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## Differential response of archaeal groups to land use change in an acidic red soil



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### HIGHLIGHTS

- Archaeal *amoA* gene numbers respond sensitively to the changes of land use.
- Soil nitrate had a significant correlation with archaeal *amoA* gene abundance.
- Archaeal community structure was significantly impacted by land use change.
- Little impact of land use was detected on the abundance of Group 1.1c.

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### ABSTRACT

Land use management, one of the most important aspects of anthropogenic disturbance to terrestrial ecosystems, has exerted overriding impacts on soil biogeochemical cycling and inhabitant microorganisms. However, the knowledge concerning response of different archaeal groups to long-term land use changes is still limited in terrestrial environments. Here we used quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) approaches to investigate the response of archaeal communities to four different land use practices, i.e. cropland, pine forest, restoration land and degradation land. qPCR analyses showed that expression of the archaeal *amoA* gene responds more sensitively to changes of land use. In particular, we observed, occurring at significantly lower numbers of archaeal *amoA* genes in degradation land samples, while the abundance of total archaea and Group 1.1c based on 16S rRNA gene copy numbers remained constant among the different treatments examined. Soil nitrate content is significantly correlated with archaeal *amoA* gene abundance, but not their bacterial counterparts. The percentage of archaea among total prokaryote communities increases with increasing depth, but has no significant relationship with total carbon, total nitrogen or pH. Soil pH was significantly correlated with total bacterial abundance. Based on results from PCR-DGGE, three land use practices (i.e. cropland, pine forest, restoration land) showed distinct dominant bands, which were mostly affiliated with Group 1.1a. Degradation land, however, was dominated by sequences belonging to Group 1.1c. Results from this study suggest that community structure of ammonia oxidizing archaea were significantly impacted by land use practices.

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### 1. Introduction

Land use management regimes have exerted a major influence on soil biodiversity and sustainability, subsequently resulting in a serial of ecological consequences such as greenhouse gas emission. Land use intensity has been increasing in China over the last 30 years, facilitated by booming economic growth (Chen, 2007; Chen et al., 2009),

and characterized by a spectrum of detrimental effects on soil quality, including reduced soil fertility, soil erosion and decline in soil biodiversity (Xu and Cai, 2007; Zhang et al., 1999). Chinese red soils are a noteworthy example, as their ability to sustain productivity has decreased as a consequence of intensive agricultural management marked by high acidity and low fertility among other symptoms (Yao et al., 2011; Zhang et al., 1999). Extensive studies have focused on biogeochemical cycling and the related bacterial communities in acidic red soil (Chen et al., 2010; He et al., 2007; Xu et al., 2010; Yao et al., 2000), while only a few focused on archaea (He et al., 2007; Ying et al., 2010; Zhang et al., 2012). Together, these microorganisms are the most important biotic factor contributing to the transformation processes of soil nutrients and thus maintenance of soil sustainability and ecosystem functions (Kemnitz et al., 2007; Leininger et al.,

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2006; Torsvik and Ovreas, 2002). A more thorough investigation of soil microbial function, particularly of the less-studied archaeal domain, their abundance, diversity and driving factors, is greatly needed for a better understanding of land use impacts on red soil ecosystems.

Archaea are the third domain of life and are evolutionarily distinct from the bacterial and eukarya domains (DeLong, 1992; Woese et al., 1990). The widespread distributions and high abundances of archaea have been well documented from extreme environments to non-extreme environments, including aquatic and terrestrial niches (Timonen and Bomberg, 2009). These studies indicate their potential roles of archaea in biogeochemical cycling and energy flow (Berg et al., 2007; Lipp et al., 2008). Environmental factors, among which soil pH and C:N ratio were found to be the driving factors in most cases (Bates et al., 2011; Cao et al., 2012; Lehtovirta et al., 2009), play an essential role in controlling the spatial distribution and diversity of archaea in soils (Auguet et al., 2010; Bates et al., 2011; Wessén et al., 2010). It is a challenge to specifically predict the effects of land use practices on microbial diversity, because the influence of soil characteristics on microbial communities is often involved in the process of land use changes (Kuramae et al., 2012; Wallenius et al., 2011). Most investigations have shown that agricultural management practice has strong impacts on soil bacteria through both direct and indirect effects of substrate modification and availability (He et al., 2007; Jesus et al., 2009; Lauber et al., 2008; Shen et al., 2010). However, the effects of land use change on archaeal groups, in particular of the emerging group of Thaumarchaea (formerly recognized as mesophilic Crenarchaea) (Brochier-Armanet et al., 2008; Spang et al., 2010), in acidic red soils are still not fully understood (Yao et al., 2011).

