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ORIGINAL ARTICLE

A comparative study of ammonia-oxidizing archaea and bacteria in acidic and alkaline purple soils

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Abstract Soil pH has been suggested as one of the most important factors affecting the ecological characteristics of soil ammonia-oxidizers (AO), which mediate the conversion of ammonia to nitrate via nitrite and contribute significantly to the leaching of nitrate to groundwater and the production of atmospheric nitrous oxide (N_2O). However, the dynamics of the AO community in acidic purple soils, which are widely distributed in Southwest China, remain largely unknown. In this study, two typical purple soils with different pH values (acidic: ACI; alkaline: ALK) were collected and studied. The abundance of *amoA* (gene encoding ammonia monooxygenase) of ammonia-oxidizing bacteria (AOB) and archaea (AOA) and that of the *cbbL* gene (encoding ribulose-1,5-biphosphate carboxylase/oxygenase) were determined by real-time PCR, and the community structures of AOB and AOA were investigated by cloning and sequencing. The results revealed that abundances of AOB and AOA were significantly lower in the ACI purple soil sample than in the ALK sample, but a higher ratio of AOA to AOB was found in the ACI purple soil sample. No significant difference in the abundance of *cbbL* was found between the two soils, but the ratio of AOB and AOA *amoA* to *cbbL* genes in the ACI soil samples was higher than that in the ALK sample. Moreover, the ALK and ACI soils harbored contrasting community compositions of AO. AOB in the ALK were dominated by cluster 3a (87 %), while the percentage of cluster 3a decreased and clusters 9 and 10 accounted for almost 77 % of the AOB community in the ACI soil. *Nitrososphaera* and *Nitrosotalea* were the major AOA phylotypes in the ALK and ACI soils, respectively. In conclusion, our results revealed the potential relations among pH, AO, and total chemoautotrophic bacteria in soil and that pH might have an essential impact on the adaptation and selection of AO in purple soils.

Keywords Purple soil · pH · Ammonia-oxidizers · *amoA* · *cbbL* · Community composition

Introduction

Ammonia oxidation, as the first and rate-limiting step in nitrification, is mainly driven by ammonia-oxidizers (AO) and is considered to be one of the most important processes in the soil nitrogen (N) cycle (Deboer et al. 1991). In the soil environment, ammonia oxidation can trigger the subsequent nitrification process, resulting in the transformation of ammonia to nitrate; the nitrate can be leached into groundwater and finally be reduced to nitrous oxide (N_2O) or nitrogen gas (N_2) by denitrification, which is an important part of the global N-cycle and can also lead to soil nitrogen losses and environmental problems (Kowalchuk and Stephen 2001; Ravishankara et al. 2009). Th importance of soil ammonia oxidation has been increasingly recognized in recent years, and increasing attention has focused on the drivers of soil AO and the relative contributions of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) to nitrification (Zhang et al. 2012; Hu et al. 2014).

AOB were considered to be the sole key drivers of soil ammonia oxidation until the discovery of AOA (Konneke et al. 2005). Subsequent studies confirmed that both AOB and AOA may be the main drivers of ammonia oxidation in

