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Short-term effects of biomass burning on soil ammonia-oxidizing bacteria and ammonia-oxidizing archaea

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Biomass burning, a form of disturbance in soil ecosystem, is frequently carried out in farmland after harvest in China, which has potential influence on soil biological properties. To study the short-term effects of biomass burning on soil ammonia-oxidizing bacteria (AOB) and archaea (AOA), a field investigation utilizing poplar branch (PB) and corn stalk (CS) as feedstock for experimental biomass burning was conducted in farmland in Yanqing County, Beijing, China (116°12'E, 40°25'N). The shifts in abundance and community composition of AOB and AOA were investigated utilizing real-time polymerase chain reaction (PCR), cloning and sequencing approaches based on *amoA* genes. Striking increase in potential ammonia oxidation rate and abundance of soil AOA and AOB after fire were observed, as well as community composition of AOA. For AOB, little changes in diversity but significant shifts in relative abundance of different Clusters were observed after fire. Phylogenetic analyses of the *amoA* gene fragments showed that all AOB clones from different treatments were affiliated with *Nitrosospira* species and grouped into 5 clusters (*3a, 3b, 3c, A* and *B*). Most of the clones were almost evenly distributed in Cluster 3a, 3b, A and B before burning. However, domination in the clones by Cluster 3a was observed after fire. All AOA clones fell within Soil cluster (soil origin). Although Group 1.1b was found dominant in the obtained AOA clones in all the treatments, the relative abundance of clones belonging to Group 1.1b decreased after fire. These findings could be fundamental to improve our understanding of the effect of biomass burning on soil AOB and AOA in agricultural ecosystems.

Key words: Biomass burning, soil ammonia-oxidizing bacteria, soil ammonia-oxidizing archaea, ammonia oxidation.

INTRODUCTION

Farmland biomass burning is a global phenomenon. To prevent the spread of crop diseases, shift cultivation and the ease disposal of residues, burning of crop residues in farmland is commonly practized by farmers (Streets and Waldhoff, 2000; Isichei et al., 1995). In China, burning of crop residues is a frequent, widely distributed phenomenon, especially in rural areas. Soil chemistry and biogeochemical cycles are substantially affected by biomass burning (Garcia-Villaraco Velasco et al., 2009; Jacobson, 2004). The majority of the reported researches con-

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Abbreviations: AOB, Ammonia-oxidizing bacteria; **AOA,** ammonia-oxidizing archaea; ***AOM,*** ammonia monooxygenase subunits; **PB,** poplar branches; **CS,** corn stalks; **Corg,** organic carbon; **PCR,** polymerase chain reaction; **BSA,** bovine serum albumin; **BLAST,** Basic Allignment Search Tool; RDA, redundancy analysis.

cerning fire-mediated changes in soil ecosystems have focused on soil chemical (Almendros et al., 1990) and biological properties (Staddon et al., 1998; Garcia-Villaraco Velasco et al., 2009). The effects of fire on soil properties depend firstly upon fire severity, which consists of intensity and duration of the fire. The pH, concentration of organic carbon, total and mineral nitrogen of surface soil were observed sharply increased after burning (Rashid, 1987). The increased soil pH is attributed to organic acids denaturation. Decreases in soil organic matter are also observed caused by distillation and oxidation (Giovannini et al., 1988). The response of soil organic nitrogen to fire is associated to the fire severity too (Certini, 2005). A considerable part of soil organic nitrogen is converted to inorganic forms (ammonium and nitrate) during fire. Striking increase in inorganic nitrogen were observes in the surface soil (Weston and Attiwill, 1990). Ammonium is a direct product of the combustion. Covington and Sackett (1992) attributed the increase in nitrate to microbial nitrification. The immediate effect of fire on soil microorganisms is a reduction of their biomass by soil heating (Prieto-Fernández et al., 1998). However, increased microbial biomass was also found after fire (Andersson et al., 2004). Interestingly, previous studies have illustra-ted that the short-term effects of fire are more significant than long-term effects. Post-fire climatic conditions showed a greater influence than biomass burning on soil microorganisms in long term investigations (Andersson et al., 2004; Garcia-Villaraco Velasco et al., 2009). Additionally, D'Ascoli et al. (2005) observed the shifts of microbial functional diversity only occurred in the first week after burning. Although there are various studies concerning the impacts of biomass burning, limited studies have occurred on the effects of biomass burning on soil nitrifying microorganisms. Therefore, the short-term effects of biomass burning on ammonia oxidizers were investigated in this study.

Nitrification, the microbial oxidation of ammonia to nitrate, occurs in a wide variety of environments and is an important process in the global nitrogen cycle. The oxidation of ammonia is considered to be the rate-limiting step of nitrification (Prosser and Embley, 2002). A wide variety of researches have focused on ammonia-oxidizing microorganisms because of their essential roles in nitrogen transformation (Kowalchuk and Stephen, 2001). Until recently, chemolitho-autotrophic ammonia-oxidizing bacteria (AOB) are considered to be responsible for the ammonia oxidation in soil (Mertens et al., 2009). However, more recent discoveries based on metagenomic and cultivation methods have revealed that archaea belonging to the *Thaumarchaeota* lineage can also perform ammonia oxidation (Brochier-Armanet et al., 2008; Spang et al., 2010). What remains less certain is the contribution to nitrification of ammonia-oxidizing archaea (AOA) relative to AOB. Autotrophic AOB affiliated with the β- and γ-*proteobacteria* have been considered the major contributors to biological ammonia oxidation in soil (Purkhold et al., 2000; Mertens et al., 2009). However, metagenomic studies revealed that AOA just like AOB contain the key functional enzyme ammonia monooxygenase subunits (*AOM*) in ammonia oxidation (Könneke et al., 2005; Treusch et al., 2005). *AOM* is encoded by the genes *amoA*, *B*, and *C* in AOB and AOA (Nicol and Schleper 2006). Both AOB and AOA are widely distributed in the majority of terrestrial ecosystems, including agricultural, grassland, forest and alpine soils (Gubry-Rangin et al., 2010). Quantification of respective *amoA* genes demonstrated that AOA are more abundant than AOB in many environments (Leininger et al., 2006). However, greater relative abundance of AOB was also observed in particular conditions (Mertens et al., 2009). Temperature, nutrient condition and soil pH are believed to influence the distribution and activity of archaeal and bacterial ammonia oxidizers (Nicol et al., 2008; Nugroho et al., 2006; Phillips et al., 2000). Previous studies have shown that the structure of both bacterial and archaeal communities significantly changed with soil pH (Nicol et al., 2008). Ammonia-oxidizing β-proteobacteria showed a trend showing domination by *Nitrosospira cluster 3* in neutral pH agricultural plots towards *Nitrosospira cluster 2* in acidic soils (Stephen et al., 1996). Additionally, archaeal *amoA* gene and transcript abundance decreased with increasing soil pH (4.5-7.5), while bacterial *amoA* gene abundance was generally lower and transcripts increased with increasing pH (Nicol et al., 2008). With regard to temperature, little evidence of changes in relative abundance or transcriptional activity of AOB with different incubation temperature (10-30°C) was observed, while AOA 16S rRNA and *amoA* genes provided strong evidence of changes in community structure of active AOA (Tourna et al., 2008). Although various studies have focused on ecology of AOB and AOA, shifts in AOB and AOA community composition and abundance in response to biomass burning have never been investigated.

