International Scholars Journals

African Journal of Botany ISSN: 3519-3824 Vol. 8 (1), pp. 001-009, January, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

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Full Length Research Paper

Abundance and diversity of ammonia-oxidizing bacteria in rhizosphere and bulk paddy soil under different duration of organic management

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Accepted 18 November, 2019

Denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR) approaches were used to assess respectively the quantity and the molecular diversity of the ammonia-oxidizing bacteria (AOB) in rhizosphere and bulk paddy soil under conventional management (2 year) and different duration(2, 3, 5 and 9 years) organic management. Phylogenetic analysis in our study based on bacterial AOB identified that *Nitrosospira* species were dominated in organic paddy soil. When the quantity of the ammonia monooxygenase gene was determined by qPCR, 3.93×10^5 to 1.09×10^6 copies/g of soil were detected. Except for organically managed soil (2 year), the copy numbers in all the organically managed soil were significantly higher than in conventionally managed soil. The abundance and diversity of ammonia oxidizing bacteria tended to increase with duration of organic management. And the highest number of ammonia monooxygenase gene copies was observed in the soil under five year organic management. In addition, Analysis of variance and canonical correspondence analysis (CCA) showed that C/N, C and N were important factors influencing the abundance and community of ammonia oxidizing bacterial.

Key words: Ammonia-oxidizing bacteria, molecular diversity, PCR-DGGE analysis, organic and conventional management, paddy soil.

INTRODUCTION

Nitrogen (N) is important nutrition for rice growth and recognized as a major factor determining rice yields. Ammonium is considered as the major form to which rice is especially adapted (Wang et al., 1993), however, it has been suggested that rice may absorb significant amounts of nitrate formed by nitrification of ammonium in the rhizosphere (Kronzucker et al., 2000). Nitrification is the conversion of the most reduced form of N (ammonia) to its most oxidized form (nitrate), and the ammonium oxidation is the initial and rate-limiting step in nitrify-cations, which is generally carried out by the chemolitho-autotrophic ammonia-oxidizing bacteria (AOB). Based on 16S rRNA sequence analysis, Well-known genera in ammonia-oxidizing bacteria group are only two *Nitrosomonas* and *Nitrosospira*. Studies conducted in rice soil suggested that *Nitrosospira* spp. were predominant on rice roots from Italian rice fields (Horz et al., 2000; Rotthauwe et al., 1997) and Japanese rice fields (Bowatte et al., 2007). By contrast, only *Nitrosomonas* spp. was observed in a Philippines rice soil (Nicolaisen et al., 2002; 2004). At present, very little is known about how the population structure of these bacteria affects nitrification in rice. It was confirmed that *Nitrosospira* spp. and *Nitrosomonas* spp. are both important and predo-minant in paddy soil, however, very little is known about the population structure and relative abundance of AOB

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Abbreviations: CM, Conventional management; **OM,** organic management; **DGGE,** denaturing gradient gel electrophoresis; **COFCC,** China organic food certification center; **CCA,** canonical correspondence analysis; **qPCR,** real-time quantitative PCR.

in organically and conventionally managed paddy soils.

Recently, some researchers showed that soils under organic management had a higher diversity of bacteria (Drinkwater et al., 1995), higher microbial activity (Mäder et al., 2002; Workneh et al., 1993) and microbial biomass (Mäder et al., 2002; Mulder et al., 2003) than conventionally managed soils. On the contrary, some studies found that there were no differences in bacterial biodiversity (Lawlor et al., 2000) or in fungal communities (Franke-Snyder et al., 2001) between organically or conventionally managed soils. As mentioned earlier, some studies have investigated the diversity of bacterial and fungal communities under organic and conventional management. However, no investigator has investigated about the diversity and abundance of ammonia-oxidizing bacteria in organically or conventionally managed paddy soils, especially in different durations of organically managed paddy soils. Undertanding the diversity and abundance of ammonia-oxidizing bacteria may reveal the nitrification in agriculture system.

The important factors influencing ammonia oxidation is the availability of ammonium and O_2 (Avrahami et al., 2003; Briones et al., 2002; Nicolaisen et al., 2004). The difference of physical, chemical and biological conditions in rhizosphere and bulk could influence the availability of ammonium and O_2 (Lu et al., 2007; Noll et al., 2005; Ratering and Schnell, 2001; Wind and Conrad, 1997). It has been suggested that oxygen released from rice roots may support aerobic microbial nitrification (Brune et al., 2000). However, a less information is available regarding the abundance, diversity and composition of Ammonia-oxidizing bacteria populations occurring in the rice rhizosphere in organically and conventionally managed paddy soils.

The objective of the present study is:

(1) Investigate abundance and community structure of ammonia-oxidizing bacteria in soils under different management regimes (organic and conventional) in paddy soils, especially the effects of different duration of organic management (2, 3, 5, and 9 year),
(2) Investigate abundance and community structure of Ammonia-oxidizing bacteria in rhizosphere and bulk paddy soil.
(3) Study the soil chemical parameters affecting the ammonia-oxidizing bacteria.

