted with 50 µl of Solution S5 (MoBio, cat. No 12800-100) and stored at -20 °C.

Real-time qPCR quantification of bacterial 16S rRNA genes in soil DNA samples was performed on an iCycler iQ5 thermocycler (Bio-

Rad, Hercules, CA) as described previously (He et al., 2007; Shen et al., 2008). The universal primers BACT1369F (5'-CGG TGA ATA CGT TCY CGG-3') and PROK1492R (5'-AAG GAG GTG ATC CRG CCG CA) were used in combination with the probe TM1389F (5'-CTT GTA CAC CGC CCG TC-3'), labeled at the 5'-end with the reporter dye FAM (6-carboxy-fluorescein), and at the 3'-end with the quencher dye TAMRA (6-carboxy-tetramethylrhodamine), for quantification using iQ Supermix (Bio-Rad). Each reaction was performed in a 25-µl volume and run using 35 cycles in order to minimize non-specific background fluorescence. The standard curve for real-time PCR was developed as described previously (He et al., 2007; Shen et al., 2008). Data analysis was carried out with iCycler software (version 1.0.1384.0 CR). The threshold cycle (C_T) of amplification for each sample was determined as the cycle number at which a statistically significant increase in the reporter fluorescence was detected.

For PCR-DGGE analysis, the primers used for 16S rRNA gene amplification were 954f-GC (5'-[CGC CCG CCG CGC GCG GCG GCG GCG GCG GCA CGG GGG G] GCA CAA GCG GTG GAG CAT GTG G-3') and 1369r (5'-GCC CGG GAA CGT ATT CAC CG-3') (Yu and Morrison, 2004). Amplifications were performed in 25 µl reaction mixtures containing 1× PCR buffer, 3.0 mM MgCl₂, 400 µM each dNTP, 2.0 U Taq DNA polymerase (TaKaRa, Otsu, Shiga, Japan), and 500 nM of each primer. The temperature cycling program consisted of an initial denaturation at 94 °C for 5 min; 10 touchdown cycles of 94 °C for 30 s, 61–0.5 °C per cycle for 30 s, and 72 °C for 60 s; 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s; and a final elongation at 72 °C for 5 min. PCR products were checked on 1% agarose gels.

2.3. DGGE analysis and sequencing

DGGE analysis of the bacterial 16S rRNA gene products was performed with the DCode Universal Mutation Detection System (Bio-Rad) as described previously (Shen et al., 2008) with slight modifications. Ten microliters of each PCR product (about 25 ng µl⁻¹) were loaded onto 6% polyacrylamide gradient gels with a denaturing gradient of 40–60% where 100% denaturant is defined as 7 M urea and 40% formamide (Muyzer et al., 1993). Electrophoreses were run at 150 V for 5 h at 60 °C. The gels were stained with 1:10,000 SYBR Gold nucleic acid gel stain (Invitrogen 20 Molecular Probes, Eugene, OR) for 30 min according to the manufacturer's instructions, and scanned using a GBOX/HR-E-M (Syngene, UK).

The dominant bands in the DGGE gel with the same mobility were excised and incubated overnight in 10 mM Tris-HCl, pH 8.0 to elute DNA. Reamplification of the eluted DNA by PCR was carried out as before except that the primers 954f did not possess a GC-clamp at the 5'-end. Purified PCR products were ligated into the pGEM-T Easy Vector (Promega, Madison, WI) according to the manufacturer's instructions. Five positive clones were checked by PCR-DGGE to confirm the mobility, and used for DNA sequencing.

2.4. Phylogenetic analysis

The obtained sequences were edited and searched against the NCBI GenBank database using the BLAST. The most similar GenBank sequences to the soil 16S rRNA gene clones (98% cutoff) were included in phylogenetic analysis. The partial 16S rRNA gene sequences were deposited in the GenBank database under accession nos. EU216572–EU216595 and EU249321.

2.5. Statistical analysis

The 16S rRNA gene copy numbers was distributed log-normally and log₁₀-transformed prior to one-way analysis of variance (ANOVA) followed by an S–N–K test, performed using SPSS ver-

Table 1

The basic chemical characteristics of Shenyang brown soil.

