Shifts in the abundance and community structure of soil ammonia oxidizers in a wet sclerophyll forest under long-term prescribed burning

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HIGHLIGHTS

- We examined how fires affected the abundances and communities of soil AOB and AOA.
- A long-term repeated forest fire experiment was investigated.
- Fires increased the abundance of bacterium *amoA* genes, but not archaeal *amoA* genes.
- Fire also modified the composition of AOA and AOB communities.
- AOB genotype was affected by soil pH and DOC, while AOA by nitrate-N and DOC.

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ABSTRACT

Fire shapes global biome distribution and promotes the terrestrial biogeochemical cycles. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) play a vital role in the biogeochemical cycling of nitrogen (N). However, behaviors of AOB and AOA under long-term prescribed burning remain unclear. This study was to examine how fire affected the abundances and communities of soil AOB and AOA. A long-term repeated forest fire experiment with three burning treatments (never burnt, B0; biennially burnt, B2; and quadrennially burnt, B4) was used in this study. The abundances and community structure of soil AOB and AOA were determined using quantitative PCR, restriction fragment length polymorphism and clone library. More frequent fires (B2) increased the abundance of bacterium *amoA* gene, but tended to decrease archaeal *amoA* genes. Fire also modified the composition of AOA and AOB communities. Canonical correspondence analysis showed soil pH and dissolved organic C (DOC) strongly affected AOB genotypes, while nitrate-N and DOC shaped the AOA distribution. The increased abundance of bacterium *amoA* gene by fires may imply an important role of AOB in nitrification in fire-affected soils. The fire-induced shift in the community composition of AOB and AOA demonstrates that fire can disturb nutrient cycles.

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1. Introduction

Fire markedly modifies much of the Earth's land surface and terrestrial biogeochemical cycles (Matson et al., 2011; Orians and Milewski, 2007). It has been predicted the frequency and intensity of wildfire would increase under global warming (Bradstock, 2002; Cary, 2002; Westerling et al., 2006). Fire influences the population and species diversity of the aboveground plants (Bond and Van Wilgen, 1996) and belowground soil properties (Certini, 2005) as well as microorganisms (Vázquez et al., 1993). Niboyet et al. (2011) found that fires increased soil nitrous oxide emission when interacting with other factors, such as elevated CO₂, precipitation and N deposition.

Being the greatest diverse organisms in terrestrial ecosystems, soil microorganisms are dominant drivers of global nutrient cycles (Fuhrman, 2009). However, effects of fire on abundance and diversity of nitrifiers remain poorly understood (Neary et al., 1999).

Lithoautotrophic ammonia-oxidizing bacteria (AOB), which gain energy from oxidation of ammonia to nitrite, have been considered as the only contributor to aerobic ammonia oxidation before isolating autotrophic ammonia-oxidizing archaea (AOA) from the marine environment (Könneke et al., 2005). Comprehensive studies have indicated AOA seemingly played a more important role than AOB in the global N cycling. It has been found that AOA widely occurred in freshwater and marine habitats (Caffrey et al., 2007; Francis et al., 2005; Herrmann et al., 2008), various soils and sediments (Di et al., 2009; He et al., 2007; Long et al., 2012; Sahan and Muyzer, 2008), as well as man-made environments (De Vet et al., 2009; Urakawa et al.,

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2008; Zhang et al., 2011). Evidence from 12 pristine and agricultural soils in three climatic zones suggested that AOA is the dominant ammonia oxidizer in soils (Leininger et al., 2006). It has been reported that microbial biomass and the proportion of AOB decreased in fire-impacted soils in comparison with unburned sites (Yeager et al., 2005). However, most studies to date have focused mainly on the impacts of fires on belowground ecosystems for single or few burn events. Few long-term studies have been conducted to evaluate the impacts of repeated burnings on ecosystems, while the impacts of repeated fires on soil AOA have not been reported yet.

A previous study in the same wet sclerophyll forest has shown that microbial biomass of soil fungi and bacteria significantly decreased in the biennially burnt plots (Campbell et al., 2008). Meanwhile, Bastias et al. (2006a, b) found that the biennially burnt treatments shifted the community composition of ectomycorrhizal fungi substantially. In this study, we examined the effect of long-term repeated prescribed burning on abundances and communities of soil AOB and AOA.