The phylogeny and ecophysiology of Thaumarchaea are of increasing concern due to their strong presence among ammonia oxidizing prokaryotes in terrestrial ecosystems, highlighting their potentially important role in the global nitrogen cycle (He et al., 2007; Leininger et al., 2006; Ochsneiter et al., 2003). Recent evidence has shown that Thaumarchaea Group 1.1b appeared to be the most dominant and ubiquitous group in soil, and sequences affiliated with Thaumarchaea Groups 1.1a and 1.1c were also found in soils but under more restricted conditions (Bates et al., 2011; Lehtovirta et al., 2009; Pester et al., 2012). For example, Group 1.1c is more likely to flourish in acidic soils, such as temperate acidic forest soil (Kemnitz et al., 2007), acidic red soils (Ying et al., 2010) and glacier foreland soils (Nicol et al., 2005) with pH below 5.0 (He et al., 2012; Lehtovirta et al., 2009; Nicol et al., 2005). The contributions of Groups 1.1a and 1.1b to nitrification process have been confirmed in most of the soils examined (Nicol and Schleper, 2006). It remains unclear, however, whether Group 1.1c is involved in soil ammonia oxidation, as no direct evidence or pure culture is available despite the widespread abundance of this group in the environment, and particularly in acidic soils (He et al., 2012). On the other hand, Group 1.1c abundance and activity may be affected by rhizosphere and vegetation (Bomberg et al., 2003; Bomberg and Timonen, 2007), and subsequently respond to land use changes (Ying et al., 2010). Furthermore, information on the response of individual archaeal groups to land use change is very scarce, and only limited knowledge of how archaeal communities change along the depth profiles are available (Cao et al., 2012).

In this study, we revisited land use experimental station in Taoyuan, China. The main objective was to assess the response of different archaeal groups to land use change by measuring abundance and community composition using a combination of quantitative PCR (qPCR) and PCR-denaturing gradient gel electrophoreses (DGGE) approaches. Although differences in archaeal communities were detected among four land utilization patterns at this experimental site in a previous study, no significant correlation was found between archaeal *amoA* gene copy numbers and potential nitrification rates (Ying et al., 2010). This finding was in opposition to the current knowledge of acidic soil ammonia oxidation, which is thought to be mainly driven by ammonia oxidizing archaea (AOA) (He et al., 2012; Zhang et al., 2012). Furthermore, the role of different Thaumarchaeal groups, especially Groups

1.1a, 1.1b and 1.1c, was still unclear in terms of ammonia oxidation along the soil depth. We hypothesized that land use change of acidic red soil would alter the community abundance and structure of archaea specific groups and consequently influence the nitrogen cycling processes they mediate.

## 2. Materials and methods

### 2.1. Study site and sampling

The soil sampling site was located at the Taoyuan Experimental Station of Agro-ecosystem Observation (28° 55' N, 111° 26' E) of the Chinese Academy of Sciences (Hunan Province, China). Detailed information about the experiment site has been described previously (Ying et al., 2010; Zheng et al., 2010). In brief, this long-term experiment started from the year of 1995 on a slightly sloped field, and five different land use types were initially set up. In this study, four different land use practices similar to a previous study were selected (Ying et al., 2010): cropland, pine forest, restoration land and degradation land. For cropland, fertilizers were applied twice a year according to local agriculture management with 2-year corn/oilseed rape rotation. No specific management was used for pine forest and restoration land, which were dominated by slash pine and herbaceous plant, respectively. For degradation land, vegetation (mainly grasses) was mowed twice per year. Ten cores for each sample were mixed, and three replicates were made for each plot at two soil depths, i.e. 0–20 cm and 20–40 cm. There were 12 samples per depth and totally 24 samples collected in May 2009. After removing stones and roots, each sample was placed in a sterile plastic bag and shipped on ice to the lab. All samples were passed through a 2.0 mm sieve, and subsamples were stored at 4 °C for analyses of soil characteristics or at –80 °C for DNA extraction.

### 2.2. Soil chemical analysis and DNA extraction

Soil pH was determined at a ratio of 1:2.5 (soil/water). Nitrate and ammonium were extracted with 2 M KCl and determined by a Continuous Flow Analyzer (SAN+++, Skalar, Holland). Soil total carbon (TC) and total nitrogen (TN) were obtained with Dumas method by an Element Analyzer (Vario EL III, Elementar, Germany). Soil DNA were isolated with MO BIO UltraClean soil DNA isolation kit (San Diego, CA, USA) according to a modified protocol and eluted with 80 µl of solution S5 (MO BIO Laboratories, cat. no. 12800-100). The purity and quality of extracted DNA were checked with a NanoDrop (ND-1000) spectrophotometer (NanoDrop Technologies, USA) and 1% agarose gel, respectively.

### 2.3. Quantitative PCR assay

qPCR was performed on an iCycler iQ 5 thermocycler (Bio-Rad, Hercules, CA, USA) as described previously (He et al., 2007). In order to reduce the PCR inhibition, soil DNA was diluted 10-fold for qPCR reactions containing bovine serum albumin (BSA). qPCR targeting bacterial and archaeal 16S rRNA and *amoA* genes were all carried out in 25 µl reactions using SYBR Premix Ex Taq (TaKaRa, Shiga, Japan). Detailed information about primer sequences, concentrations and PCR amplification conditions is listed in Table 1. Melting curve analysis from 55 °C to 95 °C was performed to confirm PCR product specificity after amplification. Due to the lower background fluorescence signal in the negative controls, we reduced the number of amplification cycles to 35 for the bacterial 16S rRNA gene quantification analysis. Data analysis was carried out with iCycler software (version 1.0.1384.0 CR).

Generation of standard curves for *amoA* and 16S rRNA gene copy numbers is described in detail in He et al. (2007) and Cao et al. (2012), respectively. Ten-fold serial dilutions of a known copy number of the plasmid DNA were subjected to qPCR in triplicates to generate an external standard curve. Absolute copy numbers of each target gene were calculated directly from these plasmid DNA standard curves.