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soil, but their relative contributions to ammonia oxidation is still under debate (He et al. 2012; Hu et al. 2014). It is assumed that soil AOA may be the primary participants in ammonia oxidation under various harsh environmental conditions, such as low pH, extremely low or high temperature, high salinity, low oxygen, and low concentrations of the ammonium cation (NH_4^+) (Erguder et al. 2009). Of these properties, soil pH, which affects the form and availability of a number of important elements [N, sulfur, phosphorus (P)] in soil, has long been considered as one of the most essential factors determining the relative contributions of AOB and AOA (Hu et al. 2014). The direct relationship between autotrophic nitrification activity and AOA in acidic soils has been verified using DNA enrichment detection with stable $^{13}CO_2$ (Lehtovirta-Morley et al. 2011; Zhang et al. 2012). More recently, the ecological characteristics of AO in soils with different pH values have attracted worldwide attention (Gubry-Rangin et al. 2011; Yao et al. 2013; Hu et al. 2014). Gubry-Rangin et al. (2011) found that soil AOA can be clustered into acidophilic, acido-neutral, and alkalinophilic groups based on the soil pH value, with the former dominant in soils with pH<5.5, acido-neutral AOA dominant in soils with pH 5.5–7.5, and the latter dominant in soils with pH>7.5. It has also been reported that in some Chinese agricultural soils pH is the most important factor controlling the community structures of AOA and AOB (Yao et al. 2013; Hu et al. 2014). Although these studies confirm the pH-dependent adaptation and selection of soil AOB and AOA, the factors affecting AOB and AOA are very complex (Prosser and Nicol 2012), and the effects of parent material should not be ignored in the investigation of ecological characteristics of soil AO. Therefore, for a better understanding of the pH-dependent adaptation and selection of soil AO, comparative studies on the community compositionthe relationship between soil carbon dioxide (CO_2)-fixing bacteria and the CO_2 budget has received much attention, and the community structure of total chemoautotrophic bacteria within this framework has been investigated by many researchers (Tolli and King 2005; Videmsek et al. 2009; Yuan et al. 2013; Wu et al. 2014; Xiao et al. 2014). However, although AO represent one of the important groups of CO_2-fixing microorganisms, the relationship between AO and total chemoautotrophic bacteria has only rarely been reported. Soil AOB and AOA have long been considered to be obligate chemoautotrophic organisms, but recent studies have demonstrated that AOA and AOB also have the ability to utilize organic carbon at low concentrations (Schmidt 2009; Tourna et al. 2011), possibly indicating that AO are not obligate chemolithoautotrophs but mixotrophs. However, despite the potential for being obligate chemolithoautotrophs or mixotrophs, both AOA and AOB genomes contain genes encoding enzymes involved in carbon fixation via the modified 3-hydroxypropionate/4-hydroxybutyrate pathway (Blainey et al. 2011) and the ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) pathway (Kusian and Bowien 1997). Therefore, as an important characteristic of AO, the functional genes related to carbon fixation should be taken into consideration in the investigations of soil AO dynamics.

Purple soils are the most representative soil type in the Sichuan basin of Southwestern China. These soils are characterized by lithologic soils without distinct pedogenic horizons and have developed from the fast physical weathering of sedimentary rocks of the Trias-Cretaceous system (He 2003). They are classified as Orthic Entisols according to the Chinese Soil Taxonomic System and as Regosols in the FAO Taxonomy or Entisols in USDA taxonomic terms. In this study, we have investigated the abundance of *amoA* (gene encoding ammonia monooxygenase) of AOB and AOA and of *cbbL* (encoding RuBisCO), as well as the community structures of ammonia-oxidizing microorganisms in two purple soils (originating from the same parent material) with distinctly different pH values. The objective was to compare the differences in abundance and community structure of AOB and AOA in purple soil with different pH values and to study the relationship between the abundance of ammonia-oxidizing microorganisms and that of the total chemoautotrophic bacteria. This knowledge could be helpful for a better understanding of effect of pH on ammonia oxidation in soils.

Materials and methods

Site description and soil sampling Soil samples were collected from two agricultural fields with a similar planting pattern (cabbage–sweet potato rotation), in the Jiangjin [28°75′N, 106°46′E; acidic (ACI) purple soil] and Fulin [29°57′N, 107°11′E; alkaline (AKI) purple soil] districts of Chongqing (China). Six soil cores (diameter 5 cm; depth 20 cm) were collected randomly from each plot and mixed thoroughly to form one composite sample. Each treatment contained three replicate soil samples. After the fine roots and visible organic debris had been removed by passage through a 2-mm sieve, the soil samples were stored at 4 °C and −80 °C for chemical analysis and DNA extraction, respectively.

Physicochemical determination of soil Soil pH was determined in a water suspension (soil:water, 2:5). Soil organic matter (OM) was measured using the $K_2Cr_2O_7$ oxidation method. Soil total nitrogen (TN), total P (TP), and total potassium (TK) content were determined by micro-Kjeldahl digestion, colorimetric analysis, and a dissolution-flame photometer, respectively (Lu 1999). Soil available N (AN), available P (AP), and available K (AK) were determined by conventional methods described by the Chinese Society of Soil Science (Lu 1999). Soil properties are listed in Table 1.