2. Materials and methods

2.1. Field site description and soil sampling

The prescribed burning experiment was conducted in the Peachester State Forest (south-eastern Queensland, Australia) (26°50'S, 152°53'E), a typical wet sclerophyll forest dominated by *Eucalyptus pilularis* Smith with other canopy species including *Corymbia intermedia*, *Eucalyptus microcorys*, *Eucalyptus resinifera*, *Syncarpia glomulifera* and *Lophostemon confertus*. Peachester State Forest has a sub-tropical climate with an average annual rainfall of 1711 mm. The long-term experiment was established in 1972, including three treatments: biennially burnt (designated as B2), quadrennial burnt (designated as B4) and never burnt (control plots, designated as B0). Prescribed fires are carried out in the winter and are generally of low intensity ($<2500 \text{ kW m}^{-1}$). No artificial and natural factors have been applied since the establishment of the burning experiment. There were four replicates for each treatment, and total 12 plots (30 m 27 m) were randomly arranged in the experimental sites. Soils are red to yellow Kandosols (Isbell, 2002), deep sandy and highly permeable with acidic to neutral pH. Further details of the site can be found in previous publications (e.g. Guinto et al., 2001). Soil samples were collected using a 7 cm diameter corer and at two depths (topsoil (0–10 cm) and subsoil (10–20 cm)). A total of 15 cores were randomly taken from each plot and bulked as one composite sample in June 2005 and July 2010, respectively. All soils were sieved through a 2.0-mm sieve to remove large organic debris and a proportion of subsamples (ca. 50 g) were stored at -80°C prior to DNA extraction, while the remaining samples were kept at 4°C prior to chemical and biological analyses.

2.2. Analysis of soil properties

Soil dissolved organic C (DOC) and N (DON) were measured in 2 M KCl extracts as described by Chen et al. (2005). In brief, soil DOC and DON were measured by extracting 5.0 g (dry weight equivalent) of air-dried soil with 50 ml of 2 M KCl, shaking on an end-to-end shaker for 1 h and filtering through a Whatman 42 paper followed by a $0.45 \mu\text{m}$ membrane. The DOC and total soluble N (TSN) concentrations in the filtrates were determined using SHIMADZU TOC-VCPH/CPN analyzer (fitted with a TN unit). Concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were determined using the LACHAT Quickchem Automated Ion Analyzer (QuikChem Method 10-107-06-04-D for $\text{NH}_4^+\text{-N}$ and QuikChem Method 12-107-04-1-B for $\text{NO}_3^-\text{-N}$). The DON in the extracts was calculated as the difference between TSN and the sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. Soil pH was determined in 1:5 (v/v) soil/water extracts using a combination glass electrode. Soil total C and N were determined using an isotope ratio mass spectrometer with a Eurovector Elemental Analyzer (Isoprime-EuroEA 3000, Milan, Italy). Microbial biomass C (MBC) and N (MBN)

were determined by a chloroform fumigation extraction method using an EC factor of 2.64 (Vance et al., 1987) and an EN factor of 2.22 (Jenkinson, 1988), respectively.

2.3. Soil DNA extraction and PCR

Total genomic DNA was extracted from 0.3 g of soil using the MoBio Powersoil DNA Isolation Kit (Carlsbad, USA). The DNA extracts were qualified using a NanoDrop-2000 UV-vis Spectrophotometer (Thermo Scientific) at 260 nm and ratios of A_{260}/A_{230} and A_{260}/A_{280} were above 1.4 and 1.7, respectively. The DNA extracts were stored at -20°C prior to further analysis. The negative effects of inhibitors from the soil were tested using a dilution series of 10-, 20- and 50-fold of the extracted DNA in the presence and absence of bovine serum albumin (BSA). A 10-fold dilution plus BSA addition decreased the negative effect of PCR inhibitors to minimum. Primer pairs amoA1F (GGG GTT TCT ACT GGT GGT)/amoA2R (CCC CTC KGS AAA GCC TTC TTC) and CrenamoA23F (ATG GTC TGG CTW AGA CG)/CrenamoA616R (GCC ATC CAT CTG TAT GTC CA) were used to amplify bacterial and archaeal *amoA* genes, respectively (Tournu et al., 2008). The PCR reaction (50 μL) contained 10 μL $5\times$ GoTaq Flexi Green Buffer (Promega), 2.5 mM MgCl_2 , 750 μM each dNTP, 100 μM each primer, 1.25 units GoTaq DNA polymerase (Promega), 0.5 μL template DNA and ultraclean water to volume. The fragment length of the PCR products was 491 and 629 bp for AOB and AOA, respectively.

2.4. Bacterial and archaeal *amoA* gene clone library construction and their genotype identification

In this study, we only examined the diversities of AOB and AOA of the soil samples collected in 2005. The *amoA* gene amplicons were purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and cloned into the pGEM-T Easy Vector System. The ligation products were used to transform JM109 Competent Cells (Promega, Madison, WI). Twenty-four *amoA* gene clone libraries were established for AOB and AOA, respectively. For each clone library, approximately 70 transformed clones were randomly obtained and screened with T7/Sp6 primers. The restriction fragment length polymorphism (RFLP) was used to assign *amoA* genotypes. The RFLP analysis of *amoA* gene amplicons was carried out by digesting AOA with the restriction endonuclease MboI (NEB) and HaeIII (NEB) and AOB with and MboI (NEB). Five microliter digested products were loaded on a 3% agarose gel, running at 100 V in $1\times$ TAE buffer for 60 min and digitally photographed using ChemiDoc XRS imaging system (Bio-Rad). Digested *amoA* gene fragment patterns were detected using the Quantity-One software (Bio-Rad). Triplicate representative genotypes from the clone libraries were selected for sequencing (3130x1 Genetic Analyzer, Applied Biosystems). Clone library rarefaction curve analysis with 95% confidence intervals was calculated using software EstimateS v8.2 (Colwell, 2009). Sequences were deposited in GenBank under accession numbers JF520841–JF520989 for AOA and JF520990–JF521023 for AOB.