Here, we report the responses of AOB and AOA after experimental biomass burning disturbance. Because of the higher significance of short-term impacts than long-term effects, we focused on the short-term effect of biomass burning on AOA and AOB. The numbers of *amoA* gene copies and the *amoA* community composition for both AOB and AOA in response to fire were measured. Additionally, to examine effects of biomass burning on soil nitrogen dynamics the soil potential ammonia oxidetion were measured too.

MATERIALS AND METHODS

Study site and combusted materials

The experimental biomass burning was conducted in farmland in Yanqing County, Beijing, China (116°12'E, 40°25'N) on July, 2011. Corn is normally cultivated in the farm land. Poplar branches (PB) and corn stalks (CS) which were gotten from the same farm were used as burning materials for experimental fires. As experimental material, the PB and CS cover a suite of taxonomy: woody/non-

woody pair, as well as a C_3/C_4 pair, and the chemical structure and major organic components are different from each other (for example, content of cellulose, hemicellulose and lignins). Due to the peak combustion temperature and duration, CS and PB burning represent different fire severity. Additionally, burning of CS is a typical local agriculture practice.

Experimental design and sampling methodology

Temperature gradients along with soil depth were produced during fire. Soil temperatures at 5 cm depth rarely exceed 150°C and the lower layer of the mineral soil remains cool during fire (DeBano, 2000). Therefore the top 5 cm soil was investigated in this study. Nine 2 m^2 treatment plots were established with each treatment plot surrounded by a 2-m buffer zone. Within each plot, soils from the 0-5 cm depth were collected using a 5 cm diameter auger before burning as initial samples from four different locations and combined to yield one mixed sample. The CS burning, PB burning and control (unburned) treatments were randomly distributed within the plots with three replicates each. After initial sampling, both CS and PB, which were nearly evenly distributed in the plots, were burned with an average value of 2.5 $kg \cdot m^{-2}$ (dry weight). The soils from 0-5 cm depth were collected from each experimental plot as the initial samples at three and nine days after burning. Each sample was placed in a sterile plastic bag, sealed and placed on ice when transported to the laboratory.

The initial samples collected from the control plots, CS burning plots and PB burning plots before burning were respectively labeled as CK0, CS0 and PB0. The samples collected from the control plots, CS burning plots and PB burning plots three days after burning were respectively labeled as CK3, CS3 and PB3 (3^{rd}-day samples). The samples collected from the control plots, CS burning plots and PB burning plots nine days after burning were respectively labeled as CK9, CS9 and PB9 (9^{th}-day samples). Each mixed soil sample was passed through a 2.0-mm sieve and equally divided into two parts after sampling. One part was stored at 4°C for soil chemical characteristics and potential ammonia oxidization analysis. The other part was stored at -80°C for total DNA extraction.

Soil chemical characteristics and potential ammonia oxidation

All samples were analyzed to determine their chemical characteristics and potential ammonia oxidation activity. The chemical characteristics of total organic carbon (Corg), total nitrogen (N), nitrate (NO_3^--N), ammonium ($NH4^+$-N) and pH of the soil samples were tested according to Carter's method (Carter 1993). Potential ammonia oxidation rate, an independent estimate of the populations of active ammonia oxidizers, was measured according to Kurola et al. (2005) with minor modifications. Briefly, 5.0 g of fresh soil was added to 50 ml centrifuge tubes containing 20 ml phosphate buffer solution (PBS) ($g \cdot l^{-1}$: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 0.2; NaH_2PO_4, 0.2; pH 7.1) with 1 mM $(NH_4)_2SO_4$. Potassium chlorate with 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 wavelength of 545 nm with N-(1-naphthyl) ethylenediamine dihydrochloride.

Extraction and preparation of soil DNA

For each sample, 0.5 g of fresh soil was used to extract genomic DNA using a FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA) according to the manufacturer's protocol. The extracted soil DNA of the three replicates of the same treatment with the same sampling time was respectively dissolved in a 30 µl TE buffer and then carefully mixed together. After that, the extracted DNA was quantified by Nanodrop® ND-1000 UV-Vis Spectrophotometer (USA) and stored at -20°C until further use.

Real-time polymerase chain reaction (PCR) assay

The real-time PCR amplification was carried out in Mx 3005P QPCR thermocycler (Stratagene, USA). And all data analysis was performed with Mx Pro QPCR software (version 3.0, Stratagene, USA). The DNA extracts were 10-fold diluted and used as template with a final content of 1–10 ng in each reaction mixture, and bovine serum albumin (BSA) was added. Bacterial 16S rRNA gene, bacterial *amoA* gene and archaeal *amoA* gene were respectively, quantified using the primer pair F357/R518 (Zwart et al., 2003), A189/*amoA*-2R' (Okano et al., 2004) and Arch-*amoA*F/Arch-*amoA*R (Francis et al., 2005). The reaction mixture contained 1× Master Mix (Fermentas, USA), 400 nM of each primer, 8% (w/v) BSA (New England Biolabs), diluted DNA extractions and double-distilled H_2O to a final volume of 25 uL. Real-time PCR assay was carried out in triplicate with the protocol for each target group as shown in Table 1.