**Table 1**  
Primers, probes and PCR conditions used for qPCR amplification.

Group	Primer and probe	Sequence (5'–3')	Conc. (nM)	Annealing temperature and time	Reference
Archaea	A364aF <sup>a</sup>	CGGGGYGCASCAGGCGCGAA	500	59 °C for 30 s	Kemnitz et al. (2005)
	A934bR	GTGCTCCCGCCAAATTCCT	500		
Thaumarchaea	771F	ACGGTGAGGGATGAAAGCT	100	54 °C for 30 s	Ochsenreiter et al. (2003)
	957R	CGGCGTTGACTCCAATTG	100		
AOA	Arch- <i>amoA</i> F	STAATGGCTGCTTAGACG	100	53 °C for 45 s	Francis et al. (2005)
	Arch- <i>amoA</i> R	GCGGCCATCCATCTGTATGT	100		
Group 1.1c	1C-200F	AGGAGAGATGGCTTAAAGGGG	100	57 °C for 30 s	Lehtovirta et al. (2009)
	1C-385R	GGATTAACCTCRTCACGCTTTCC	100		
Bacteria	BACT1369F	CGGTGAATACGTTTCYCGG	1000	56 °C for 1 min	Suzuki et al. (2000)
	PROK1492R	GGWTACCTTGTTACGACTT	1000		
	TM1389F	CTTGTACACACCGCCCGTC	1500		
AOB	<i>amoA</i> -1F	GGGGTTTCTACTGGTGGT	100	55 °C for 45 s	Rotthauwe et al. (1997)
	<i>amoA</i> -2R	CCCCTKGSAAAGCTTTCTC	100		

<sup>a</sup> A 40-bp GC-clamp (CGCCCGCGGCCCGCGCCCGCCCGCGCCCGCCCGCCCG) was attached to the 5' end of the primers.

Amplification efficiencies for all the target groups were 80–98% with R<sup>2</sup> values greater than 0.99 for all curves.

#### 2.4. Community structure analysis by DGGE

PCR products for DGGE analysis were amplified with the primer pair A364aF–GC/A934bR targeting archaeal 16S rRNA gene (Table 1). The reactions were performed in triplicate in 50 µl reaction containing 1× PCR buffer, 3.0 mM MgCl<sub>2</sub>, 400 µM each dNTP, 2.5 U Taq DNA polymerase (TaKaRa, Shiga, Japan), 0.5 µM of each primer, for which 35 amplification cycles were used.

Each soil DNA sample was amplified in triplicate to reduce PCR biases then combined for DGGE analysis using the DCode Universal Mutation Detection System (Bio-Rad laboratories, Hercules, USA). PCR products from archaeal 16S rRNA gene amplification reactions were loaded onto polyacrylamide gradient gels (6% polyacrylamide; 1.0 mm thick; 1× TAE; 37.5:1 acrylamide–bisacrylamide) with a denaturing gradient of 30% to 50% (100% denaturant contains 7 M urea and 40% formamide). Gels were run at 90 V for 12 h for archaea. After DGGE, the gels were stained with 1:10,000 SYBR Gold Nucleic Acid Gel Stain (Invitrogen Molecular Probes, Eugene, USA) for 30 min, then rinsed twice with sterilized water, and scanned by a GBOX/HR-E-M (Gene Company Limited, Syngene, UK).

#### 2.5. Sequencing and phylogenetic analysis

The DGGE image was analyzed with Quantity One software (Bio-Rad Laboratories, Hercules, USA), and dominant bands were excised using 10-µl sterile pipette tips. Excised bands were eluted from acrylamide by incubation in 30 µl sterilized water overnight. The PCR products were re-amplified with the primers A364aF/A934bR without GC clamp and ligated into the pGEM-T Easy Vector (Promega, Madison, WI, USA), and transformed into *Escherichia coli* JM109 competent cells growing in Luria–Bertani broth at 37 °C overnight. Inserts in several positive clones were amplified using the same primers with a GC clamp, and checked by DGGE. Inserts with the correct migration profile on DGGE gels were selected for Sanger sequencing.

Sequence chromatograms were manually edited with DNASTar and DNAMAN version 6.0. Phylogenetic analyses were conducted using MEGA version 5.0, and a neighbor-joining tree was constructed using Kimura 2-parameter distance with 1000 bootstraps (Tamura et al., 2011).

#### 2.6. Statistical analysis

Copy numbers were log-transformed as necessary to normalize distributions prior to statistical analysis. One-way analysis of variance (ANOVA) followed by S–N–K-test was used to check for quantitative differences between treatments using SPSS version 16.0 (SPSS Inc.,

Chicago, IL). Spearman's correlation coefficients were calculated to assess significant relations between gene copy numbers and soil chemical parameters (SPSS 16.0).  $P < 0.05$  was considered to be statistically significant. The band intensities on DGGE gels were digitized and used for downstream statistical analysis. Non-metric Multidimensional Scaling (NMDS) analysis was applied to graphically visualize 16S rRNA community composition patterns based on DGGE band profiles using the package "MASS" in the R (version 2.13.2) statistical programming environment with Euclidian distance measure.

#### 2.7. Sequence accession numbers

The archaeal 16S rRNA gene sequences have been deposited in GenBank under accession numbers JN671920 to JN671942.