MATERIALS AND METHODS

Study site and field design

We sampled soil from YingFengWuDou organic farms in ChongMing Island, Shanghai, China (31'38N, 121'39E). There were five different types of fields, four of which have received organic management for different durations (2, 3, 5 and 9 years) and one conventional management. All organic fields were COFCC (China Organic Food Certification Center) certified and thus used no chemical fertilizer, pesticides or genetically modified organisms for at least the last 5 years (3 years of conversion). The farmer's cropping practice consists on one rice crop annually. Rice fields are flooded during the growing season and drained to plant green manure, normally Chinese milk vetch (*Astragalus sinicus* L.), in organic fields. Wheat is planted in conventional paddy rice. Fertilizers were applied as described previously during the previous seasons following farmer's practice (Wang et al., 2011).

Rhizosphere soil and bulk soil were collected on the 28th of October of 2008 at 10:00, twenty days before the rice was harvested. Bulk soil was sampled from the surface soil (0-10 cm), about 15 cm away from the plants uprooted for rhizosphere soil collection. Bulk soil samples were taken from five points and five plants were randomly selected in every field. The roots were shaken vigorously to separate soil as rhizosphere soil, not tightly adhered to the roots. Three replicates were collected from both, bulk and rizhosphere soil. All the samples were transported in plastic bags in containers with ice. Upon arrival in the laboratory, soil samples were thoroughly mixed and sieved through a 0.5 cm mesh and plant parts and earthworms were removed. Homogenized manually by thorough mixing, soil samples (50 g) for gene analysis were stored at -80°C until extraction of DNA and subsamples were dried in a thin layer for three days and stored at room temperature in plastic bags for chemical analyses.

Soil chemical analysis

The dried soil samples were ground into powder and analyzed: TDN (Total Dissolved Nitrogen), $NO3^-$ and $NH4^+$ content were $CaCl_2$ extracted and analyzed with a SmartChem200 Analyzer (Alliance Corp, France).Total N and total C content were determined with a CHNS/O Analyzer (Elementar, Germany). Organic N was calculated as DON (Dissolved Organic Nitrogen) = TDN - (NO_3 + NH_4). Soil pH and conductivity were measured in soil water extract (10 g of soil and 50 ml of water were mixed and left to stand overnight at room temperature). Moisture was assessed by drying soil at 80°C to constant weight.

PCR for DGGE analysis

For DGGE analysis, DNA was extracted and purified using E.Z.N.A. TM Soil DNA Kit (D5625-01, Omega, USA) as recommended by the manufacturer and amplified with the primer CTO189f and CTO654r (Alvey et al., 2003), set specific for AOB. The sequence of primers are (5'-ACGGGGGGAGGAAAGTAGGGGATG-3') and (5'-CTAGCYTTGTAGTTTCAAACGC-3'), and a GC-clamp (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-3') (Myers et al., 1985) was attached to the 5' end of all the primers. The reaction mixture was 50 µl volumes containing 50 ng of template DNA, 5 mM of each primer, 10×PCR buffer for HotStarTaq, 0.2 mM of each dNTP, 1.5 mM of $MgSO_4$, and 1U of HotStarTaq DNA polymerase. PCR was performed with a Thermal Cycler Dice (PTC-200, Bio-Rad, USA) using HotStarTaq DNA polymerase (Qiagen, USA). The thermal profile for amplification was: 15 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 46°C, and 60 s at 72°C; and final 8 min at 72°C. Three replicate amplifications were carried out for each sample and all the amplified PCR products were analyzed by 1% (w/v) agarose-TAE gel electrophoresis with ethidium bromide staining, to examine product integrity and estimate yield.

Quantitative real-time PCR assays

The copy numbers of ammonia monooxygenase gene in all samples were determined in triplicate using an Applied Biosystems 7500 Fast Real-Time PCR System (ABI, USA). The quantification

was based on the fluorescent dye SYBR-Green I, which binds to double-stranded DNA during PCR amplification. And the pair of primers (CTO189f and CTO654r) without GC clamp was used. The thermal profile for amplification was: 5 min at 95°C; 40 cycles of 25 s at 95°C, 30 s at 46°C, and 40 s at 72°C. Each reaction was performed in a 25 µl volume containing 1–10 ng of DNA, 0.2 mM of each primer and 12.5 µl of ABI SYBR 2x Mix (ABI, USA). Product specificity was confirmed by melting curve analysis (65–95, 0.5°C per read, 5 s hold) and visualization in agarose gels, which showed specific product bands at the expected size of 465 bp.