Standard curve for real-time PCR were completed according to He et al. (2007). To make the 16S rRNA gene standard, the almost full-length 16S rRNA gene was PCR-amplified from extracted DNA with the primers 27F/1492R (Dees and Ghiorse, 2001) and gel-purified with TaKaRa Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa). Then the purified PCR products were ligated into the pGEM-T Easy Vector (Promega, Madison, WI), and the resulting ligation products were utilized to transform *Escherichia coli* DH5α competent cells following the instructions of the manufacturer. After re-amplification with the vector-specific primers M13F and M13R, the positive clones were selected to extract plasmid DNA with a BioMed Mini Plasmid Kit (BioDev-tech, Beijing, China). The plasmid DNA concentration was determined on a Nanodrop® ND-1000 UV-Vis Spectrophotometer (USA) and the copy numbers of the 16S rRNA gene were calculated directly from the concentration of the extracted plasmid DNA. Ten-fold serial dilutions of a known copy number of the plasmid DNA were subjected to real-time PCR assay in triplicate to generate an external standard curve. For the same purpose, bacterial and archaeal *amoA* gene were respectively, PCR-amplified from extracted DNA with the assay primers (Table 1) and cloned. The resulting clones containing a proper insert of the target gene were selected to extract plasmid DNA and then to make standard curves as described for the bacterial 16S rRNA gene.

Construction of *amoA* gene fragment libraries of AOB and AOA and statistical analyses

For the construction of clone library, DNA extracts from the soil with the same treatment sampled three and nine days after burning (for example, CK3 and CK9) were mixed and served as the template of PCR. Polymerase chain reaction products of bacterial and archaeal *amoA* genes amplified with the primers for the real-time PCR assays were purified and cloned as described above. Colonies were randomly chosen for PCR re-amplification using M13 primers. The right-inserted recombinant clones were sequenced. The sequences obtained were examined for chimeras with Mallard software (http://www.bioinformatics-toolkit.org) and aligned with Basic Allignment Search Tool (BLAST) similarity search program in order to find the most similar sequences from the GenBank database. The best matching sequences were obtained from the database and analyzed with CLUSTAL X (Thompson et al., 1994). Phylogenetic analyses of bacterial and archaeal *amoA* genes were conducted using MEGA version 4.0 and the neighbour-joining tree was constructed using Kimura 2-parameter distance with 1000 replicates to produce bootstrap values (Tamura et al., 2007).

Table 1. Primers and PCR conditions used in this study.

Target group	Primer	Sequence (5'-3')	Length of amplicon (bp)	Thermal profile	Reference
16S rRNA	27F	AGAGTTTGATCMTGGCTCAG	1400	94°C for 5 min, followed by 35 cycles of 45 s at 94°C, 45 s at 56°C and 90 s at 72°C, and 10 min at 72°C for the last cycle	(Suzuki et al., 2000)
	1492R	GGWTACCTTGTTACGACTT			
	F357	CCTACGGGAGGCAGCAG	160	94°C for 5 min, followed by 30 cycles of 45 s at 94°C, 45 s at 50°C, and 45 s at 72°C, and 10 min at 72°C for the last cycle	(Zwart et al., 2003)
	R518	ATTACCGCGGCTGCTGG			
AOB amoA	A189	GGHGACTCCCAYTTCTGG	670	95°C for 5 min, followed by 40 cycles of 60 s at 94°C, 45 s at 57°C and 45 s at 72°C, and 10 min at 72°C for the last cycle	(Okano et al., 2004)
	amoA-2R'	CCTCKGSAAAGCCTTCTTC			
AOA amoA	Arch-amoAF	STAATGGTCTGGCTTAGACG	635	94°C for 2 min followed by 40 cycles of 45 s at 94°C, 1 min at 53°C, 45 s at 68°C, plate read at 83°C for Q-PCR.	(He et al., 2007)
	Arch-amoAR	GCGGCCATCCATCTGTATGT			

Table 2 Chemical properties (Corg, N, NO_3^--N, NH_4^+-N and pH) of the soil samples. Values are mean ± standard error (n=3). Treatment: fire-free (CK), CS burning (CS), and PB burning (PB).

Parameter	Corg (g/kg)	N (g/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	pH
CK0	2.03±0.11	6.57±0.23	5.38±0.26	25.58±2.05	7.13±0.04
CK3	2.16±0.23	5.85±0.19	6.02±0.13	26.93±1.45	7.32±0.06
CK9	1.85±0.25	6.32±0.22	5.72±0.16	28.85±0.36	7.21±0.05
CS0	2.07±0.14	6.98±0.41	6.09±0.21	30.03±1.66	7.17±0.06
CS3	1.86±0.09	6.37±0.25	6.17±0.14	38.55±1.58	7.75±0.03
CS9	1.88±0.31	7.06±0.41	6.18±0.16	42.03±2.08	7.94±0.07
PB0	1.98±0.18	5.88±0.21	5.81±0.13	27.75±1.66	7.21±0.02
PB3	1.78±0.08	6.06±0.33	5.71±0.11	33.56±2.48	7.88±0.03
PB9	1.84±0.19	6.01±0.25	6.06±0.29	36.61±3.44	7.92±0.06

Rarefaction analysis and two additional nonparametric richness estimators, Chao1, Simpson and the Shannon diversity index were performed in DOTUR based on a 3% gene sequence distance cutoff (Schloss and Handelsman 2005). The relationships between physicochemical characteristics and those diversity parameters and abundance of *amoA* gene for AOA and AOB were determined through Redundancy analysis (RDA) utilizing CANOCO 4.5.

Sequence accession numbers

All *amoA* gene sequences have been deposited in the GenBank nucleotide sequence database under Accession numbers JQ735231 to JQ735264 affiliated with AOA and JQ735265 to JQ735440 affiliated with AOB.

RESULTS

Soil chemical properties and potential ammonia oxidation

Increase in soil ammonium and pH were observed after fire (Table 2). Decrease in organic matter content while slight increase in soil nitrate concentration was found (Table 2). Soil total nitrogen was little changed after fire. Potential ammonia oxidation rates, which provide an independent estimate of the abundance of ammonia oxidizers, were distinctly increased in 9th-day samples (Figure 1). With regard to the potential ammonia oxidation rate in the 3rd-day samples, increase and decrease were respectively, observed after CS and PB burning (Figure 1).