2.5. Quantitative PCR analyses of 16S rRNA genes and *amoA* genes of bacteria and archaea

The population sizes of microbial genes were determined using quantitative PCR (Mastercycler thermocycler, Eppendorf) by monitoring the SYBR Green I fluorescence. The transformed plasmid DNA was extracted and their concentrations were measured with a NanoDrop 2000 UV-vis Spectrophotometer (Thermo Scientific). Q-PCR standard curves were generated in duplicate with eight serial 10-fold dilutions of the transformed environmental sample plasmid DNA. Plasmid DNA extracted from clone 05PC-A01-03 (JF520842) and 05PC01-60 (JF520991) was used for AOA and AOB Q-PCR standard curve, respectively. The Q-PCR reaction system comprised 10 μL SYBR Premix Ex Taq (Perfect Real Time) (Takara Bio), 200 μM of each primer, 2 μL of

template DNA and ultraclean water to 20 μL volume. Bacterial primer pairs 338f (ACT CCT ACG GGA GGC AG)/518r (ATT ACC GCG GCT GCT GG) and archaeal primer pairs A109f (ACK GCT CAG TAA CAC GT)/A334r (TCG CGC CTG CTG CTC CCC GT) were used to perform the Q-PCR (Sun et al., 2009). Q-PCR cycling for the bacterial 16S rRNA gene was used with the following conditions: 95 °C for 2 min; 40 cycles of 95 °C for 20 s, 55 °C for 15 s, 72 °C for 30 s, 80 °C for 20 s; and final extension at 72 °C for 60 s, followed by a default melting curve cycle. Only the annealing temperature was increased to 57 °C for archaeal 16S rRNA gene Q-PCR protocol. Primer pairs for *amoA* genotype identification were also used to perform the Q-PCR as above. The thermal protocol for the *amoA* gene Q-PCR was the same as in the previous publication (Long et al., 2012). The generated standard curve had a slope of -3.3 , a correlation coefficient >0.999 , and amplification efficiency >0.94 . Q-PCR was carried out for soil samples both collected in 2005 and 2010.

2.6. Statistical and phylogenetic analysis

The differences of soil chemical, biochemical properties and microbial community size under the three prescribed burning regimes and two soil sampling depths were assessed by analysis of variance (ANOVA) with SPSS 18.0. Correlations between soil properties and the abundance of microbial community were analyzed using the Pearson correlation test. All variables showed either a normal or log-normal data distribution with Shapiro–Wilk W test. Least significant difference (LSD) and Dunnett's T3 tests were performed for the homogeneous and non-homogeneous variance, respectively, according to the test of homogeneity of variances. Molecular variance analyses of *amoA* genes based on the RFLP genotypes were performed using Arlequin 3.5 (Excoffier and Lischer, 2010). We evaluated the frequency and distribution of the haplotypes using 1000 permutations. The effects of soil properties and prescribed burning on the *amoA* genotypes distribution were analyzed by canonical correspondence analysis (CCA) using the community ecology package 'vegan' (Oksanen et al., 2010). The standard R function STEP was used to select environmental variables with Akaike's information criterion (AIC). Canonical correspondence analysis (CCA) ordination diagrams were performed using 1000 times permutation tests and p -values ≤ 0.05 was considered statistically significant. The phylogenetic trees of *amoA* genes were based on the evolutionary distance (maximum likelihood algorithm with Jukes–Cantor model, 1000 bootstrap repetitions performed) using MEGA5 (Kumar et al., 2008).

3. Results

3.1. Soil properties

Results from one-way ANOVA have shown that most soil properties (except for nitrate-N, ammonium-N, and MBN) significantly decreased in the topsoil of B2 plots (Table 1). Soil pH was 0.5 units higher in the

B2 and B4 plots than that of B0 plots ($p < 0.05$). Soil total C and soil total N were significantly lower in the B2 plots compared with the other treatments, but the C:N ratio showed no significant difference among the treatments. Mean DOC and DON both significantly decreased by ca. 30% in the B2 plots compared to the B0 plots from 77.4 to 46.8 mg kg^{-1} ($p < 0.05$) and 18.0 to 12.6 mg kg^{-1} ($p < 0.01$), respectively (Table 1). Mean MBC significantly decreased from 780 mg kg^{-1} in the B0 plots to 554 mg kg^{-1} in the B2 plots ($p < 0.01$), while there was no significant difference in MBN between the B0 and B2 plots. There were no significant differences in most soil properties measured between the B0 and B4 plots, except for soil pH which was lower in the B0 plots than in the B4 plots (Table 1).