The fragments were cloned as described above respectively, and clones that had the right gene inserts were chosen as the standards for real-time PCR. Plasmid DNA was extracted with Plasmid Kit (TaKaRa), and the plasmid concentration was measured with a spectrophotometer (Nanodrop). As the sequences of the vector and PCR inserts were known, the copy numbers of ammonia mono-oxygenase gene were calculated directly from the concentration of extracted plasmid DNA. Ten-fold serial dilutions of a known copy number of plasmid of the clone from the soil were generated to produce the standard curve over seven orders of magnitude (6×10^3 to 6×10^7 copies of template) per assay. High amplification efficiencies of 101.1–103.4% were obtained for ammonia mono-oxygenase gene quantification with R^2 values between 0.97 and 0.99 and slopes from -3.2 to -3.5. The parameter Ct (threshold cycle) was determined as the cycle number at which a statistically significant increase in the reporter fluorescence was detected.

DGGE analysis

DGGE analysis was performed using a D-Code universal mutation system (Bio-Rad Laboratories, USA) according to the instruction manual. The conditions for separation were as follows: running at 75 V for 14 h in an 8% polyacrylamide gel with the denaturing gradient from 30 to 60% at a constant temperature of 60°C. The 100% denaturant solution contained 7 M urea, 40% (v/v) formamide, 8% acrylamide/bis-acrylamide (37.5:1) and 0.5×Tris-acetate-EDTA (TAE) buffer (pH 8) in ultrapure water. The 0% denaturant solution contained 8% acrylamide/bis-acrylamide (37.5:1) and 0.5×TAE buffer (pH 8) in ultrapure water. After electrophoresis, gels were stained with SYBR™ Green I nucleic acid gel stain for 45 min following the manufacturer's instructions, and the bands were visualized in UV light with the Gel Doc 2000 System (Bio-Rad). Major bands of interest were excised from the gel and boiled at 95 °C for 5 min or suspended in 10 µl of sterile Milli-Q water (24 h) and then re-amplified under the same conditions, except that the forward primer had no GC clamp attached. The PCR products were again subjected to DGGE to ensure that the products contained single bands and showed electrophoretic mobility identical to that of the original bands. PCR products were purified with the MinElute PCR purification kit (Qiagen, USA), ligated into the pGEM-T Vector (Promega, USA) and transformed into Trans*5α* Chemical competent cell (TransGen Biotech, Shanghai, China). PCR amplification with primers SP6 and T7 was performed directly on selected white colonies. Then, the strains shook at 37°C overnight for sequencing. Sequences were analyzed using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in the GenBank (DDBJ, EMBL, and PDB at NCBI) to find the closest sequence matches. Sequences generated in this study have been deposited in GenBank with accession No. GU097354-GU097377.

Statistical analysis

DGGE gel images were analyzed using Quantity One software (version 4.6, Bio-Rad Laboratories), Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) were carried out with MVSP 3.1 software (http://www.kovcomp.co.uk/Mvsp/). Analysis of variance was done using Excel 2007. We used the algorithm BLAST-N to identify the most similar sequences and then aligned with representative nitrogenase sequences obtained from the same database using the software package CLUSTAL X (Thompson et al., 1997). The aligned DNA sequences were translated into amino acid sequences using MEGA 4 and were used for construction of neighbor-joining phylogenetic trees by using Poisson correctiondistances and pairwise deletion of gaps and missing data. Bootstrapping (500 replicate reconstructions) was used to estimate the reliability of phylogenetic reconstructions.

RESULTS

The chemical properties of the soils

Total carbon (C), nitrogen (N), TDN, ammonia, nitrate, Moisture, EC and pH were determined for triplicate samples taken from different fields (Table 1). According to Table 1, Total C and N content in OM soils increased gradually with duration of organic management, and were significantly higher than in CM soils. Regardless of organic and conventional management soil, total C and N content in rhizosphere soil were significantly higher than in bulk soil. C/N ratio, however, decreased gradually in both rhizosphere soil and bulk soil. TDN, DON, NH_4-N and Moisture in CM soil (including bulk and rhizosphere) were significantly lower than those in all OM soil. But the pH in CM soil (including bulk and rhizosphere) were much higher than those in all OM soil. Moreover, NO_3-N in CM bulk soil were also significantly higher than those in OM bulk soil whereas NO_3-N in rhizosphere bulk soil were lower than those in OM rhizosphere soil.

Quantitative real-time PCR assay

The ammonia monooxygenase gene copy in paddy soil ranged from 3.93×10^5 to 1.09×10^6 copies/g of soil (Figure 1). No significant difference was observed in CM and OM-2 soil including bulk and rhizosphere soil. This suggests that short-term organic management soil, compared to conventionally managed soil, have no effects on the abundance of nitrogen fixing bacteria. Except for OM-2 soil, the ammonia monooxygenase gene copy numbers in all the organic soil (OM-3, OM-5 and OM-9 soil) was significant higher than those in CM soil respectively. Moreover, the ammonia monooxygenase gene copy numbers both in bulk and rhizosphere soil, increased from OM-2 soil to the yielding point OM-5 soil, then went down. In addition, the ammonia mono-oxygenase gene copy numbers in the OM-3 and OM-5 rhizosphere soil were significantly higher than those in the bulk soil respectively. In addition, correlation analysis based on abundance of AOB show that there was a positive correlation with N (R^2=0.85, P<0.001) and C (R^2=0.85, P<0.001), whereas negative correlation C/N (R^2=0.77, P<0.001).