Abundance of total bacteria in the soil

The bacterial 16S rRNA gene abundance, measured by real-time PCR, ranged from 3.73×10^9 to

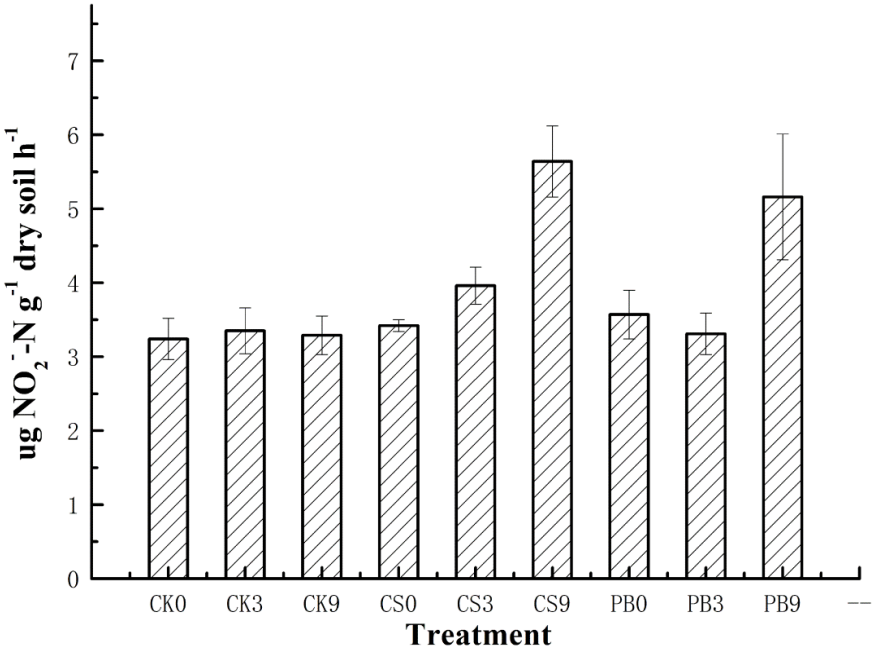

Figure 1. Potential ammonia oxidization rates of the samples with different treatments. Treatment: fire-free (CK0), CS burning (CS), and PB burning (PB).

6.26×10^9 copies g (dry weight) of $soil^{-1}$ (Figure 2a). The 16S rRNA gene abundance was highest in CS9 and lowest in PB3. For unaffected soil samples, the 16S rRNA gene abundance did not differ significantly from each other (Figure 2a). However, the 16S rRNA gene abundance decreased in the 3rd-day fire affected samples (Figure 2a). Nine days after burning, the abundance of total bacteria recovered in CS treatment plots. However, no significant recovery was found in PB treatment plots.

Abundance of AOB and AOA in the soil

The AOB and AOA *amoA* gene abundance, measured by real-time PCR respectively, ranged from 4.34×10^8 to 2.30×10^9 and 2.53×10^6 to 5.37×10^6 copies g (dry weight) of $soil^{-1}$ (Figures 2b and 2c). The AOB and AOA *amoA* gene copy numbers in the unaffected soil samples were relatively constant (Figures 2b and 2c). Increase in AOA *amoA* gene abundance was observed in fire affected samples (Figure 2). With regard to AOB, increase and decrease in *amoA* gene abundance was respectively, observed in 3rd-day CS and PB fires affected samples. Striking increase in AOB and AOA *amoA* gene abundance was found in 9th-day fires affected samples. Additionally, significantly higher abundance of AOA than AOB was found in the samples.

Community analysis of AOB and AOA

Great shifts in the abundances of AOB and AOA after fire were illustrated by the results based on real-time PCR. Therefore, we prompted our investigation into the composition of AOB and AOA populations using the *amoA* gene clone libraries. Overall, six clone libraries including 3 bacterial *amoA* gene clone libraries and 3 archaeal *amoA* gene clone libraries were generated. 60 clones were sequenced in each archaeal *amoA* gene clone library and recovered 6, 11 and 11 OTUs (based on a 3% cutoff) from CK, CS and PB treatment plots, respectively. 115 clones were sequenced in each bacterial *amoA* gene clone library and recovered 59, 65 and 59 OTUs (based on a 3% cutoff) from CK, CS and PB treatment plots, respectively (Figure 3).

The Chao1 diversity estimator, a conservative one for species richness, showed higher variability with AOA than AOB (Table 3) at a 3% identity difference. The Shannon index, which takes into account both species richness and evenness, also showed a similar variability difference between AOA and AOB at a 3% identity difference (Table 3). Both the Chao1 diversity estimator and the Shannon diversity index indicated that AOA diversity was significantly affected by burning.

The obtained sequences were subjected to BLAST search in the GenBank database, which confirmed that all sequenced clones represented *amoA*-like sequences. Phylogenetic analyses of bacterial *amoA* gene sequences at the 3% nucleotide cut-offs showed that all AOB sequences were affiliated with *Nitrosospira* species, and were grouped into *Cluster 3a*, *3b*, *3c*, *A* and *B* (Figure 4). The definition of the *amoA Clusters 3a*, *3b* and *3c* was mainly based on the previous study (He et al., 2007; Avrahami and Conrad, 2003). The *Cluster A* and *B* was tentatively defined in this study in order to classify these sequences, which may be redefined in future when more