Treatment	pH	DOC ^a (mg kg ⁻¹)	TOC ^b (g kg ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)
CK	6.34	104	9.42	1.46	0.16
N	4.90	83.4	7.85	2.71	0.44
M	6.24	136	10.6	0.31	0.17
M + N	5.82	104	11.2	1.96	0.41

^a Dissolved organic carbon.^b Total organic carbon.

sion 11.5 (SPSS Inc., Chicago, IL). The banding patterns of DGGE gels were analyzed using the software Quantity one 4.5.2 (Bio-Rad). The resulting band profiles were exported and used in statistical analysis as 'species' presence-absence matrices, and all detected bands were evaluated categorically with absence scored as 0 and the presence as 1. To compare normalized bacterial DGGE patterns and test the influences of soil characteristics on the bacterial community structure, canonical correspondence analyses (CCA) were carried out in Canoco for Windows 4.5 (ter Braak and Šmilauer, 2002). A Monte-Carlo permutation test based on 199 random permutations was used to analysis the relationship between bacterial community and environmental properties. The resulting ordination biplot approximated the weighted average of each species (bands, in this case) with respect to environmental variables, which were represented as arrows. The length of the individual arrow indicated the relative importance of soil characteristics in explaining variation in bacterial profiles. Environmental variables with inflation factor over 20 were not included in the CCA analysis.

3. Results

3.1. Abundance of bacteria in the soil

The abundance of total bacteria detected with real-time PCR by targeting universal bacterial specific 16S rDNA regions showed significant differences among the four fertilization treatments (Fig. 1). The 16S rRNA gene copy numbers in the N treatment at 7.4×10^9 per gram of dry soil was significantly lower than those in other treatments. One-way ANOVA of qPCR results showed that the bacterial 16S rRNA gene copy numbers in all the treatments had a significant correlation with soil pH ($r = 0.723$, $n = 16$, $P < 0.01$) and soil TOC

content ($r = 0.557$, $n = 16$, $P < 0.05$), while no significant correlation with other soil properties (i.e. soil DOC, NH₄⁺, NO₃⁻).

3.2. DGGE profiling of bacterial 16S rRNA gene

The community structure of bacteria was analyzed by PCR-DGGE targeting sequence variation present in the 16S rDNA community (Fig. 2). There was a treatment effect and variance within treatments was much lower than variance among treatments based on the DGGE patterns. Twenty-eight dominant DGGE bands in total were detected and numbered (Fig. 2). The DGGE profile of bacteria in the N treatment was unique relative to the other treatments. For example, bands 17, 22, 24 and 26 were unique to the N treatment, while bands 4–6, 8–10, 12, 13, 21, 23, 25, 27 and 28 were all detected in other three treatments, i.e. the CK, M and N + M treatments (Fig. 2 and Table 2). In addition, bands 1–3 appeared with higher intensity in the N treatment DGGE gels than in the other treatment. Although some bands were present in the profiles of all treatments, their intensity varied among treatments. For example, band 10 was strong in the CK treatment, while slight in the treatments with manure (i.e. treatments M and M + N) and absent in the N treatment.

CCA analysis of the DGGE band patterns showed that the N treatment clustered separately from other treatments, while the M and M + N treatments were more closely grouped (Fig. 3). It is clear that bacterial community diversity in the manure amendment treatments (Fig. 3) was distributed along the soil carbon gradient, and that the treatments not receiving manure amendment (CK and N) clustered separately. Based on the partial Monte-Carlo permutation test, our results indicated that both axes explained a highly significant proportion of the variation in the data ($P = 0.002$). The variation within bacterial community structure among the treatments explained 74.6% and 19% of the variation on the first two axes (Table 3), respectively. Species-environment correlation coefficients using CCA for the first two axes were more than 0.99 and indicated a significant relationship between bacterial community structure and the selected environmental variables. The first CCA axis was found to be strongly correlated with pH, DOC, TOC, NO₃⁻ and NH₄⁺ content, and the second axis was correlated with TOC and NH₄⁺ content (Table 3), which showed that soil properties under long-term fertilization influenced the bacterial community composition.