### 3. Results

#### 3.1. Variances of soil characteristics among different land use practices

Soil chemical properties are listed in Table 2. There was no evident variation in pH among different land use practices and soil core depths, except in degradation land, which had a significantly lower pH in surface samples (0–20 cm) than in subsurface samples (20–40 cm). Interestingly, total carbon (TC) and total nitrogen (TN) in the surface layers of three land use practices (i.e. cropland, restoration and degradation land) were significantly higher than those of subsurface samples, while no difference was observed in the pine forest. The highest TC and TN values were both recorded in the surface samples of cropland. Land use practice had significant impact on soil ammonium and nitrate contents (Table 2). Regardless of the depth, soil nitrate content generally decreased in the order of pine forest > cropland > degradation land, restoration land. One-way ANOVA identified significant variations in soil pH, TC and TN ( $n = 24$ ,  $P < 0.01$ ) as a function of land use practice.

#### 3.2. The abundance of archaeal groups based on 16S rRNA and *amoA* genes

In order to make a comprehensive investigation of archaeal groups, we quantified the abundance of archaeal 16S rRNA and *amoA* genes, as well as bacterial 16S rRNA and *amoA* gene copy numbers using qPCR. Archaeal 16S rRNA gene copies accounted for 6–11% of total prokaryotes (bacterial and archaeal 16S rRNA genes) in surface soils, while in the subsurface they accounted for 10–23%. There was no significant difference in the abundance of archaea, Thaumarchaea and Group 1.1c among different land use practices as assessed by copy numbers of 16S rRNA genes, although archaeal and Thaumarchaeal abundances were generally one to two orders of magnitude higher than Group 1.1c at both depths investigated (Fig. 1). The highest bacterial 16S rRNA gene abundance was observed in surface soil of restoration land

**Table 2**  
Soil basic chemical properties and bacterial 16S rRNA and *amoA* gene copy numbers among different land use practices.

Land type	Depth (cm)	pH (H <sub>2</sub> O)	TC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	Bacterial 16S rRNA gene	Bacterial <i>amoA</i> gene
							Log number of gene copies g <sup>-1</sup> dry soil	
Cropland	0–20	4.04 ± 0.06ab <sup>a</sup>	14.81 ± 0.06a	1.26 ± 0.01a	20.87 ± 0.84ef	10.57 ± 0.56c	8.67 ± 0.17ab	4.75 ± 0.10ab
	20–40	3.72 ± 0.17b	7.50 ± 0.18g	0.85 ± 0.04f	20.51 ± 0.15f	10.93 ± 0.96c	7.96 ± 0.16d	4.55 ± 0.02ab
Pine forest	0–20	3.97 ± 0.05ab	10.78 ± 0.09d	1.05 ± 0.01d	32.16 ± 0.93b	12.70 ± 0.97b	8.34 ± 0.11bc	3.99 ± 0.24b
	20–40	3.90 ± 0.24b	10.64 ± 0.15d	1.07 ± 0.01d	23.13 ± 1.16d	21.88 ± 1.21a	8.56 ± 0.17bc	4.55 ± 0.58ab
Restoration land	0–20	3.96 ± 0.06ab	11.52 ± 0.04c	1.11 ± 0.01c	29.93 ± 0.25c	4.44 ± 0.27e	8.92 ± 0.02a	5.22 ± 0.50a
	20–40	3.89 ± 0.14b	7.97 ± 0.16e	0.87 ± 0.01ef	33.93 ± 0.30a	4.96 ± 0.15e	8.29 ± 0.20c	4.13 ± 0.22b
Degradation land	0–20	4.22 ± 0.01a	13.93 ± 0.09b	1.14 ± 0.02b	21.82 ± 1.12def	6.28 ± 0.33d	8.68 ± 0.14ab	4.62 ± 0.33ab
	20–40	3.87 ± 0.03b	7.71 ± 0.05f	0.89 ± 0.00 e	22.50 ± 1.10de	3.87 ± 0.50e	8.39 ± 0.03bc	4.49 ± 0.20ab

<sup>a</sup> Different letters in the same column indicate statistical differences (*P* < 0.05) among land use practices.

with  $8.28 \times 10^8$  copies per g of dry soil, while the lowest abundance was found in subsurface cropland soil with  $9.63 \times 10^7$  copies per g of dry soil (Table 2).