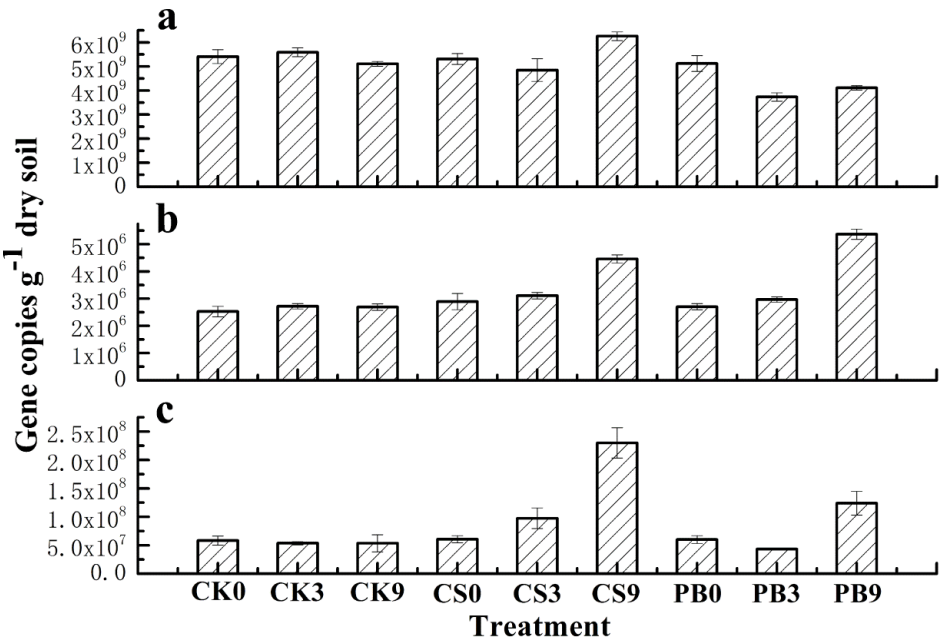

Figure 2. Gene copy numbers in initial, 3rd-day and 9th-day soil samples with different treatment. *a*, bacterial 16S rRNA gene copy numbers; *b*, archaeal *amoA* gene copy numbers; *c*, bacterial *amoA* gene copy numbers. Treatment: Fire-free (CK0), CS burning (CS), and PB burning (PB).

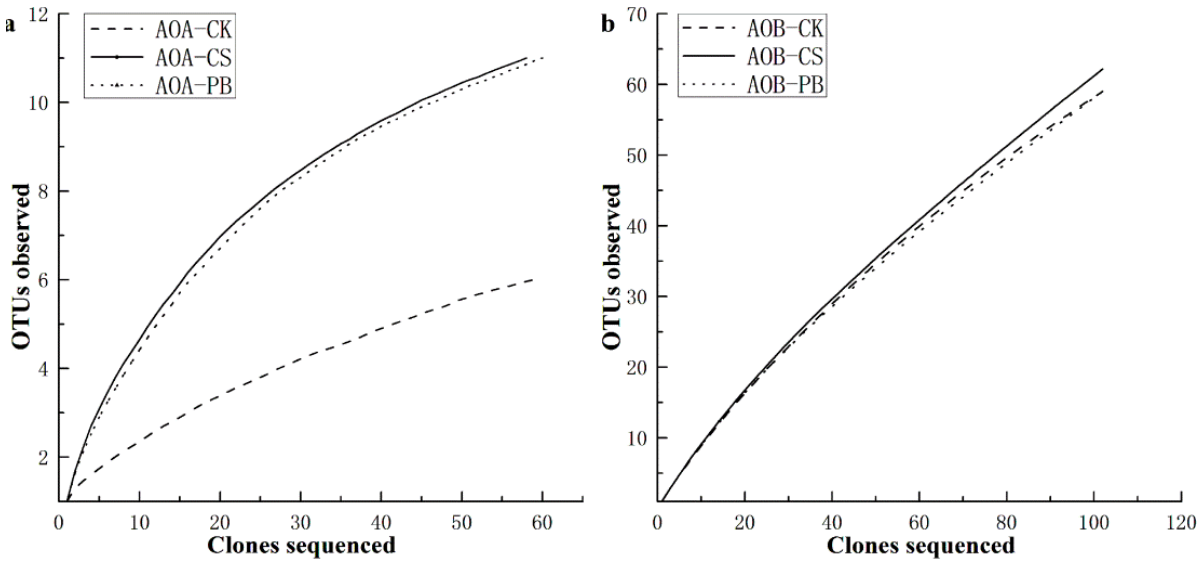

Figure 3. Rarefaction curves indicating archaeal (*a*) and bacterial (*b*) *amoA* richness within clone libraries from DOTUR analysis using furthest neighbor assignment algorithm with AOA and AOB *amoA* gene sequences. OTUs were defined as groups of sequences differing by ≤3% at the DNA level. Treatment: fire-free (CK), CS burning (CS), and PB burning (PB).

pure cultures and clones are available. Most of the sequences recovered from CK plots were evenly distributed in *cluster 3a*, *3b*, *A* and *B* while only a few clones fell into *Cluster 3c* before burning. However, after CS and PB burning, a trend showing increase in *Cluster 3a* and decrease in *Cluster A* and *Cluster B* in *Nitrosospira* clones were detected. Therefore, a significantly higher proportion of the obtained clones appeared in *Cluster 3a*. Compared to CK, substantial decrease in abundance of the clones affiliated with *Cluster A* and *B* were observed after fire (Figure 4).

Phylogenetic analysis of archaeal *amoA* gene showed

Table 3 Diversity and richness indices of the amoA gene sequences from the clone libraries. Treatment: fire-free (CK), CS burning (CS), and PB burning (PB).

Sample	Clone number		OTUs		Simpson index		Shannon index		Chao1 index	
	AOA	AOB	AOA	AOB	AOA	AOB	AOA	AOB	AOA	AOB
CK	59	102	6	59	0.72	0.04	0.64	3.34	7.5	55.3
CS	58	108	11	65	0.31	0.04	1.65	3.31	11	62.46
PB	60	102	11	59	0.37	0.04	1.53	3.33	14	60.09