3.3. Phylogenetic analyses

The BLAST analysis of the 16S rDNA sequences obtained from DGGE gel bands classified the sequences into seven main groups: *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Alphaproteobacteria*, *Actinobacteria*, *Gammaproteobacteria* and *Firmicutes* (Table 2). All these seven bacterial groups could be detected in all the treatments, except the N treatment, in which no *Gammaproteobacteria* were observed (Table 2). Most of the bands in the CK, M and M + N treatments had highest similarity database hits to the *Acidobacteria* (20% of sequenced bands), *Sphingobacteria* (20% of sequenced bands), and *Gemmatimonadetes* (20% of sequenced bands), while

Fig. 1. Abundance of bacterial 16S rRNA gene copies under different fertilization treatments. Treatments: control (CK), fertilizer nitrogen (N), manure (M), manure plus half fertilizer nitrogen (M + N). The different letter on the top of the column showed a statistically significant difference between the treatments at $P = 0.05$ level using one-way ANOVA.

Fig. 2. DGGE profile of amplified 16S rDNA fragments from Shenyang soil samples. Band position was highlighted with a numbered arrow. Treatments: control (CK), fertilizer nitrogen (N), manure (M), manure plus half fertilizer nitrogen (M+N).

in the N treatment the proportion of *Acidobacteria* were 23% of sequenced bands. Band 7 fell within the phylum *Bacillales* and was detected in all treatments.

4. Discussion

In this study, culture-independent methods qPCR and PCR-DGGE were applied to investigate the abundance and community structure of soil bacteria after 20 years of different fertilizer management regimes. qPCR has recently become a powerful technique

for quantitative analysis of bacteria from environmental samples (Suzuki et al., 2000; Fierer et al., 2005). In this study, significant differences in the bacterial 16S rRNA gene copy numbers were obtained between N treatment and other treatments, which may be explained by the low pH, TOC and DOC in the N treatment (Liang et al., 2009). We observed previously that the abundance of bacteria in N fertilizer treatment were lower than CK and NPK+OM treatments in a red Chinese soil (He et al., 2007). Long-term N fertilizer application could result in a net increase in concentration of protons in the soil, and thus soil acidification. In this experiment, the

Table 2

Tentative identification of dominant DGGE bands in DGGE gels and the closest match to the sequence from GenBank database with BLAST and taxonomic affiliation.

Band number	Accession no.	Origin (treatment)	Closest relative (accession no.)	Alignment, % similarity	Taxonomic affiliation
Band 1	EU216574	CK, N, M, M+N	Uncultured soil bacterium (GU359109)	453/457, 99	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 2	EU216574	CK, N, M, M+N	Uncultured soil bacterium (GU359110)	453/457, 100	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 3	EU216575	CK, N, M, M+N	<i>Bacterium Ellin5032</i> (AY234449)	457/459, 99	<i>Alphaproteobacteria</i>
Band 4	EU216586	CK, M, M+N	Uncultured soil bacterium (EF551932)	447/458, 97	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 5	EU216587	CK, M, M+N	Uncultured bacterium (FJ592639)	456/457, 99	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 6	EU216587	CK, M, M+N	Uncultured bacterium (FJ592640)	456/457, 100	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 7	EU216588	CK, N, M, M+N	Uncultured bacterium (FJ683738)	455/456, 99	<i>Bacillales</i>
Band 8	EU216595	CK, M, M+N	<i>Pedobacter</i> sp. BZ42 (GQ161990)	435/466, 93	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>
Band 9	EU216575	CK, M, M+N	<i>Bacterium Ellin5032</i> (AY234449)	457/459, 99	<i>Alphaproteobacteria</i>
Band 10	EU216589	CK, M, M+N	Uncultured gamma proteobacterium (EF142045)	455/455, 100	<i>Gammaproteobacteria</i>
Band 11	EU216590	CK, N, M, M+N	Uncultured bacterium (FJ479543)	458/458, 100	<i>Alphaproteobacteria</i>
Band 12	EU216576	CK, M, M+N	Uncultured bacterium (FJ525299)	460/462, 99	<i>Gammaproteobacteria</i>
Band 13	EU216592	CK, M, M+N	Uncultured Rhizobiaceae bacterium (FM201296)	457/459, 99	<i>Alphaproteobacteria</i>
Band 14	EU216577	CK, N, M, M+N	Uncultured Oxalobacteraceae bacterium (EF019646)	428/465, 92	<i>Gemmatimonadetes</i>
Band 15	EU216593	CK, N, M, M+N	Uncultured bacterium clone (GQ095375)	453/459, 98	<i>Acidobacteria</i>
Band 16	EU216573	CK, N, M, M+N	Uncultured bacterium (EU421850)	458/458, 100	<i>Acidobacteria</i>
Band 17	EU216578	N	Uncultured proteobacterium (EF019498)	453/457, 99	<i>Acidobacteria</i>
Band 18	EU216578	CK, N, M, M+N	Uncultured proteobacterium (EF019499)	453/457, 100	<i>Acidobacteria</i>
Band 19	EU216579	CK, N, M, M+N	Uncultured Acidobacteriales bacterium (EU193015)	454/455, 99	<i>Acidobacteria</i>
Band 20	EU216581	CK, N, M, M+N	Uncultured bacterium (AY917636)	447/452, 98	<i>Actinobacteria</i>
Band 21	EU216594	CK, M, M+N	Uncultured bacterium (GQ402578)	440/458, 96	<i>Acidobacteria</i>
Band 22	EU216578	N	Uncultured proteobacterium (EF019498)	453/457, 99	<i>Acidobacteria</i>
Band 23	EU216582	CK, M, M+N	Uncultured bacterium (EU729911)	451/459, 98	<i>Gemmatimonadetes</i>
Band 24	EU216580	N	<i>Catenulispora acidiphila</i> DSM44928 (CP001700)	452/455, 99	<i>Actinobacteria</i>
Band 25	EU249321	CK, M, M+N	Uncultured bacterium (AB486649)	455/459, 99	<i>Gemmatimonadetes</i>
Band 26	EU216572	N	Uncultured bacterium (EU239936)	460/460, 100	<i>Acidobacteria</i>
Band 27	EU216584	CK, M, M+N	Uncultured bacterium (GQ069903)	440/464, 94	<i>Gemmatimonadetes</i>
Band 28	EU216585	CK, M, M+N	Uncultured bacterium (EU133743)	438/454, 96	<i>Sphingobacteria</i> , <i>Bacteroidetes</i>