Table 1. Soil physicochemical characteristics.

	Total N (g/kg)	Total C (g/kg)	TDN (mg/kg)	DON (mg/kg)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	Moisture (%)	pH	EC (μs)	C/N	DON/TN (%)
CM-B	1.06±0.01g	15.56±0.40f	37.10±0.40h	34.41±0.40i	0.15±0.02d	2.49±0.02g	18.91±0.05g	8.37±0.04a	317.67±2.52e	14.68±0.25a	3.30±0.21c
OM-2-B	1.39± 0.00f	19.30±0.16e	87.22±0.68a	85.32±0.28a	0.25±0.01b	2.24±0.10h	26.06±0.02e	8.34±0.01a	572.33±3.21c	13.89±0.12b	6.35±0.20a
OM-3-B	1.51±0.03d	20.79±0.29c	77.19±0.51e	75.39±0.41e	0.22±0.02c	2.26±0.07h	31.79±0.10a	8.18±0.02c	655.33±2.52a	13.74±0.12c	4.72±0.30b
OM-5-B	2.51±0.03b	28.52±0.53b	81.29±0.32c	78.38±0.23d	0.23±0.01c	2.30±0.01h	30.50±0.02b	8.10±0.03d	266.00±2.00f	11.36±0.11d	3.32±0.19c
OM-9-B	2.42±0.06c	28.93±0.74b	63.53±0.35g	60.25±0.19h	0.47±0.01a	2.96±0.05f	25.88±0.02e	8.23±0.02b	238.00±2.00h	11.91±0.12c	2.42±0.16d
CM-R	1.11±0.01g	16.11±0.32f	66.75±0.21f	63.47±0.31f	0.11±0.01e	3.08±0.08e	18.24±0.01h	8.36±0.02a	223.67±3.21i	14.51±0.18a	5.60±0.28a
OM-2-R	1.46±0.02e	20.05±0.06d	86.96±0.76a	83.44±0.26b	0.15±0.01d	3.45±0.05d	27.05±0.01d	8.10±0.02d	566.33±2.52d	13.76±0.18b	5.56±0.43a
OM-3-R	1.52±0.02d	20.94±0.14c	79.01±0.31d	75.54±0.25e	0.15±0.01d	3.56±0.04c	21.94±0.05f	8.16±0.02c	577.00±3.00b	13.81±0.20b	4.79±0.34b
OM-5-R	2.85±0.03a	32.87±0.60a	85.67±0.25b	81.48±0.21c	0.23±0.03bc	3.76±0.05b	29.25±0.04c	8.07±0.03d	246.00±3.00g	11.52±0.13d	2.78±0.17cd
OM-9-R	2.51±0.05b	29.23±0.47b	67.53±0.47f	62.67±0.26g	0.48±0.01a	3.95±0.03a	29.28±0.03c	8.20±0.02bc	217.33±3.06j	11.63±0.03cd	2.57±0.26d

CM (second year rice with conventional management); OM-2 (second year rice with organic management); OM-3 (third year rice with organic management), OM-5 (fifth year rice with organic management); OM-9 (ninth year rice with organic management), and B (bulk soil); R (rhizosphere soil) ;means ± sd followed by the same letter are not significantly different ($P < 0.01$) using t-Test (LSD).

DGGE analysis

Denaturing gradient gel electrophoresis analysis was performed on ammonia monooxygenase gene PCR products obtained from soil (Figure 2). Reproducible profiles were obtained from both templates for all soil treatments, and the numbered bands with two replicates with the same mobility in the DGGE gels were excised for sequencing. Generally, a greater diversity of ammonia monooxygenase gene fragments (band richness) was observed in organic management soils than in conventional management soils. In addition, the AOB community present in the rhizosphere was higher than in bulk soil, since the band richness or intensity were increased.

The community compositions of AOB, as revealed by the DGGE profiles, were studied by PCA. PCA plot (Figure 3A) explained PC1 (60.21%) and PC2 (17.66%) of the variance of AOB community respectively. Significant difference was observed between AOB community in organic and conventional soil. In addition, the community compositions of AOB in long-term organic (OM-5, OM-9) soil were similar and located in the right part of the plot, were different with those derived from organic (OM-2, OM-3) soil.