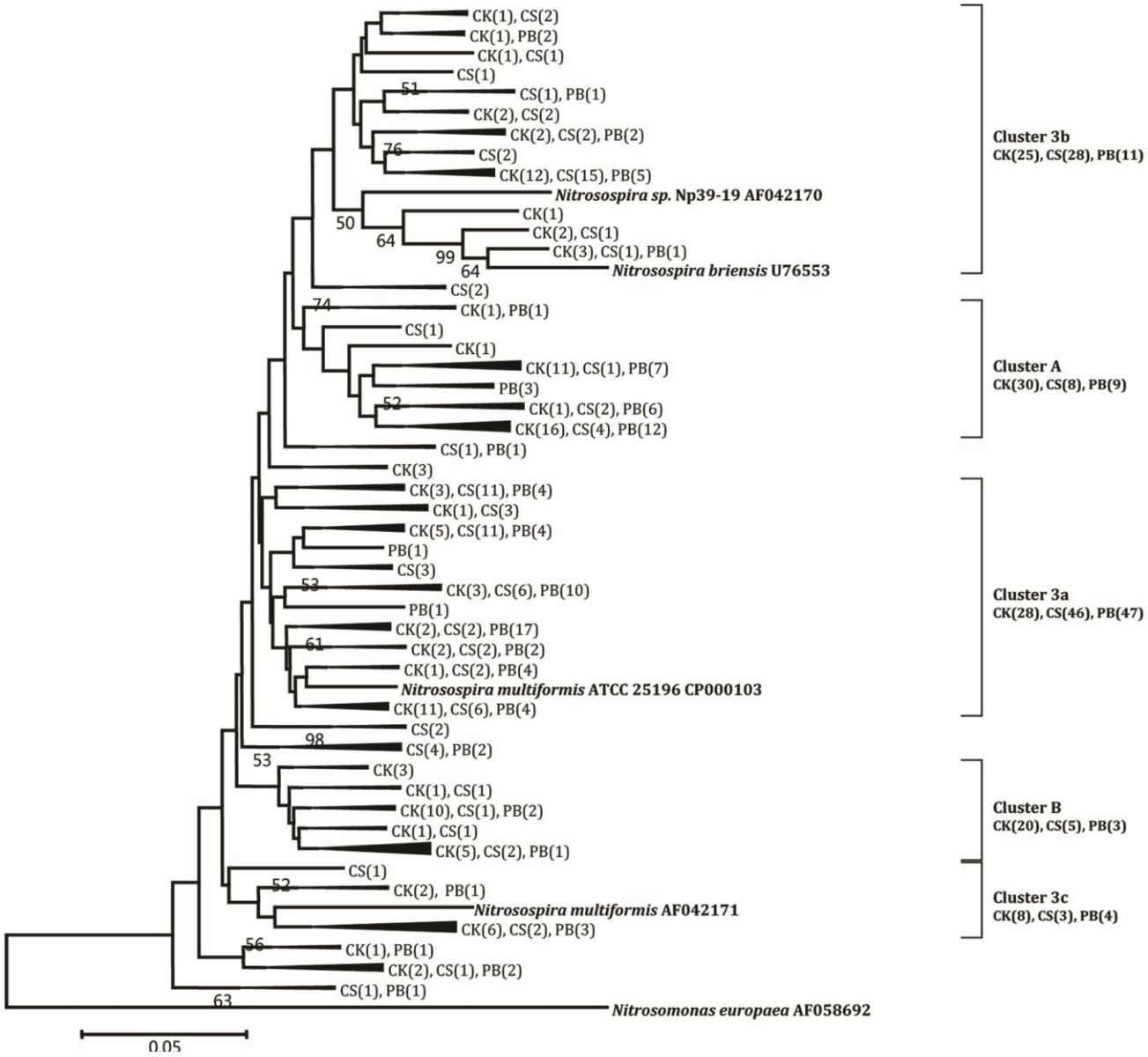

Figure 4. Phylogenetic relationships among bacterial *amoA* sequences retrieved from CK, CS burning, and PB burning treatments. The Arabic numbers in brackets following each clone mean the total number of clones represented by the clone in each library. Bootstrap values (≥50%) are indicated at branch points. The scale bar represents 5% estimated sequence divergence. Treatment: Fire-free (CK), CS burning (CS), and PB burning (PB).

that all sequences fell into *Soil cluster* (soil origin) (Figure 5). Additionally, 84.7, 63.8 and 56.3% of the clones respectively recovered from CK, CS and PB plots fell into *Group 1.1b* (Figure 5) which was demonstrated to be