Fig. 3. Canonical correspondence analysis (CCA) ordination diagram of DGGE data, with soil environmental characteristics (pH, DOC, TOC, NO_3^- and NH_4^+) as arrows. Circle symbols represent the CK treatments, star symbols represent the N treatments, left-triangle represent the M+N treatments, and X-mark symbols represent the M treatments. Values on the axes indicate % of total variation explanation by the corresponding axis. DOC: dissolved organic carbon; TOC: total organic carbon.

sampled soil pH originating from loess-like parent materials was 6.0 before the experiment (Liang et al., 2009), and decreased to pH 4.9 after 20 years of urea-N fertilizer application (Table 1). Significant correlation between bacterial abundance and soil pH also indicated that soil pH could have a major effect on the population sizes of bacteria.

The changes in soil pH caused by urea-N fertilization in the N treatment and its striking influence on bacterial communities has been documented in a number of studies using various molecular methods and traditional approaches (Fierer and Jackson, 2006; Fierer et al., 2007; He et al., 2007). In particular, a recent study by Lauber et al. (2009) showed that soil pH significantly correlated with differences in overall bacterial community composition, and could be used as a predictor of soil bacterial community structure based on the analysis of 88 soils from across North and South America by bar-coded pyrosequencing. Variables that also correlated with the variation in soil bacterial community diversity were soil TOC, DOC, and NH_4^+ -N.

The DGGE band patterns in CK, M and M+N treatments judged by the naked eye were clearly more similar to each other than to those of soils with only N fertilizer application, which suggested that manure amendment, even in the presence of urea-N fertilizer application, circumvents the changes in bacterial community structure and abundance observed in the plots treated with urea-N fertilizer. The incorporation of organic manure may provide a conducive environment for microbial proliferation, which could maintain an even distribution of bacterial species with the com-

munity (Sun et al., 2004). This result corroborates previous studies showing that bacterial community shifts occurred after long-term application of inorganic-N fertilizer (Dolfing et al., 2004; Sun et al., 2004; Shen et al., 2008), while no such shifts occurred over the short-term (Marschner et al., 2003). Our results are in accordance with other studies showing a decrease in DGGE-identifiable bacterial band under long-term N fertilizer management regimes (Bruns et al., 1999). However, some studies have demonstrated that the bacterial community structures of N fertilizer treatments were more similar to the unfertilized treatment (CK) (Chu et al., 2007; Ge et al., 2008). This discrepancy may be explained by the differences in methodologies (such as clone library, sequencing and DGGE), but it is most likely due to the higher soil pH measured in the above-mentioned studies (Chu et al., 2007; Ge et al., 2008).