To investigate the factors influence on the community composition, the relationship between the characterization of soil and the DGGE profiles of AOB was evaluated by CCA (canonical correspondence analysis). The CCA (Figure 3B) showed that C/N, C, and N had a significant impact on AOB community in all soil. In addition, DON/N, TON/N, Moisture and NH_4 were also important factors. The order of the predominant factors according to the degree of effect on the AOB community was C/N, C, N, DON/N, TON/N and NH_4.

Phylogenetic analysis

In total, 25 bands were retrieved from the DGGE gels, reamplified, and sequenced. BLAST-N analyses revealed that most bands were closely related to ammonia monooxygenase genes of uncultured bacteria, On the basis of the BLAST-N results, highly similar GenBank sequences and AOB reference sequences have been added to the data set. Multiple sequence alignment was performed and the phylogenetic distance tree shown in Figure 4. After editing, each sequence included in the final tree consisted of 423 bases. A neighbour-joining tree was constructed using sequences of DGGE bands and the related sequences deposited in GenBank. Most of the band sequences appeared to be the genus *Nitrosospira*, In addition, there were seven clusters, six of which were belonged to *Nitrosospira* and one was relative to *Nitrosomonas*.

DISCUSSION

The aim of this study was to investigate the abundance and diversity of AOB in the rhizosphere

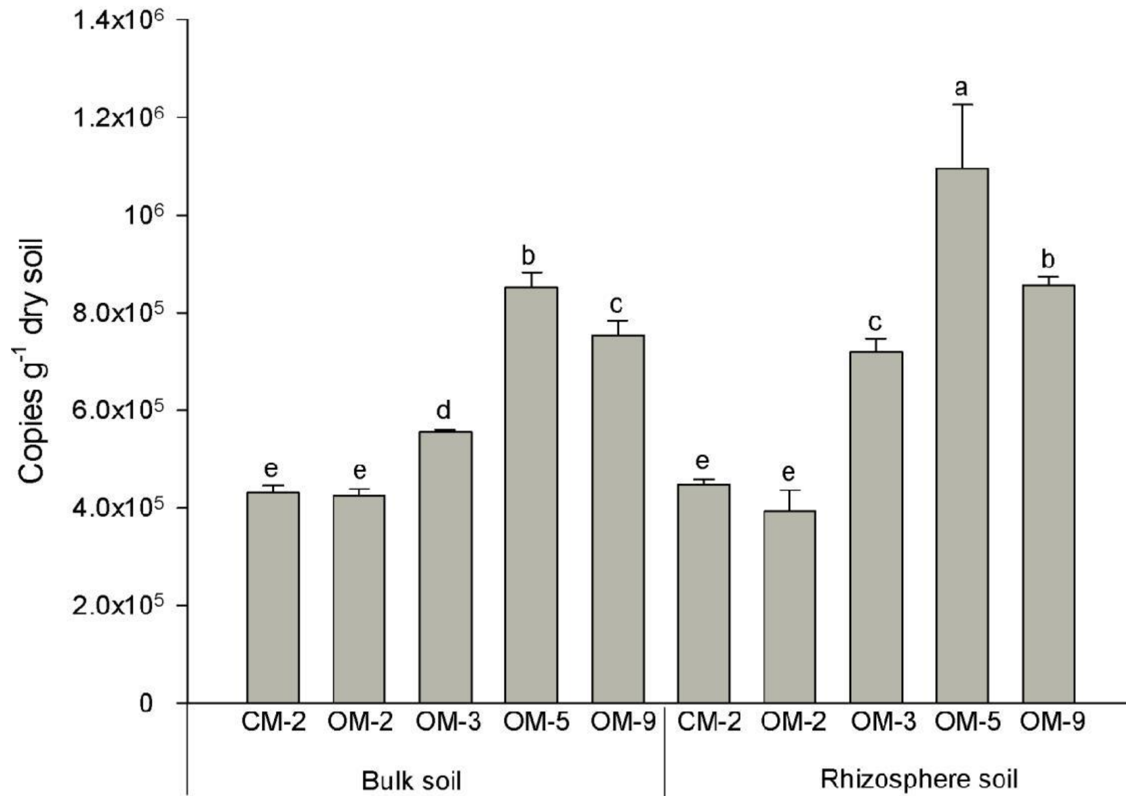

Figure 1. Abundance of AOB in paddy soil under conventional and different duration of organic management expressed as DNA-targeted copy numbers per gram dry weight of soil respectively. Error bars indicate standard deviation. B, bulk; R, rhizosphere. means (mean ± SE, n = 3) followed by the same letter are not significantly different at $P < 0.05$.