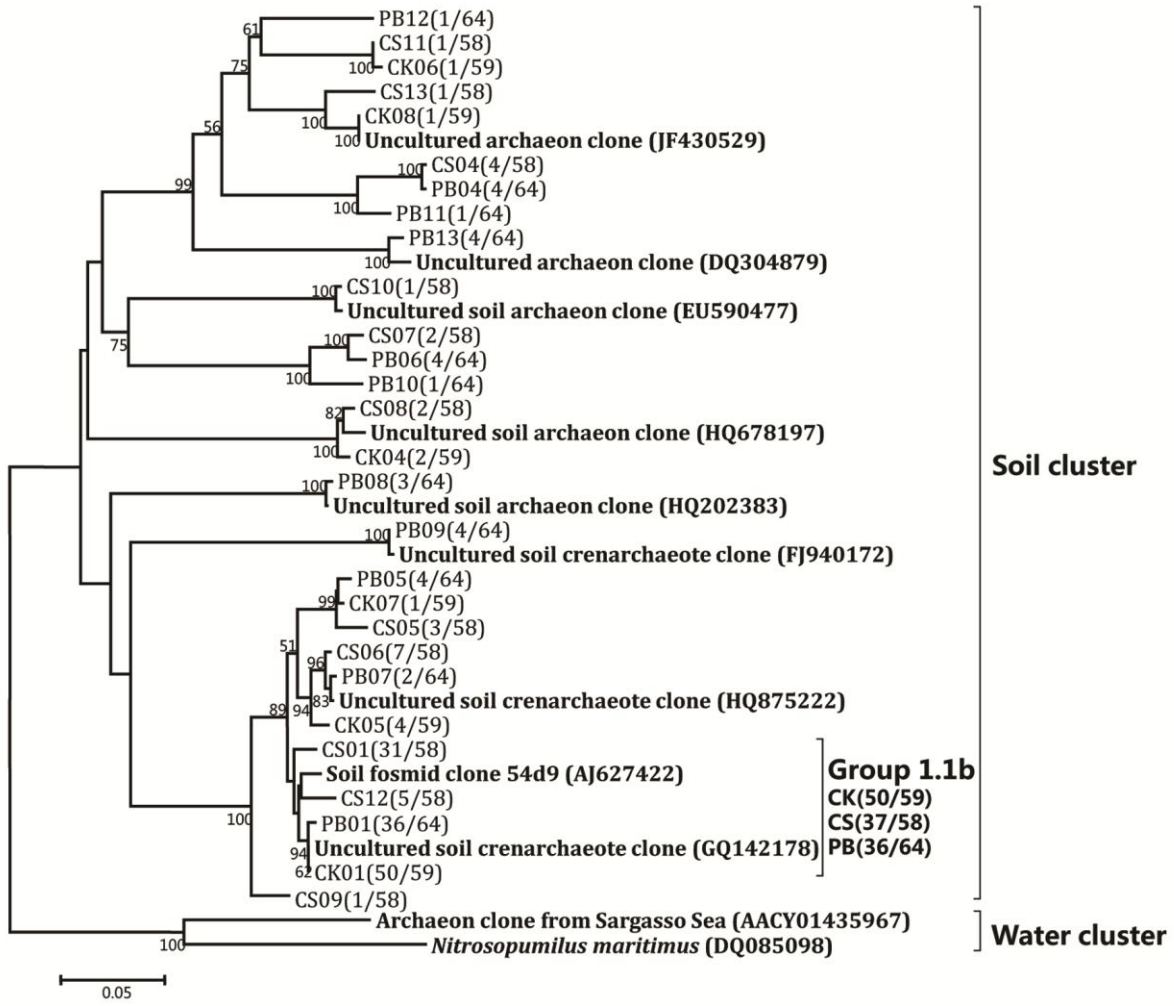

Figure 5. Phylogenetic relationships among archaeal *amoA* sequences retrieved from CK, CS burning, and PB burning treatments. The Arabic numbers in brackets following each clone mean the relative abundance of clones represented by the clone in each library. Bootstrap values (≥50%) are indicated at branch points. The scale bar represents 5% estimated sequence divergence. Treatment: Fire-free (CK), CS burning (CS), and PB burning (PB).

dominant in AOA communities in most soil environments (Schleper et al., 2005). After fire, a trend that decreases in relative abundance of archaeal species affiliating to *Group 1.1b* was observed.

Environmental factors to affect the diversity of the AOA and AOB

Correlations of the bacterial and archaeal *amoA* assemblages with environmental parameters analyzed by RDA indicated that the three samples were significantly separated via RDA axis 1 and 2 (Figure 6). Both the bacterial and archaeal RDA ordination plots indicated that the concentration of total nitrogen and inorganic nitrogen could contribute to the diversity of AOA and AOB after CS burning treatment. In addition, the pH value could contribute to the diversity of AOA and AOB after PB burning treatment (Figure 6).

DISCUSSION

Biomass burning has been well demonstrated that it has substantial impacts on soil chemical and biological properties (Certini, 2005; Macdonald et al., 2009). In this study, the short-term effects of biomass burning on abundance, activity, diversity and community composition of the AOB and AOA in soil were studied.

The chemical properties shifted in present study are attributable to soil heating and external inputs of chemical from biomass burning residues. In a previous study on the immediate effects of biomass burning on soil chemical and biological properties (unpublished), we concluded that the decrease in the soil organic matter is mainly due to distillation and decomposition. The increase in soil inorganic nitrogen should be attributable to the pyrolysis of macromolecular materials contenting nitrogen and external inputs of cellulosic biomass pyrolysis. The increase in pH might be attributable to the release of the

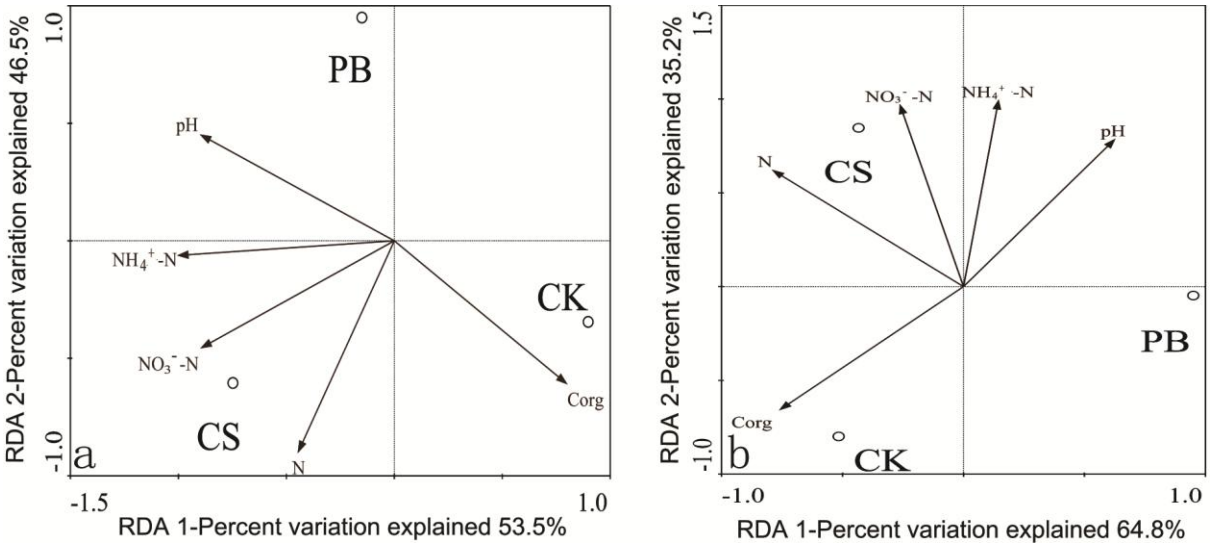

Figure 6. RDA ordination plots for the environmental factors and the ammonia-oxidizing bacterial and archaeal lineages representing by amoA gene sequences analyzing from the CANOCO software. Treatment: fire-free (CK), CS burning (CS), and PB burning (PB).

free acids and alkaline cations (K, Ca, Na and Mg) in the process of burning. Significant shifts in potential ammonia oxidation rate, bacterial 16S rRNA gene copy numbers, *amoA* gene copy numbers and community composition of AOA and AOB were observed after biomass burning. In addition, microbial diversity of AOA was greatly increased after burning while the microbial diversity of AOB was not apparently affected by the treatments (Table 3).