Table 1 Basic properties of the alkaline and acidic purple soils

Samples[a]	pH	OM (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	AN (mg kg^{-1})	PNR (μg NO_2^--N g^{-1} dry soil h^{-1})
ACI soil	5.10[b]	5.11[b]	0.55[b]	0.21[b]	10.60[b]	3.80[b]	82.00[a]	37.70[b]	0.07[b]
ALK soil	7.50[a]	14.20[a]	1.27[a]	0.64[a]	18.30[a]	9.40[a]	72.50[a]	76.30[a]	0.36[a]

Values are the mean of 3 measurements (n=3), and values within the same column followed by the different lowercase letters indicate significant differences at $P<0.05$

OM, Organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium; PNR, soil potential nitrification rate

[a] ACI, Acidic purple soil; ALK, alkaline purple soil

Extraction of soil DNA Soil DNA was extracted from 0.5 g of fresh soil using a Fast DNA® SPIN kit for soil (Q BIOgene, USA) according to the manufacturer's protocol. The extracted DNA was checked by electrophoresis on a 1 % agarose gel and then stored at −20 °C prior to use.

Measurement of potential nitrification rates Soil potential nitrification rate (PNR) was measured by the chlorate inhibition method (Kurola et al. 2005). Briefly, $(NH_4)_2SO_4$ was added to catalyze the formation of nitrite, and $KClO_3$ was added to inhibit the last step in which the nitrite is transformed to nitrate. The accumulation of nitrite was then measured to calculate the PNR value.

Quantification of bacterial and archaeal *amoA* and *cbbL* genes Real-time PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, USA), and the standard curves for real-time PCR assays were developed as described previously (He et al. 2007). Amplification was performed in 25-μL reaction mixtures using SYBR® Premix Ex Taq™ following the manufacturer's instructions (Takara Biotechnology, Japan), with the original DNA as template in each reaction mixture. The primer sets (AOB: *amoA*-1 F/*amoA*-2R; AOA: Arch-*amoA*F/Arch-*amoA*R; *cbbL*: K2f/V2r, specific to all chemolithotrophs in the bacterial domain) and thermal profiles used in the amplification of each target gene are listed in Table 2. As the temperature increased from 55 to 95 °C during the thermal cycling step, a melting curve analysis was performed to assess the specificity of the PCR products for each real-time PCR amplification by measuring the fluorescence continuously as the temperature increased from 55 °C to 95 °C. Using iCycler software (version 1.0.1384.0 CR), the parameter C_t (threshold cycle) was determined as the cycle number at which the start of exponential increase in the reporter fluorescence was detected.

Construction and analysis of bacterial and archaeal *amoA* clone libraries Bacterial and archaeal *amoA* gene clone libraries were constructed based on purified PCR products, using the primer sets of Arch-amoAF/Arch-amoAR and amoA1F/amoA2R, respectively (Table 2). PCR reactions (50 μL) contained 5 μL of 10× PCR buffer (Mg^{2+} plus), 4 μL of 2.5 mM dNTPs, 0.5 μL of Ex Taq polymerase (5 U μL^{-1}; Takara Biotechnology), 1 μL of each primer (10 μM), and 2 μL of DNA template (1–10 ng). Each PCR product was gel-purified with the Wizard® SV Gel and PCR Clean-Up System (Promega Corp., USA). The purified PCR products were ligated into the pGEM-T Easy Vector (Promega Corp.) and then transformed into *Escherichia coli* JM109 (Takara Biotechnology) following the manufacturer's instructions. The positive clones (110) were randomly selected from each clone library and sequenced by a DNA sequencer (ABI 3730XL; Applied Biosciences, USA). The homology analysis of the obtained sequences was performed by the software

Table 2 Primer sets and thermal profiles used in PCR amplification

Target group	Primer set	Sequence (5′-3′)	Thermal profile	Reference
AOB	*amoA*-1 F *amoA*-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C,30 s at 55 °C and 1 min at 72 °C, and 5 min at 72 °C for the last cycle	Rotthauwe et al. 1997
AOA	Arch-*amoA*F Arch-*amoA*R	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C,30 s at 53 °C and 1 min at 72 °C, and 5 min at 72 °C for the last cycle	Francis et al. 2005
cbbL	K2f V2r	ACCAYCAAGCCSAAGCTSGG GCCTTCSAGCTTGCCSACCRC	5 min at 94 °C, followed by 38 cycles of 30 s at 94 °C,30 s at 57 °C and 1 min at 72 °C, and 5 min at 72 °C for the last cycle	Tolli and King 2005

cbbL, Gene encoding ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO)