3.2. Abundances of soil 16S rRNA genes and *amoA* genes

In 2005 samples, the abundance of soil bacterial 16S rRNA genes ranged from 1.90×10^8 to 5.47×10^8 copies g^{-1} dry soil weight (dsw), 10-fold higher than that of archaeal 16S rRNA genes (0.08×10^7 to 2.36×10^7 copies g^{-1} dsw) (Fig. 1a) ($p < 0.01$). The abundance of archaea was positively correlated with soil DOC and DON at both soil depths ($p < 0.05$) (Table 2). However, the abundance of both soil bacteria (2.48×10^7 to 8.65×10^7 copies g^{-1} dsw) and archaea (1.23×10^5 to 2.27×10^6 copies g^{-1} dsw) was lower in 2010 samples. The abundance of AOA *amoA* genes (2.48×10^6 to 5.41×10^6 copies g^{-1} dsw) was over 10-fold higher than that of AOB (0.76×10^5 to 1.15×10^5 copies g^{-1} dsw) in 2005 samples (Fig. 1c). The abundance of AOB *amoA* gene was significantly higher in the B2 plots compared with the B0 plots ($p < 0.05$) in both 2005 and 2010 samples (Figs. 1c and 2c). However, copy numbers of archaeal *amoA* gene in the B0 treatment were over 7-fold higher than that of the B2 treatment of 2010 samples (Fig. 2c).

3.3. Community composition and phylogenetic analysis of AOB and AOA

The diversity analyses of bacterial and archaeal *amoA* genes were only carried out in soil samples collected in 2005. Cloning and restriction fragment length polymorphism (RFLP) were performed to identify the community composition of AOB and AOA (Table 3). Clones displaying unique RFLP patterns were designated operational taxonomic units (OTUs) or genotypes, and representative clones were selected for sequencing. According to unique RFLP patterns, 21 OTUs of AOA and 6 OTUs of AOB were obtained. The rarefaction curve (Fig. 3) indicated that the clone libraries were sufficient to cover the *amoA* gene diversity. Phylogenetic affiliation analysis showed that *amoA* genes were classified as the *Nitrosospira* type for bacteria and the soil-related cluster for archaea (Figs. 4 and 5). Three bacterial *amoA* gene clusters (2, 9 and 11) were detected; cluster 2 is the dominant group (Table 3). Most genotypes of bacterial *amoA* genes were distributed in all treatments, except for cluster 11. In addition, an increase in cluster 2 was accompanied by a decrease in cluster 11 in the B2 plots (Table 3).

Table 1
Soil pH, ammonium N ($\text{NH}_4^+\text{-N}$), nitrate N ($\text{NO}_3^-\text{-N}$), total carbon (TC), total nitrogen (TN), ratio of TC to TN (C:N), dissolved organic C (DOC), dissolved organic N (DON), ratio of DOC to DON (DOC:DON), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) under different prescribed burning regimes at the Peachester site, southeast Queensland.

Treatment ^a	pH	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	TC (%)	TN (%)	C:N	DOC (mg kg^{-1})	DON (mg kg^{-1})	DOC:DON	MBC (mg kg^{-1})	MBN (mg kg^{-1})
<i>0–10 cm</i>											
B0	4.6 $\pm$ 0.2 ^b	6.5 $\pm$ 2.7 ^a	1.9 $\pm$ 2.4 ^a	6.5 $\pm$ 2.1 ^a	0.3 $\pm$ 0.1 ^a	24.3 $\pm$ 1.4 ^a	77.4 $\pm$ 8.1 ^a	18.0 $\pm$ 3.0 ^a	4.4 $\pm$ 0.6 ^a	780.1 $\pm$ 146.3 ^a	73.7 $\pm$ 16.4 ^a
B2	5.1 $\pm$ 0.1 ^a	3.5 $\pm$ 1.5 ^a	4.4 $\pm$ 3.0 ^a	3.5 $\pm$ 0.3 ^b	0.1 $\pm$ 0.0 ^b	25.9 $\pm$ 2.0 ^a	46.8 $\pm$ 7.7 ^b	12.6 $\pm$ 1.1 ^b	3.7 $\pm$ 0.4 ^a	553.9 $\pm$ 136.7 ^b	68.5 $\pm$ 16.4 ^a
B4	5.2 $\pm$ 0.3 ^a	4.2 $\pm$ 3.6 ^a	3.6 $\pm$ 4.3 ^a	6.6 $\pm$ 0.5 ^a	0.3 $\pm$ 0.0 ^a	24.0 $\pm$ 1.5 ^a	74.4 $\pm$ 20.9 ^a	19.5 $\pm$ 2.8 ^a	3.8 $\pm$ 0.6 ^a	880.8 $\pm$ 43.0 ^a	89.1 $\pm$ 4.3 ^a
<i>10–20 cm</i>											
B0	4.9 $\pm$ 0.2 ^a	4.5 $\pm$ 2.0 ^a	1.9 $\pm$ 1.8 ^a	4.1 $\pm$ 1.4 ^a	0.2 $\pm$ 0.1 ^a	23.6 $\pm$ 0.6 ^a	40.9 $\pm$ 5.3 ^a	16.5 $\pm$ 3.0 ^a	2.5 $\pm$ 0.6 ^a	419.6 $\pm$ 128.8 ^a	44.4 $\pm$ 6.3 ^a
B2	5.1 $\pm$ 0.1 ^a	4.1 $\pm$ 1.3 ^a	4.0 $\pm$ 2.4 ^a	2.8 $\pm$ 1.0 ^a	0.1 $\pm$ 0.1 ^a	24.4 $\pm$ 2.4 ^a	33.4 $\pm$ 7.5 ^a	11.5 $\pm$ 1.4 ^b	2.9 $\pm$ 0.5 ^a	430.5 $\pm$ 88.3 ^a	50.8 $\pm$ 8.7 ^a
B4	5.2 $\pm$ 0.4 ^a	3.2 $\pm$ 2.4 ^a	3.5 $\pm$ 2.8 ^a	4.0 $\pm$ 0.4 ^a	0.2 $\pm$ 0.0 ^a	22.8 $\pm$ 1.6 ^a	40.5 $\pm$ 6.4 ^a	17.8 $\pm$ 2.6 ^a	2.3 $\pm$ 0.1 ^a	552.2 $\pm$ 83.7 ^a	52.6 $\pm$ 9.6 ^a