A study by Sun et al. (2004) demonstrated using band-sharing coefficients that bacterial communities in manure-treated soil were more closely related to community structure in untreated soil than in soils treated with inorganic-N fertilizers. Interestingly, our results from CCA analysis of relative DGGE band intensity showed that the M and M+N treatments grouped closely together but distinctly from the CK and N, which grouped separately from each other. CCA has been proven more effective in illustrating the relationship between bacterial community composition and environmental parameters than univariate methods (Salles et al., 2006; Zeng et al., 2009).

Phylogenetic analysis of bacterial 16S rRNA gene suggested that seven major phyla, *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Proteobacteria*, *Actinobacteria*, *Gammaproteobacteria* and *Firmicutes* were identifiable by PCR-DGGE in this study. These phyla have consistently been detected as the main bacterial divisions in soil environments (Janssen, 2006), such as a century-old manure-treated agroecosystem (Sun et al., 2004), and forest soil under long-term residue management (He et al., 2006). Three of these six divisions, *Acidobacteria*, *Bacteroidetes* and *Gemmatimonadetes*, were dominant in this study. The wide distribution and diversity of *Acidobacteria* has been demonstrated in numerous surveys from environments that vary greatly in physical and biogeochemical characteristics, including soil, marine samples and sediments (Barns et al., 1999; Sun et al., 2004; Janssen, 2006; Kielak et al., 2009). More recently, three genomes from the phylum *Acidobacteria* revealed putative functions which are important in the environment (Ward et al., 2009). The phylum-level distribution of bands in this study showed that a higher proportion of *Acidobacteria* were detected in the N treatment, which lowered the ratio between the *Proteobacteria* and the *Acidobacteria* to 0.43. This ratio might be indicative for the nutrient status in the soil system, as reported ratios at 0.46 in a low-nutrient system (Smit et al., 2001) and 0.87 in a high-input agricultural system (McCaig et al., 1999), while the confirmation of this point will require much more data.

In conclusion, the application of real-time PCR and PCR-DGGE in combination with CCA provides a useful means for detection of differences in soil bacteria communities and abundance. The results of this study clearly demonstrated that the bacteria abundance and community composition were significantly affected by management practice, particularly in soils receiving long-term N fertilizer application. Microbial communities in soils play a key role in residue decomposition, nutrient cycling and soil symbioses, all of which are good indicators of environmental changes and sustainability of soil ecosystems (Naeem and Li, 1997; Cardinale et al., 2006). The change in soil pH due to urea-N fertilizer application was an important factor for bacterial community structure in this study, which is consistent with our previous studies of ammonia oxidizer communities in Chinese acid and alkaline soils receiving variable amendments of N fertilizer (He et al., 2007; Shen et al., 2008). However, research showed that soil and site characteristics may influence microbial community at local scale (Buckley and

Table 3
Summary results of the canonical correspondence analysis (CCA).

	Axis 1	Axis 2
Eigenvalue	0.66	0.164
Species–environment correlations	0.993	0.981
Cumulative percentage variance of species data	72.9	91.0
Cumulative percentage variance of species–environment relation	75.2	93.9
Weighted correlation		
pH	–0.919	0.295
DOC	–0.669	–0.200
TOC	–0.825	–0.532
NO_3^-	0.678	–0.002
NH_4^+	0.548	–0.523

Schmidt, 2003), while soil pH is a better predictor at the continental scale (Fierer and Jackson, 2006). Future investigations of environmental factors on microbial diversity in Chinese soils should thus also be conducted on larger scales to address the issue of local versus continental effects.

Acknowledgements

The soil samples were collected from the long-term fertilization experimental site of Shenyang Agriculture University, China. The authors thank Prof. Jingkuan Wang for technical assistance. This research was financially supported by the Chinese Academy of Sciences (KZCX2-YW-JC401 and KSCX2-YW-G072) and the Natural Science Foundation of China (No. 40901121).

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