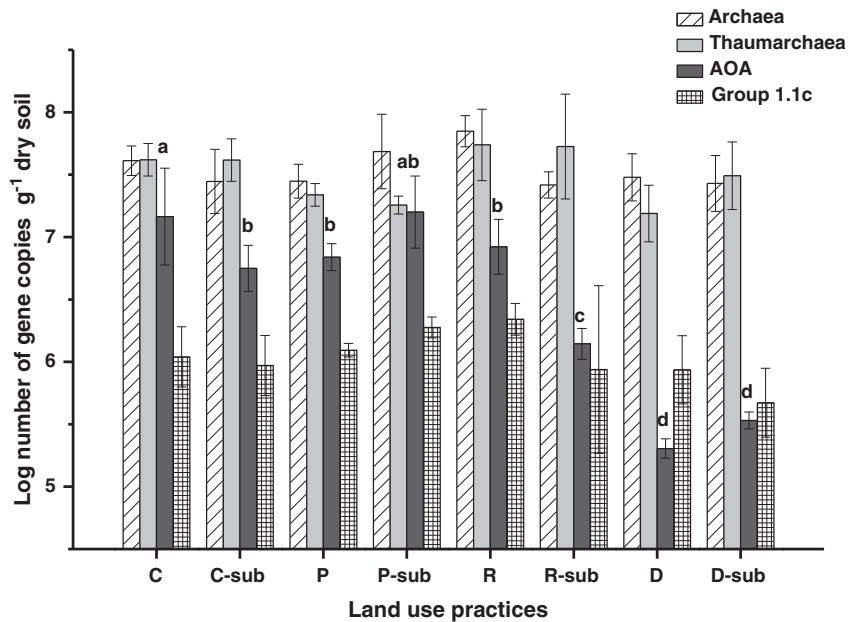
By contrast, we did observe significant impact of land use practice on the abundance of archaeal *amoA* genes, ranging from  $2.04 \times 10^5$  copies per g of dry soil in degradation land to  $3.24 \times 10^7$  copies per g of dry soil in cropland. It was interesting to note that archaeal *amoA* gene copy numbers at both depths of degradation land, and in subsurface of restoration land, were dramatically lower than those in other soils. In terms of depth, archaeal *amoA* gene copy numbers were significantly higher in the surface soils of cropland and restoration land than those in the subsurface samples, while the opposite trend was observed for the other two land use practices (Fig. 1). No significant differences in bacterial *amoA* gene copy numbers were detected between the two depths examined, except in restoration land for which the surface layer has a higher abundance than the sub-surface (Table 2). The highest ratio of archaeal to bacterial *amoA* gene copy numbers was recorded in surface soil of pine forest (approx. 729:1), while the lowest was found in surface soil of degradation land (6:1).

Spearman's correlation coefficients between gene abundance and soil chemical properties are listed in Table 3. The copy number of archaeal *amoA* gene was significantly positively correlated with NO<sub>3</sub><sup>-</sup> and TN content, but not with other soil chemical properties. Bacterial 16S rRNA gene copy numbers were significantly correlated with soil

pH, TC and TN content, whereas archaeal 16S rRNA gene copy numbers was significantly correlated with soil TN.

### 3.3. Community structure of archaea revealed by DGGE analyses

Effects of land use practices on community composition of archaea were identified by employing a PCR-DGGE approach targeting archaeal 16S rRNA gene. Banding patterns with clear differences in the number of dominant bands showed a differential response of archaeal groups to change in land use practice (Fig. 2). For example, bands 7 and 8 were the major DGGE bands observed for cropland, while bands 1–8 were dominant in the pine forest samples. Degradation land samples gave weak DGGE banding patterns, however, band 9, for example, was barely detectable by SYBR-staining. Based on pairwise dissimilarity of the relative intensity of DGGE bands for each sample, the results of NMDS clearly showed that land use practice was the main factor regulating the archaeal community structure, while regulation by depth was less pronounced (Fig. 3). Clone library analysis and sequencing techniques were applied to identify the bacterial or archaeal taxa corresponding to each main band (Fig. 4). The bands 2–8 present in the upper part of the gel were affiliated with Group 1.1a, while band 1 clustered with Group 1.1a. Most of the bands (bands 9, 11–13) at the lower part of the gel were placed within Group 1.1c, except for band 10, which clustered with Group 1.1c associated.



**Fig. 1.** Quantification analysis of total archaea, Thaumarchaea AOA and Group 1.1c among different land use practices and at two soil depths. The abundance of archaea, Thaumarchaea and Group 1.1c was detected using general primers for the 16S rRNA gene detection and AOA-specific *amoA* primers (Table 1). Different letters above black columns indicate statistical differences (*P* < 0.05) among land use practices. Treatment: C (cropland 0–20 cm); C-sub (cropland 20–40 cm); P (pine forest 0–20 cm); P-sub (pine forest 20–40 cm); R (restoration land 0–20 cm); R-sub (restoration land 20–40 cm); D (degradation land 0–20 cm); and D-sub (degradation land 20–40 cm).

**Table 3**

Spearman's linear correlation coefficients between copy numbers of targeted genes and soil chemical properties.

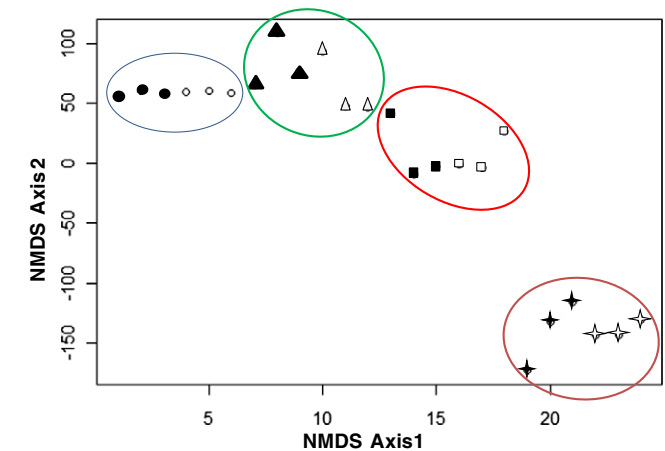
	pH	TC	TN	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>
Archaea	ns	ns	0.440*	ns	ns
Thaumarchaea	ns	ns	ns	ns	ns
AOA	ns	ns	0.414*	ns	0.602**
Group 1.1c	ns	ns	ns	ns	ns
Bacteria	0.463**	0.575**	0.793**	ns	ns
AOB	ns	ns	ns	ns	ns

Significance levels: ns:  $P > 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ .

## 4. Discussion

### 4.1. Impacts of land use management on abundances of archaea

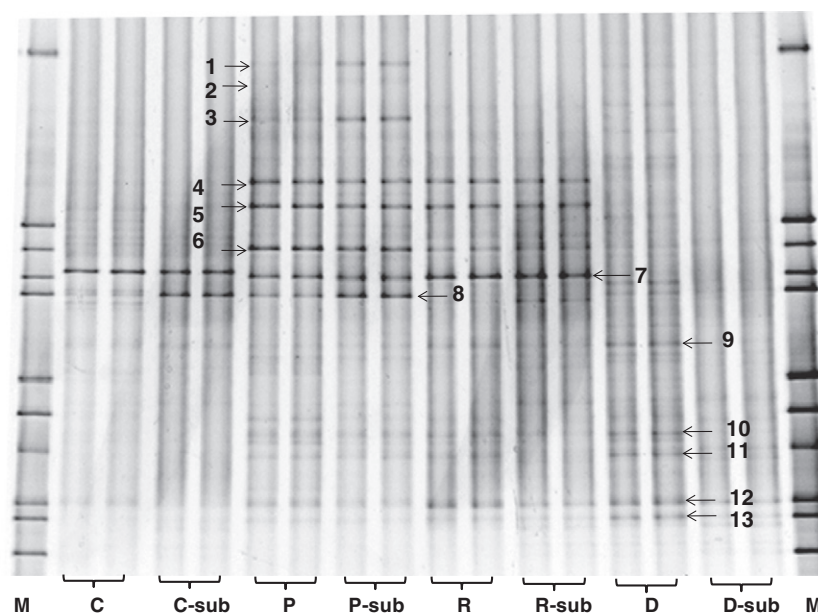
In this study, we have shown that variations in the abundance of archaeal and bacterial groups reflected the long-term impact of different land use practices on the associated terrestrial microbial communities. The abundance of archaea is comparable to that of Thaumarchaea in our samples, indicating that Thaumarchaea might be the dominant archaeal group in upland soils (Nicol et al., 2003). The changes in archaeal abundance as a function of sampling depth are quite similar to what has been observed in varied soil profiles collected from Northern to Southern China (Cao et al., 2012). Despite significant lower TC and TN concentrations at subsurface of all soil samples except pine forest, the archaeal abundance was not considerably lower compared with surface layer. This was due to the adaptive characteristic of archaea to chronic energy stress, enabling them to better survive under unfavorable niches (Valentine, 2007). Contrary to the observed variability in bacterial abundances, archaeal abundances remained relatively stable among different land use practices, irrespective of depth with the exception of a slight decrease in Group 1.1c in the degradation land samples (Fig. 1). These results indicate relatively low long-term impacts of land use on abundance of the archaeal groups examined here. Cao et al. (2012) also showed that archaeal 16S rRNA gene copy numbers did not vary significantly between different samples of the same soil type, although they did vary among four different soil types. A study on Amazonian soils also arrived at similar conclusion (Taketani and Tsai, 2010), although



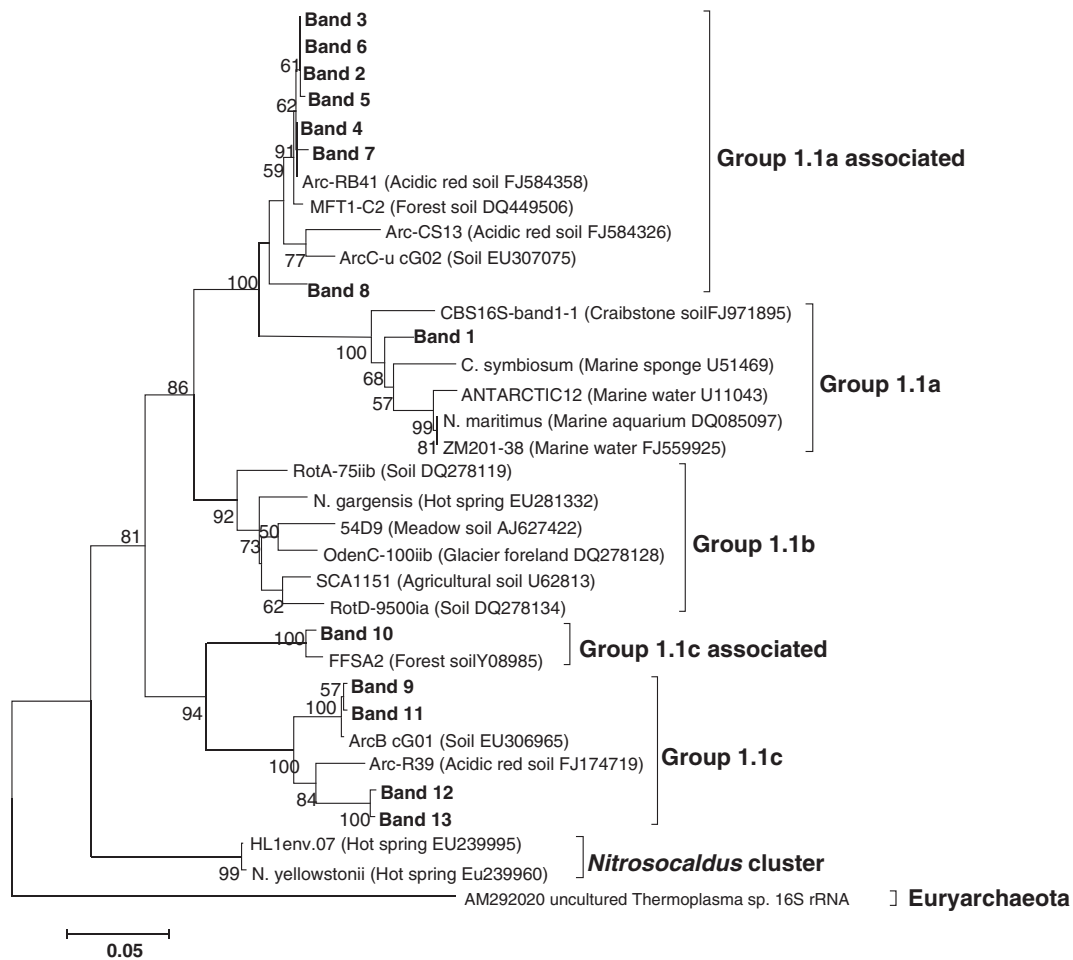
**Fig. 3.** Non-metric MDS comparison of DGGE band intensity profiles for archaea. Stress = 0.15; close circle symbols (●) represent the treatment of cropland 0–20 cm; open circle symbols (○) represent cropland 20–40 cm; close triangle (▲) represents pine forest 0–20 cm; open triangle (△) represents pine forest 20–40 cm; close square (■) represents restoration land 0–20 cm; open square (□) represents restoration land 20–40 cm; close star (★) represents degradation land 0–20 cm; open star (☆) represents degradation land 20–40 cm.