^a B0: Never burnt; B2: biennially burnt; B4: quadrennially burnt.

^b Difference is significant at the 0.05 level (LSD).

Fig. 1. Bacterial and archaeal 16S rRNA gene copy numbers under different treatments (a: 0–10 cm) and (b: 10–20 cm), ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) *amoA* gene copy numbers under different treatments (c: 0–10 cm) and (d: 10–20 cm) in 2005. B0, never burnt; B2, biennially burnt; B4, quadrennially burnt.

Twenty-one thaumarchaeal *amoA* genotypes fell into two clusters, designated as soil clusters 2 and 5. According to the reference sequences (JN227489, EF207225, FR773159), 14 thaumarchaeal *amoA* genotypes revealed a pH-associated distribution from acido-neutral ($5 \leq \text{pH} < 7$) to acidic soils ($\text{pH} < 5$). However, most thaumarchaeal *amoA* genes associated with *Candidatus Nitrososphaera* (FR773159) were found in the control plots. RFLP analysis results showed that the dominant soil cluster 2 (group 1.1b) decreased, while soil cluster 5 (group 1.1a) increased in the burnt plots (Table 3).

3.4. Communities of AOB and AOA in response to environmental factors

Canonical correspondence analysis (CCA) was performed to investigate the environmental factors influencing the community composition, evaluating the relationship between the soil parameters (Table 1) and the dominant *amoA* gene RFLP genotypes (Table 3). According to the AIC, five different factors were selected to interpret

the distribution of bacterial *amoA* genes and archaeal *amoA* genes in both topsoil and subsoil. Two axes of the CCA explained 39.3% of the total variance in the bacterial *amoA* genotype composition and 98.1% of the cumulative variance of the genotype–environment relationship in topsoil (Fig. 6a). Only soil DOC significantly influenced the distribution of AOB *amoA* genotypes ($F = 4.28$, $p = 0.01$, 1000 permutations). For subsoil, two axes of the CCA explained 49.9% of the total variance in the bacterial *amoA* genotype composition and 79.7% of the cumulative variance of the genotype–environment relationship (Fig. 6b). Soil pH significantly affected the bacterial *amoA* gene composition among the treatments ($F = 3.47$, $p = 0.01$, 1000 permutations), followed by TC ($F = 2.48$, $p = 0.07$, 1000 permutations). On the other hand, more than half of the total variance (53.7%) and the majority of the cumulative variance (78.7%) of the archaeal *amoA* gene composition versus environmental variables were explained by the first two axes of the CCA in topsoil (Fig. 6c). The CCA results indicated that nitrate-N ($p < 0.01$), MBN ($p = 0.03$) and DOC ($p = 0.06$)

Table 2

Pearson's correlations (r) between soil properties (pH, TC, TN, C:N, DOC, DON, DOC:DON, NO_3^- -N, NH_4^+ -N, MBC, MBN, and MBC:MBN) and community size (qAOB, qAOA, qBacteria and qArchaea) measured at each individual sampling point ($n = 12$).

	0–10 cm				10–20 cm			
	qBacteria	qArchaea	qAOB	qAOA	qBacteria	qArchaea	qAOB	qAOA
pH	0.32	0.45	0.54	−0.56	0.48	0.62*	0.17	0.19
TC	0.33	0.50	−0.20	−0.14	0.14	0.36	−0.03	0.21
TN	0.31	0.43	−0.17	−0.11	0.11	0.30	−0.10	0.21
C:N	0.50	0.14	0.40	0.60*	0.03	−0.13	0.57	0.04
DOC	0.36	0.67*	−0.47	−0.43	0.18	0.65*	0.35	−0.15
DON	0.45	0.73**	−0.25	−0.37	0.19	0.70**	−0.02	0.20
DOC:DON	0.02	0.15	−0.63*	−0.18	−0.02	−0.22	0.34	−0.32
NO_3^- -N	−0.33	−0.56	−0.27	0.48	−0.47	−0.68*	−0.55	0.03
NH_4^+ -N	0.49	0.69*	0.31	−0.34	0.17	0.41	0.22	0.10
MBC	−0.14	0.27	−0.36	−0.59*	0.10	0.33	−0.07	−0.01
MBN	−0.32	0.14	−0.14	−0.81**	−0.06	−0.02	0.11	−0.14
MBC:MBN	0.19	0.21	−0.34	0.17	0.18	0.49	0.03	0.12

TC, total carbon; TN, total nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea.