A significant and positive correlation between potential ammonia oxidation rate and the abundance of AOB were observed which indicated that AOB in this soil might contribute greatly to the ammonia oxidation activity. The dramatic increase in rates of potential ammonia oxidation in burning affected plots indicated increased activity of ammonia oxidizers. 16S rRNA genes and *amoA* genes were quantified in the present study. Contrary to previous observations (He et al., 2007; Leininger et al., 2006), AOB strikingly outnumbered AOA in the soil. Similar results were also observed in previous studies (Di et al., 2009; Bernhard et al., 2010). This might be attributable to the high-nutrient condition of the soil samples. Certain AOA has been demonstrated to have dramatically higher ammonia availability than known AOB in oligotrophic environments (Martens-Habbena et al., 2009). Additionally, some Crenarchaeota have been found be able to grow heterotrophically or mixotrophically (Ouverney and Fuhrman, 2000). However, AOB undergo a strict autotrophic ammonia-oxidation mechanism (Kowalchuk and Stephen, 2001). Thus AOA gain the higher adaptability and competitive advantage in oligotrophic conditions. Nonetheless, certain archaeal ammonia oxidizing activity was inhibited by high ammonia concentration (Hatzenpichler et al., 2008; Martens-Habbena et al., 2009). By contrast, the high ammonia concentration in soil might have dramatically benefited the growth of AOB. Thus AOB grows better than AOA in this condition.

The abundance of total bacteria in fire affected samples decreased 3 days after burning which should be attributable to the direct sterilization by soil heating. The decrease trend in microbial abundance has been frequently observed in precious studies (Prieto-Fernández et al., 1998). Interestingly, restoration in the abundance of total bacteria was observed 9 days after CS burning. This might be due to the decreased microbial competition. Additionally, the striking increase in the abundance of AOA and AOB in CS9 samples partly contributed to the restoration.

With regard to AOA abundance, slight but dramatic increase in 3rd-day samples and 9th-day samples was respectively observed which indicated high resistence of AOA to fire. The explanation to the resistence is due to their thermophilic characters. Until recently, all cultivable Crenarchaeota are thermophiles (Könneke et al., 2005). Additionally, the favorable impact of fire on AOA is attributable to the increased bioavailability of nitrogen, for example, ammonia. In the process of burning, parts of soil organic carbon and nitrogen were transformed into inorganic forms, which dramatically benefited the chemoautotrophic AOA. The explanation to the response of AOB was similar to that of AOA. RDA analysis indicated that pH value, total nitrogen and inorganic nitrogen contributed greatly to the shift of AOA and AOB community composition after CS burning while the pH value could influence the diversity of AOA and AOB after PB burning treatment (Figure 6). Previous studies have indicated that soil pH may be a critical factor controlling the abundances of AOA and AOB communities (Nicol et al., 2008). It has been demonstrated that the optimal pH for nitrification is 7–8 (Jiang and Bakken, 1999). In this study, soil pH increased from ~7.13 to ~7.94 and might benefit certain AOA and AOB species, AOB *Cluster 3a* for exam-

ple. Ratios of AOA to AOB decreased from 4.76×10^{-2} to 1.94×10^{-2} and from 4.49×10^{-2} to 4.33×10^{-2} nine days after CS and PB burning, respectively. Ratios of ammo-nia oxidizers (AOA and AOB) to total bacteria increased from 1.20×10^{-2} to 3.75×10^{-2} and 3.15×10^{-2}, nine days after CS and PB burning, respectively. The observation indica-ted ammonia oxidizers gain the competitive advantage over the other bacteria after fire.

It has been demonstrated that the phylogeny of the *amoA* gene largely correspond to the phylogeny of the 16S rRNA gene in ammonia oxidizers (Kowalchuk and Stephen, 2001). Thus, many studies have utilized *amoA* gene for investigating the community composition of ammonia oxidizers and resulted valuable information (Jia and Conrad, 2009; Leininger et al., 2006). In this study, phylogenetic analyses of bacterial *amoA* genes showed all *amoA* gene clones belonged to the genus *Nitrosospira*, no clones were closely aligned with the *Nitrosomonas* species. Similar results were observed before in arable soil (He et al., 2007; Avrahami and Conrad, 2003). Although, little difference in the OTU number of AOB was observed between different treat-ments (Figure 3), significant shifts in the different *Nitrosospira* clusters were observed. The species belonging to *Cluster A* and *B* dramatically reduced after fire. By contrast, the species belonging to *Cluster 3a* strikingly increased after fire. With regard to *Cluster 3b*, little influence and decrease were respectively observed after CS and PB burning. This observation might be explained by soil heating and pH influences. Soil tempe-rature and pH has been demonstrated to be two of the most important factors that influence ammonia oxidizer populations (Belser 1979; Nicol et al., 2008). It has been demonstrated that, compared with other *Nitrosospira* clusters, the species grouped in *Nitrosospira Cluster 3a* have a high adaption to high temperature and slightly alkaline conditions (pH 7.9) (Avrahami and Conrad, 2003). Therefore the species grouped in *Nitrosospira Cluster 3a* adapted to the fire and gain the competitive advantage after fire. Additionally, the species grouped in *Nitrosospira Cluster 3b* were also observe to adapt the fire to some extent. The species grouped in *Nitrosospira Cluster A, B* and *3c* were sensitive to fire in this study.

With regard to AOA, dramatic increase in OUTs was observed after fire which meant significant increase in AOA community composition. Phylogenetic analysis showed that *Soil cluster Group 1.1b* dominated in all the treatments. This observation was in line with previous studies which found *Group 1.1b* dominate and represent 1–5% of prokaryotic 16S rRNA genes in most soil samples (Ochsenreiter et al., 2003). As a stand clone for *Group 1.1b*, the soil fosmid clone 54d9 has been demon-strated as non-thermophilic Crenarchaeota (Schleper et al., 2005). Additionally, the relative abundance of the sequences fell into *Group 1.1b* respectively decreased from 85% to 64% and 56% after CS and PB burning.

In summary, the activity, abundance and community composition of AOA and AOB were substantially affected in the process of burning. Bacteria rather than Archaea dominate microbial ammonia oxidation in this agricultural soil. Although short-term impacts have been illustrated to be more significant, long-term investigations in future stu-dies will be important to construct a comprehensive awareness of biomass burning effects on soil ammonia oxidizers.

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