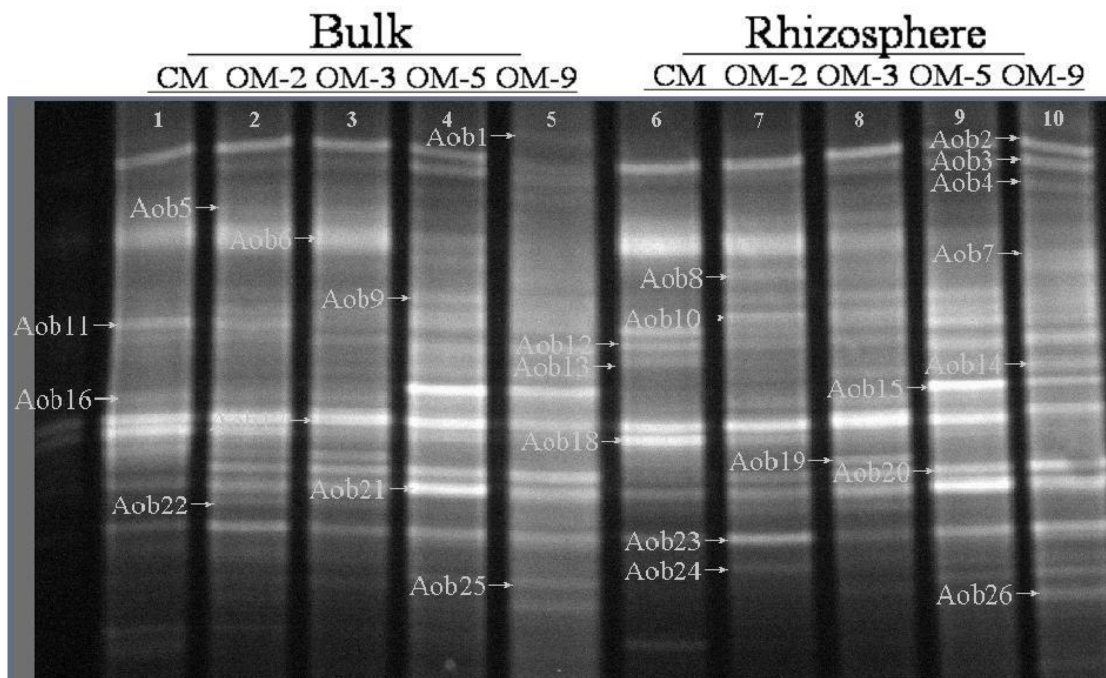

Figure 2. Denaturing gradient gel electrophoresis (DGGE) analysis of the AOB obtained from the paddy soil under conventional and different duration of organic management respectively. B, bulk; R, rhizosphere.