Values in bold are statistically significant. ** $p < 0.01$; * $p < 0.05$.

Fig. 2. Bacterial and archaeal 16S rRNA gene copy numbers under different treatments (a: 0–10 cm) and (b: 10–20 cm), ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) *amoA* gene copy numbers under different burning treatments (c: 0–10 cm) and (d: 10–20 cm) in 2010. B0, never burnt; B2, biennially burnt; B4, quadrennially burnt.

significantly affected the distribution of AOA *amoA* genotypes. Only 31.5% of the total variance and 58.9% of the cumulative variance of the archaeal genotype–environmental relationship were explained by the first two axes of the CCA in subsoil (Fig. 6d). Soil TC ($p = 0.09$) significantly affected the distribution of AOA *amoA* genes (Fig. 6d).

3.5. Molecular variance of *amoA* gene sequences

The haplotypic form of RFLP data was used to evaluate the molecular variance of *amoA* gene among the different treatments. Hierarchical genetic structures of archaeal *amoA* genes were detected among the populations within the treatment (0–10 cm: $F_{SC} = 0.37$ and 10–20 cm: $F_{SC} = 0.07$) and within populations across all samples (0–10 cm: $F_{ST} = 0.36$ and 10–20 cm: $F_{SC} = 0.11$). Replicate variation of each treatment contributed 38.18% (0–10 cm) and 12.75% (10–20 cm) to the total genetic variation, while variation in the populations of all samples accounted for ca. 64.04% (0–10 cm) and 85.16% (10–20 cm) (Table 4). AMOVA results indicated that the main variation of AOB *amoA* gene came from the populations among all samples (0–10 cm: 88.75% and 10–20 cm: 96.55%). Despite a small contribution of the variation in populations among the treatments to total genetic variation

(0–10 cm: 2.09% and 10–20 cm: 1.98%), there were significant differences in bacterial *amoA* genes among the treatments (0–10 cm: $p < 0.05$; 10–20 cm: $p < 0.10$) (Table 4).

4. Discussion

Lower pH observed in the prescribed burning plots (B2 and B4 plots) compared with the control plot (B0) (Table 1) was due to the increased inputs of ashes containing oxides, hydroxides and carbonates of alkaline elements (K, Na, Ca and Mg) (Khanna et al., 1994; Ulery et al., 1993). Soil TC, TN, DOC, DON, and MBN were lower in the more frequent burning plots (B2 plots) compared with the B0 and B4 plots. This may be associated with reduced inputs of organic matter and increased erosion loss of organic matter (e.g. Knicker, 2007; Wardle et al., 2008). However, there were no significant differences in most soil properties between the B0 and B4 plots, indicating that the ecosystem processes could recover in less frequent burning treatments (B4 plots). In addition, there were no significant differences in soil properties at the 10–20 cm among the three treatments, indicating that the effect of fires was confined to the topsoil.

Table 3

Fragment patterns of *amoA* genes and distribution of sequence types affiliated with *amoA* clusters in the clone libraries (%).

Treatment	AOA				AOB				
	Pattern number ^a	Soil cluster 2 ^b	Soil cluster 5	Ratio of soil cluster 2/5	Pattern number	Cluster 2	Cluster 9	Cluster 11	Ratio of cluster 2/11
<i>0–10 cm</i>									
B0	9	99.6%	0.4%	249.0	5	75.4%	0.8%	23.8%	3.2
B2	4	78.9%	21.1%	3.7	4	89.9%	1.5%	8.6%	10.5
B4	8	87.3%	12.7%	6.9	4	71.5%	0.5%	28.0%	2.6
<i>10–20 cm</i>									
B0	7	90.7%	9.3%	9.8	5	70.1%	0.4%	29.5%	2.4
B2	8	80.4%	19.6%	4.1	5	83.2%	0.9%	15.9%	5.2
B4	8	74.7%	25.3%	3.0	5	78.7%	1.0%	20.3%	3.9

^a Pattern numbers are numbers of molecular fragments following digestion with HaeIII and MboI.

^b Proportion of sequences is calculated as proportion of analyzed clones affiliated with the fragment patterns belonging to one of the clusters among the clone numbers used in the digestion analysis in one clone library.

Fig. 3. Rarefaction curves of cumulative *amoA* genes of AOB (a: 0–10 cm and b: 10–20 cm) and AOA (c: 0–10 cm and d: 10–20 cm) clones with 95 % upper and lower confidence intervals (error bars) for three prescribed burning treatments (never burnt, B0; biennially burnt, B2; quadrennially burnt, B4) at the Peachester site.