the composition of archaeal communities were found to be heterogeneous (Fierer et al., 2007; Nicol et al., 2003; Sliwinski and Goodman, 2004) and mostly associated with roots and fungi (Bomberg and Timonen, 2007). These studies have provided insight into archaeal community structures in different soil habitats, however, they do not investigate archaeal abundances. The low variation in abundances of archaea across different land use programs as observed in this study cannot exclude the possibility that land use practice may change the structure of archaeal communities. We therefore examined the composition of archaeal groups in order to determine if archaeal community structures reflected differences in land use practices.

Considering the importance of ammonia oxidizing archaea in soil nitrification, we further quantified the abundance of *amoA* genes encoding ammonia monooxygenase subunit A, which is the most commonly-used phylogenetic marker for AOA. A strong correlation was found between archaeal *amoA* gene and archaeal 16S rRNA



**Fig. 2.** Denaturing gradient gel electrophoresis (DGGE) profiles of archaeal 16S rRNA genes in soils from sites with different land use practices. Band position is indicated with numbered arrows. Lane number: C (cropland 0–20 cm); C-sub (cropland 20–40 cm); P (pine forest 0–20 cm); P-sub (pine forest 20–40 cm); R (restoration land 0–20 cm); R-sub (restoration land 20–40 cm); D (degradation land 0–20 cm); D-sub (degradation land 20–40 cm); and M: marker.



**Fig. 4.** Phylogenetic relationships among archaeal 16S rRNA gene sequences retrieved from soil samples under different land use practices. Band numbers in bold correspond to DGGE band position as in Fig. 2. Bootstrap values (>50%) are indicated at branch points. The scale bar represents 5% estimated sequence divergence.

gene abundances as determined by qPCR ( $r = 0.508$ ,  $n = 24$ ,  $P < 0.05$ ), with the former being approximately one to two orders of magnitude lower than the latter (Fig. 1). This is consistent with the findings in the deep Northern Atlantic ocean (Agogue et al., 2008) as well as in aquarium biofilter (Sauder et al., 2011), where the abundance of Thaumarchaeal 16S rRNA gene copies was 100 to 1000 times higher than that of archaeal *amoA* genes in the same samples. One possible explanation is that not all Thaumarchaea possess *amoA* genes, as evidenced by one molecular study on two representative Thaumarchaeal cultures (Muller et al., 2010). Additionally, it is still unclear whether all the Thaumarchaea in possession of an *amoA* gene have the ability to perform ammonia oxidation (Pester et al., 2011), such as Group 1.1c. Group 1.1c accounted for 1–9% of total archaea in the soils investigated in this study, which is similar to findings from a range of soils with low pH ( $pH < 6.5$ ) (Lehtovirta et al., 2009). The above study further suggested that Group 1.1c abundance declined along an increasing pH gradient, while total Thaumarchaea showed no clear trend, indicating the role of pH in specifically regulating the abundance of Group 1.1c (Lehtovirta et al., 2009). Group 1.1c has been found in a wide range of soils, the majority of which are acidic or rich in organic matter (Jurgens and Saano, 1999; Kemnitz et al., 2007; Timonen and Bomberg, 2009). The four different land use types here are of the same acidic soil type, with pH ranging from 3.72–4.22, potentially providing favorable growth conditions for the acidophilic Group 1.1c. This narrow pH range might have limited our ability to reveal a significant correlation between pH and Group 1.1c abundance. Additionally, no significant correlation was found between the abundance of Group 1.1c and nitrate content,

indicating the contribution of this group to ammonia oxidation might be minimal. However, further evidence may be required to support this point.

