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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.

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 CO_2, 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 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 μm 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 NH_4^+-N and NO_3^--N were determined using the LACHAT Quickchem Automated Ion Analyzer (QuikChem Method 10-107-06-04-D for NH_4^+-N and QuikChem Method 12- 107-04-1-B for NO_3^--N). The DON in the extracts was calculated as the difference between TSN and the sum of NH_4^+-N and NO_3^--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 (Tourna et al., 2008). The PCR reaction (50 μL) contained 10 μL 5× 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×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