4.1. Effects of long-term prescribed burning on the abundance of 16S rRNA and *amoA* genes

It has been reported that fire stimulated the growth of bacteria due to nutrient input via ash deposition and simultaneously reduced the growth of fungal populations (Bissett and Parkinson, 1980; Pietikäinen and Fritze, 1995). Vázquez et al. (1993) had found a short-term stimulation of fire on the microbial population size. However, this beneficial effect of burning disappeared after 1 year. They supposed that in the long term gradual leaching of ash minerals to lower soil layers and a loss of structure due to burning made the microbial population sizes return to normal (Vázquez et al., 1993). In our study, insignificant effects of fire on the abundance of bacteria and archaea may be partly explained by the relatively constant ratio of soil C:N and

DOC:DON among the treatments in both soil depths. It has been suggested that soil nutrient stoichiometry controlled the metabolism and growth of microorganisms (Elser and Hamilton, 2007; Sterner and Elser, 2002). In general, Kandosols have a coarse-texture with low fertility and poor water holding capacity. Plants or other microbes rapidly seized the brief nutrient pulses and niche vacancy following fire. In addition, the positive correlation between archaeal abundance and soil DOC or DON indicated a more effective usage of available C or N sources than bacteria at this site, which favoured their adaptation to the environmental stresses such as fire.

On the contrary, a study in the pine forest in Japan found that the abundance of culturable microorganisms was the lowest in the soil samples collected immediately after fire, followed by the samples collected 2 years after fire and the unburned samples (Mabuhay et al.,

Fig. 4. Maximum likelihood distance tree based on an alignment of 413 bp bacterial *amoA* gene sequences. The sequences obtained in this study are indicated in bold including the following information: clone name, accession number in GenBank and treatments in the parentheses. *Nitrosomonas halophila* was used as the outgroup sequence. Bootstrap values (>60%) are indicated at branch points. The scale bar represents 5% sequence divergence.

Fig. 5. Maximum likelihood distance tree based on an alignment of 559 bp archaeal *amoA* gene sequences. The sequences obtained in this study are in bold including the following information: clone name, accession number in GenBank and treatments in parentheses. *Nitrosopumilus maritimus* SCM1 was used as the outgroup sequence. Bootstrap values (>60%) are indicated at branch points. The bar shows a relative distance of 0.05.

2003). Moreover, Mabuhay et al. (2004) investigated the microbial abundance of one unburned site and another 5 different post-fire sites (2 months and 3, 6, 9 and 25 years) and found that the highest abundances of Gram-positive, Gram-negative bacteria and actinomycetes in the control site.

Our results showed a positive impact of the fire on the abundance of AOB in the topsoil of the B2 plots in comparison with the B0 plots, possibly due to the increase in soil pH. Similar results were found in a ponderosa pine forest, where Ball et al. (2010) speculated that the growth of AOB was stimulated by the pH improvement and adsorption

of soil nitrification inhibitors by the fire-generated charcoal. However, no significant changes in the abundance of AOB between the B4 plots and the B0 plots indicated that soil pH was not the only dominant environmental factor on the growth of AOB in this study. It has been suggested that soil heating favors the growth of autotrophic nitrifiers in competition with heterotrophs for ammonium and additional nutrients from ash (Bauhus et al., 1993). However, the effects of fire on AOB appeared to be weak in a California annual grassland (Docherty et al., 2011), and negative in a mixed conifer forest (Yeager et al., 2005). These could be attributed to the competition between

Fig. 6. Canonical correspondence analysis (CCA) ordination plots for the first two dimensions of CCA of the relationship between: the distribution of AOB OTUs with environmental factors (a: 0–10 cm, b: 10–20 cm) and the distribution of AOA OTUs with environmental factors (c: 0–10 cm, d: 10–20 cm). The optimal CCA models represented by the diagrams were produced with standardisation explanatory variables by weighting and scaling of scores focussed on inter-species correlations via F statistic significant tests. Correlations between environmental variables and CCA axes are represented by the length and angle of lines (environmental factor vectors).

Table 4
Arlequin AMOVA analyses of *amoA* gene sequences of Peachester forest soils by RFLP genotype frequencies.

0–10 cm					
AOA	Source of variation	Variance components	% of variation	F-statistics	p
	Among treatments	−0.04 Va	−2.22	$F_{CT} = -0.02$	0.65 ± 0.01
	Replicates/treatment	0.66 Vb	38.18	$F_{SC} = 0.37$	0.00 ± 0.00
	Among all samples	1.10 Vc	64.04	$F_{ST} = 0.36$	0.00 ± 0.00
AOB	Source of variation	Variance components	% of variation	F-statistics	p
	Among treatments	0.12 Va	4.79	$F_{CT} = 0.05$	0.04 ± 0.00
	Replicates/treatment	0.16 Vb	6.46	$F_{SC} = 0.07$	0.00 ± 0.00
	Among all samples	2.24 Vc	88.75	$F_{ST} = 0.11$	0.00 ± 0.00
10–20 cm					
AOA	Source of variation	Variance components	% of variation	F-statistics	p
	Among treatments	0.05 Va	2.09	$F_{CT} = 0.02$	0.15 ± 0.01
	Replicates/treatment	0.30 Vb	12.75	$F_{SC} = 0.13$	0.00 ± 0.00
	Among all samples	2.02 Vc	85.16	$F_{ST} = 0.15$	0.00 ± 0.00
AOB	Source of variation	Variance components	% of variation	F-statistics	p
	Among treatments	0.05 Va	1.98	$F_{CT} = 0.02$	0.07 ± 0.01
	Replicates/treatment	0.04 Vb	1.47	$F_{SC} = 0.01$	0.04 ± 0.01
	Among all samples	2.41 Vc	96.55	$F_{ST} = 0.03$	0.00 ± 0.00