No significant difference was observed in AOB among different land use at the same depth in the present study (Table 2). A higher ratio of archaeal *amoA* gene copy numbers to bacterial *amoA* gene copy numbers was obtained, consistent with previous studies (He et al., 2007; Leininger et al., 2006). This observation indicates the significant role of AOA in nitrification of acidic red soils (Gubry-Rangin et al., 2011; Yao et al., 2011). The finding was further supported by the significant correlation between nitrate content and archaeal *amoA* gene copy numbers (Table 3), but not bacterial *amoA* genes or archaeal 16S rRNA genes. It is more likely that the archaeal *amoA* gene responds more sensitively to land use change and may be a better indicator of disturbance. The noteworthy removal of ground surface vegetation from degradation land may alter the bio-availability of organic carbon, and consequently make conditions unfavorable for the mixotrophic and heterotrophic modules of AOA metabolism (Walker et al., 2010; Wessén et al., 2010). The alteration of community AOA feed back into the quantity of N supply available to plants, thereby influence plant nutrient uptake and productivity (Schimel and Bennett, 2004). Moreover, the variation in AOA abundance in degradation land may originate from mineralization of organic matter (Stopnisek et al., 2010). Previous studies have demonstrated that rates and dynamics of nitrogen mineralization are lower in older secondary succession forests or older pastures than in undisturbed forests (Carney et al., 2004; Keller et al., 2005). The low values of nitrate found in degradation land samples as compared with other three land use practices corroborate these results.

Variation in the abundance of bacterial 16S rRNA gene copies between different land use soils generally agrees with findings of previous studies, in which changes in abundance were mainly attributed to changes in soil characteristics (Hansel et al., 2008; Jesus et al., 2009; Kemnitz et al., 2007; Pesaro and Widmer, 2002). In this study, we identified a significant correlation between bacterial 16S rRNA gene abundance and soil pH, TC and TN (Table 3). Numerical studies have shown that the abundance of bacteria in a variety of land use types is strongly related to soil pH on a local scale (Rousk et al., 2010) and continental scale (Fierer and Jackson, 2006; Lauber et al., 2008). Long-term differentiation in soil use and management alter the resource availability and eventually influence the growth of microorganisms, leading to the variation in soil biodiversity and abundance (Attard et al., 2010; Hartman et al., 2008).

#### 4.2. Impacts of land use practices on community compositions of archaeal groups

The patterns of DGGE profiles using 16S rRNA gene clearly identified differences in archaeal community structure across different land use practices. Although the number of dominant DGGE bands varied among cropland, pine forest and restoration land, the gene sequences retrieved from dominant bands mainly from these sample types belonged to Group 1.1a archaea, which were not identified from dominant bands in the degradation land samples (i.e. Group 1.1c). Group 1.1a sequences have also been retrieved from other acidic soils (Lehtovirta-Morley et al., 2011; Pester et al., 2012). This group may thus represent a common phylotype contributing to ammonia oxidation in these habitats, as evidenced by global, regional and local scale studies on the genetics and physiology of cultivated isolates (Gubry-Rangin et al., 2011; Lehtovirta-Morley et al., 2011). For the interpretation of results for archaeal 16S rRNA and *amoA* gene abundance, it is likely that Group 1.1a decreased dramatically in degradation land with the result that archaeal *amoA* abundance varied greatly in these samples relative to the other three land use types. The community shift observed between these land use practices (irrespective of depth) can be mainly attributed to land use change (Fig. 4), consistent with a previous result obtained at the same location but at a different slope position (Ying et al., 2010). Degradation land harbored AOA that were compositionally distinct from those in other three land use practices (Fig. 2), which may be related to rates of N cycling among these systems. Previous study has indicated that clear-cutting of forests resulted in distinct soil community restriction profiles compared with untreated standing forest (Jurgens and Saano, 1999). Differences in community structure of archaea were also observed between managed and natural grassland in Scotland, although the gene sequences were mostly associated with Group 1.1b (Nicol et al., 2003). In this study, no sequences affiliated with Group 1.1b were detected, which could possibly be due to the biases caused by primer choice (Baker et al., 2003) or DGGE detection (Teske and Sorensen, 2008). However, this group appears to prefer soils with pH higher than 5 (Timonen and Bomberg, 2009), though it has been retrieved in more studies than other common archaeal groups (i.e. Group 1.1a associated and Group 1.1c in this study). The soil pH for all samples in this study were below 4.3, which favor the growth of Group 1.1c as evidenced by molecular studies associated with acidic biomes including forest soils and agriculture soils (Cao et al., 2012; Kemnitz et al., 2007; Lehtovirta et al., 2009; Nicol et al., 2005; Stopnisek et al., 2010). Future studies might test for the presence of Group 1.1b in these soils using high-throughput sequencing methods like 454-pyrosequencing.

## 5. Conclusions

In conclusion, land use change had clear impacts on the abundance of archaea and bacteria based on quantification analysis of 16S rRNA and *amoA* genes. Archaeal 16S rRNA gene abundances were less

influenced by land use practices than bacterial abundance, indicating that archaea may be more resistant to land use-associated disturbance in terms of abundance, but not community structure. Through the numerical comparison of different archaeal groups based on 16S rRNA and *amoA* genes, AOA may be valuable as potential indicators for assessing the influence of soil disturbance on terrestrial microorganisms (Wessen and Hallin, 2011). This study also demonstrated good correlations between soil nitrate content and archaeal, but not bacterial, *amoA* gene copy numbers, which combined with the higher ratio of AOA/AOB suggest an important role of AOA in ammonia oxidation in these soils. No specific factor was identified in shaping archaeal community structures, which were distinct among the different land use types. Other factors like soil phosphorus and texture were not examined in this study but might affect the abundance and community structure of archaea, and should be considered for future analyses.

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