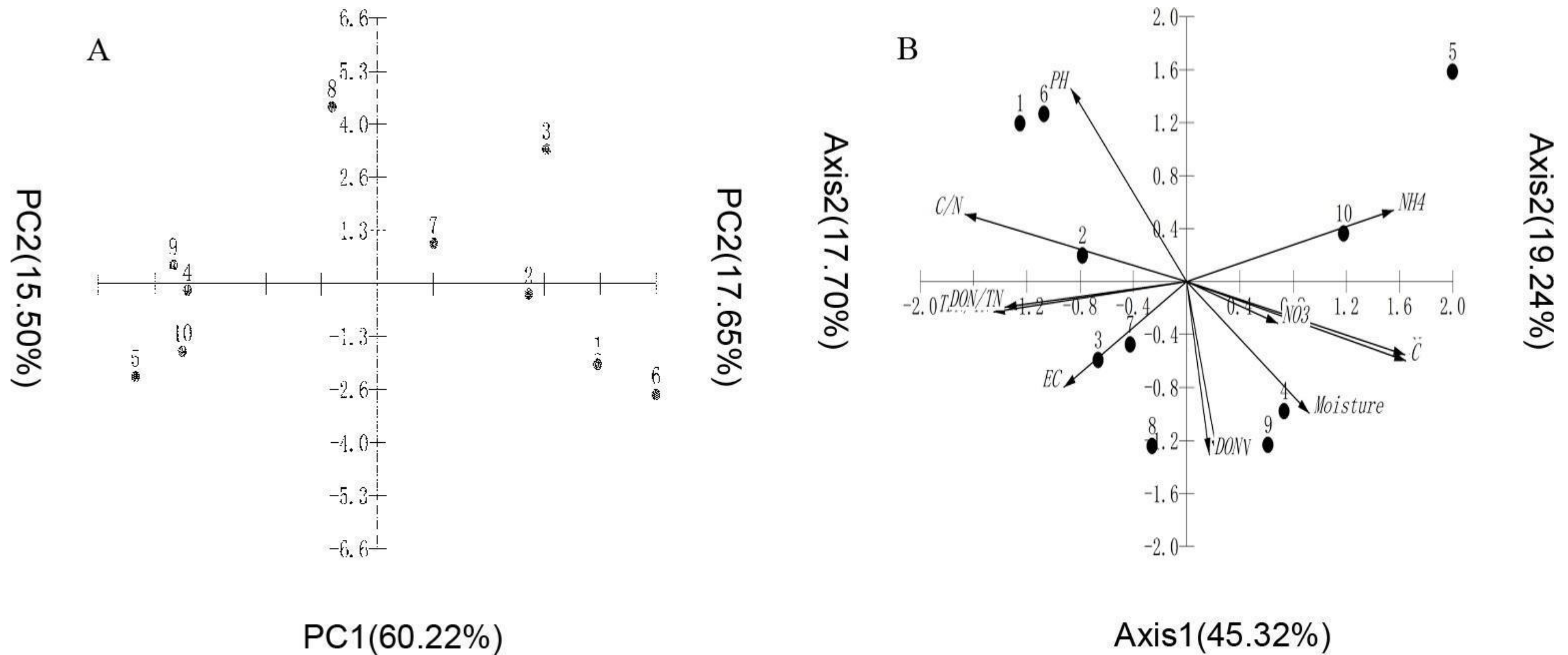

Figure 3. A, PCA score plot of PCR-DGGE patterns obtained from ten soil samples respectively. B, Canonical correspondence analysis (CCA) elucidates the relationships between AOB of species and their chemical parameter. Number 1-10 correspond with CM, OM-2, OM-3, OM-5, and OM-9 (bulk soil and rhizosphere) respectively.

and bulk of paddy soil under conventional and different duration of organic management, using qPCR and DGGE analysis. Our results showed that the DNA-targeted bacterial ammonia monooxygenase gene copy number ranged from 3.93×10^5 to 1.09×10^6 copies/g in accordance with some studies in agricultural soil by other PCR-based methods (Mendum et al., 1999). Higher abundance and were found in organic managed soils where the highest abundance was three times higher than that in conventional managed soil. In addition, the higher diversity (richness, evenness) was found in organic managed soils (data not showed). Interestingly, the abundance and diversity of AOB increased with duration of organic management but decrease after 5 years of organic management. Organic fertilizer amendment during long-term application thus appears to lead to an increasing number and diversity of ammonia oxidizers in the soil community. This is not only a higher N level than that supplied by the mineral fertilizers, but also the N is offered to the microorganisms mainly organically. The N is liberated by ammonification, which explains the high AOB cell numbers.

The difference of physical, chemical and biological conditions in rhizosphere and bulk could influence the availability of ammonium and O_2 (Avrahami et al., 2003; Briones et al., 2002; Nicolaisen et al., 2004). The abundance of AOB in the conventional and organic paddy soils (including rhizosphere and bulk) varied a lot. Correlation analysis based on abundance of AOB