plants and plant-associated arbuscular mycorrhizal fungi (AMF) with AOB for nutrients inputs by the ash, and a slow recovery rate of microbial biomass caused by low soil moisture content (Docherty et al., 2011; Yeager et al., 2005). A decreasing abundance of AOA in 2010 samples and a negative relationship between the abundance of AOA and soil pH in 2005 samples indicated an impact of fire on the growth of AOA. In a recent study, O'Sullivan et al. (2012) also found the abundance of AOA was reversely interrelated to soil pH in two agricultural systems with 360 kg and 65 kg N per ha per year in Australia.

4.2. Effects of long-term prescribed burning on AOB and AOA community composition

The fire greatly affected the ratio of clusters 2–11 of the AOB community structure at both soil depths, which increased from 2.4–3.9 in the B0 and B4 plots to 5.2–10.5 in the B2 plots (Table 3). The shift in AOB community structures might be due to burning-associated increases in soil pH and ammonium-N level. The similar results have been reported in a mixed conifer forest ecosystem with dominant soil cluster 3a in burned soils (Yeager et al., 2005) and a ponderosa pine forest with dominant soil clusters 3 and 4 in recent-fire soils (Ball et al., 2010). However, the cluster 3a sequence of AOB was not detected in this study. The alteration of AOB populations in the fire-impacted plots was confirmed by the results of phylogenetic tree analysis and AMOVA. For example, one genotype (JF5210004) of cluster 11 was only detected in the burned plots and the molecular variance among the treatments was significant in both soil depths.

Phylogenetic analyses of bacterial *amoA* genes showed that *Nitrosospora* cluster 2 was predominant among the treatments (Fig. 4), in agreement with previous findings that *Nitrosospora* cluster 2 sequences were dominant in acidic forest soils (Nugroho et al., 2005). *Nitrosospora* cluster 11 possessed nearly one-quarter ratio in the clone library and four *amoA* genotypes indicated cluster 11 as an important contributor to nitrification at our study site (Fig. 4, Table 4). In addition, CCA results indicated that soil DOC was positively correlated with the dominant genotypes of AOB (JF520991, JF521005 and JF521000) in the topsoil (Fig. 6a). Previous studies at the same study area have demonstrated that fungal community composition in the B2 plots was significantly different from that in B0 or B4 plots due to the change in soil C and N (e.g. Bastias et al., 2006a). Meanwhile, soil pH and TC were found to have significant effects on the AOB community composition in the topsoil.

The investigation of wildfire or prescribed burning on the soil ammonium-N and nitrate-N had demonstrated that both inorganic N increased immediately after the fire, but these improvements were dissipated in 1 year or more post-fire (Covington et al., 1991; Covington and Sackett, 1992; Smith et al., 2008). The CCA results showed a positive correlation between soil nitrate-N and dominant AOA genotypes (JF520847, JF520868 and JF520885) (Fig. 6c), which were only detected in the soil cluster 2. Interestingly, the impact of the fire on the AOA community composition not only manifested in the decrease of the ratio of soil clusters 2–5 under B2 or B4 plots but also different genotype numbers of AOA among the treatments (Table 3). For example, there were 10 genotypes of soil cluster 2 in the topsoil of B0 plots but 4 in the B2 or B4 plots. In particular, six (JF520971, JF520968, JF520978, JF520976, JF520973 and JF520980), one (JF520841) and three (JF520915, JF520904 and JF520910) unique genotypes were detected in the B0, B2 and B4 plots, respectively (Fig. 5). In addition, most genotypes of soil cluster 5 were detected in the topsoil of B4 plots except for JF520895 and JF520948 (Fig. 5). Therefore, the shift of AOA genotypes between the control (B0) and the B4 plots indicated that the 4 year interval fire could affect the AOA community composition.

5. Conclusions

In summary, results from this study have demonstrated that prescribed fires did not change the abundance of bacteria and archaea, while more frequent burning (B2 plots) significantly increased the abundance of bacterial *amoA* gene, but tended to decrease archaeal *amoA* genes. This may imply a more important role of AOB in nitrification in this acidic soil after the long-term prescribed burning. Fire has also modified the composition of AOA and AOB communities, which could be explained by changes in the key soil parameters (e.g. DOC, MBN, pH and TC) due to the fires. Further studies are needed to confirm the relationship between the nitrification rate and the AOA and AOB abundances and community structures at the study site and to explore the implication of shifts in AOA and AOB communities due to the fires for N management in forest ecosystems.

Conflict of interest

The authors have declared that there were no conflicts of interests involved in this work.

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