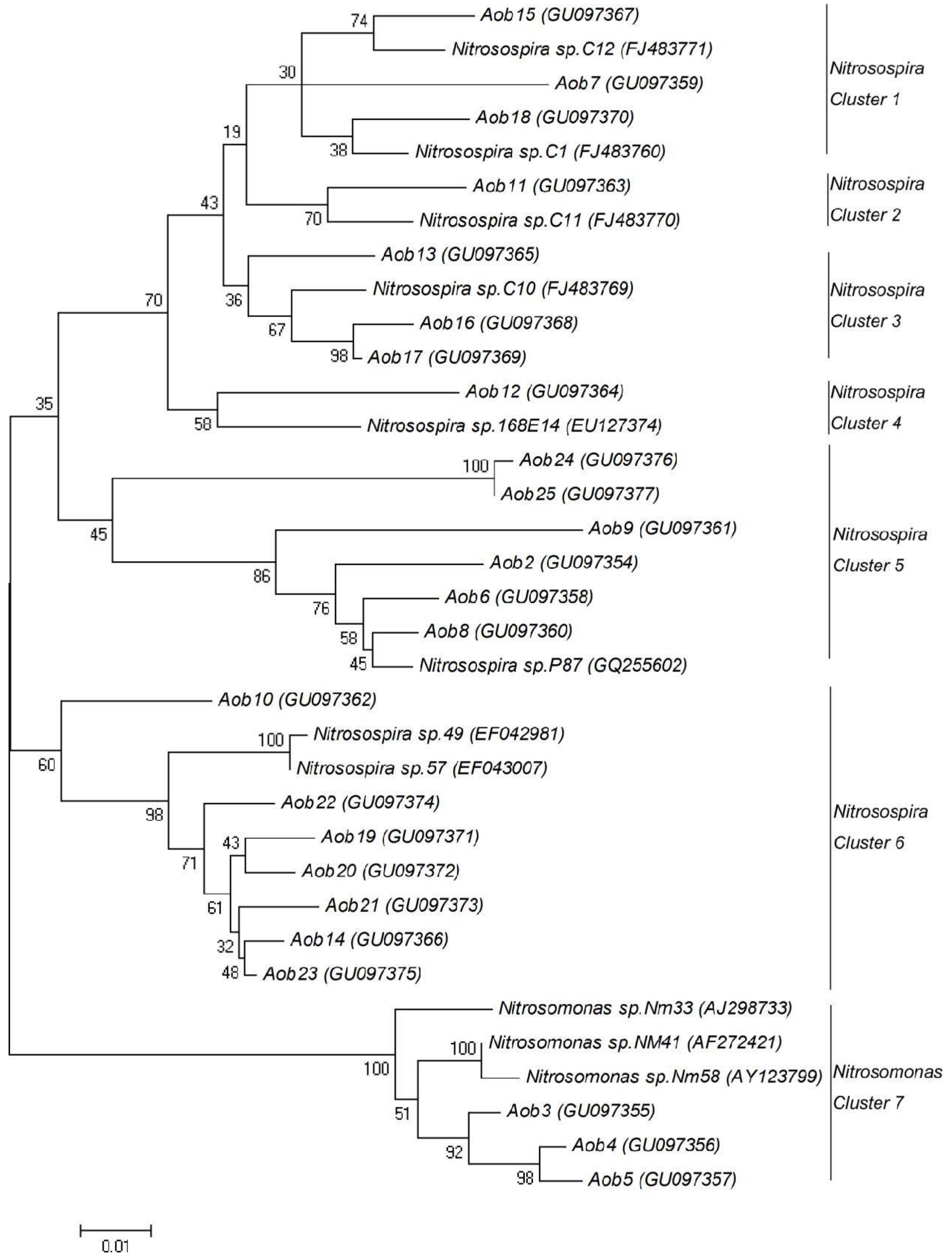

Figure 4. Phylogenetic analyses of ammonia monooxygenase genes recovered from DGGE bands of paddy soil under different management. The bar indicates an estimated 1% sequence divergence. Bootstrap values are given for 1000 replicate trees.

shows that there was a positive correlation with N (R^2=0.85, P< 0.001) and C (R^2=0.85, P<0.001), whereas negative correlation C/N (R^2=0.77, P< 0.001). In addition, an analysis of the DGGE patterns using CCA showed that C/N, C, and N had the strongest influence on diversity of the AOB community. These results from our study indicates that soil C and N content influence the abundance and community structure of AOB bacterial. This is not surprising as it may be expected that higher organic N contents favour the growth of ammonia oxidizers. Higher total C and N content in the organic soils in our study seems to be driven by application of organic fertilizers.

Phylogenetic analysis in the our study based on bacterial AOB identified that *Nitrosopira* species were dominated in organic paddy soil, These results differ from a previous study in Philippine rice soil (Nicolaisen et al., 2004), but were consistent with other study (Chen et al., 2008). Moreover, in the Ikenaga study (Ikenaga et al., 2003), all the AOB detected from the rice roots also belonged to *Nitrosospira*. Here, *Nitrosospira* species may also be the predominant AOB in this experimental paddy field, which was in agreement with a previous study in paddy soil (Bowatte et al., 2006a). Both culture-dependent and independent techniques have suggested the predominance of *Nitrosospira* over *Nitrosomonas* in AOB communities in terrestrial ecosystems (Kowalchuk et al., 2000; Avrahami et al., 2003), whereas

Nitrosomonas were often detected in high N environments, such as wastewater treatment plants (Geets et al., 2006). Interestingly, we found that *Nitrosospira* were abundant in organic management paddy soils which the content of nitrogen was higher. These results were inconsistent with earlier studies which show that the predominance of *Nitrosomonas* in rice soils was due to high N application (Nicolaisen et al., 2004). The relative importance of each of these factors was unknown, although pure-culture studies indicated that ammonia oxidizer cell numbers remain stable throughout a range of environmental stresses (Johnstone and Jones, 1988; Jones and Morita, 1985). It had showed that different environmental parameters like the content of soil organic carbon, total nitrogen and altitude could affect the diversity of soil bacteria (Zhang et al., 2006). Total C, N, NH_4 and C/N were the major fact impacted on the AOB community by CCA analysis in our study indicated that multiple factors resulted from different management might play a part in common.

This study includes analysis of community structure and abundance of AOB in different organic management. However, considering that this work was based on PCR amplification using total DNA extracted from soil, it could not be confirmed that ammonia monooxygenase genes were actually expressed. Therefore, in future studies, it is necessary to investigate activity of ammonia oxidizers bacteria in paddy soil under different regime by analyzing soil RNA. Moreover, different period of rice growth should be considered to better understand the variation of ammonia oxidizers bacteria in rhizosphere and bulk paddy soil.

ACKNOWLEDGEMENTS

This research was funded by Key Projects of Science and Technology Research (108141, Ministry of Education of the People's Republic of China) and Shanghai international cooperation research program (072307011, Shanghai Science and Technology Committee). The research was also supported by University Innovation Team of Landscape Architecture from Yunnan Province, Southwest Forest University (23002802), Ornamental Plant and Horticulture key disciplines of Yunnan Province, Southwest Forest University (500017) and Ornamental Plant and Horticulture key lab of Yunnan Province, Southwest Forest University (000703)

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