DNAMAN (version 6.0.3.48;, Lynnon Biosoft, USA). The sequences displaying >97 % identity with each other were classified into the same operational taxonomic units (OTUs), and one representative sequence of each OTU was used to construct the phylogenetic tree. The phylogenetic analysis based on the sequences (obtained from this study and reference sequences from GenBank) was conducted with the software MEGA (version 4.0), and the neighbor-joining tree was constructed using Kimura two-parameter distance with 1,000 replicates to produce bootstrap values (Tamura et al. 2007). Analytic Rarefaction version 1.3 software (http://www.uga.edu/strata/software.hml) was used for the rarefaction analysis of each clone library. The sequences obtained in this study have been submitted to the GenBank nucleotide sequence database with the accession numbers KJ433520–KJ433548 (AOB) and KJ433492–KJ433519 (AOA).

Statistical analysis A one-way analysis of variance followed by a Student–Newman–Keuls test was performed using SPSS version 11.5 software (IBM Corp., USA) to check for quantitative differences between treatment groups. A *P*value of <0.05 was considered to be significant.

Results

Soil properties and PNR

Soil properties and PNR are listed in Table 1. The pH value of the ALK purple soil and ACI purple soil samples was 7.5 and 5.1, respectively. Except for there being no significant difference in soil AK, the contents of OM, TN, TP, TK, AP, and AN were all lower in the ACI soil than in the ALK soil. A significantly higher PNR value was detected in the ALK soil sample, which was almost fivefold higher than that of the ACI sample.

Abundance of archaeal, bacterial *amoA* and *cbbL* genes

In the ALK soil sample, the copy number of AOA *amoA* gene (1.09×10^7 g^{-1} dry soil) was higher than AOB *amoA* gene (1.93×10^6 g^{-1} dry soil) or *cbbL* (1.47×10^6 g^{-1} dry soil), and no difference was found for the copy numbers of AOB *amoA* and *cbbL* genes (Fig. 1). In the ACI sample, the copy numbers of these three functional genes were in the order of AOA (3.91×10^6 g^{-1} dry soil)>*cbbL* (2.13×10^6 g^{-1} dry soil)> AOB (1.72×10^5 g^{-1} dry soil). Additionally, no difference in the copy number of *cbbL* was found between these two purple soils, but the abundance of both the AOA and AOB *amoA* genes in the ALK soil were higher than those in the ACI soil sample. Moreover, the ratio of AOA to AOB in the ALK soil (5.65) was lower than that in the ACI soil (22.79), and the ratios of AOA and AOB *amoA* to *cbbL* gene in the ALK soil (7.44 and 1.32, respectively) were higher than those in the ACI sample (1.84 and 0.08, respectively).

Results of cloning and sequencing

To analyze the structure of the AO communities in the ALK and ACI soils, we randomly selected and sequenced 110 positive clones from each clone library. The rarefaction curves of the four libraries tended to approach the saturation plateau indicating that the positive clones selected in each library adequately covered the diversity of the AO (Fig. 2). Ultimately, 73 and 72 available AOB sequences were obtained for the ALK and ACI clone libraries, respectively, and used to construct the phylogenetic tree of AOB (Fig. 3a). The AOB sequences in the ALK and ACI libraries can be binned into 12 and 16 OTUs, respectively, but the community structures of the AOB sequences of these two libraries were very different. The AOB OTUs in ALK belonged to clusters 9, 3b, 3a.1, 3a.2, and *Nitrosomonas*, and the composition analysis (Fig. 3b—a) indicated that the dominant OTUs in ALK were cluster 3a.2 (54 %) and 3a.1 (34 %). In contrast, the AOB taxa in ACI belonged to clusters 10, 9, 3a.1, and 3a.2 (Fig. 3a), and the dominant OUTs were clusters 9 (24 %), 3a.2 (15 %), and 10 (53 %) (Fig. 3b—b).

For AOA, 78 (15 OTUs) and 65 (14 OTUs) available AOA sequences were obtained for the ALK and ACI clone library, respectively (Fig. 4a). The composition of the AOA communities in ALK and ACI was different, as *Nitrososphaera* subcluster1.1 was the sole community shared by the two communities (Fig. 4b). The dominant OTUs in ALK were *Nitrososphaera* subclusters 9–11 (73 %) and 5.1 (14 %) (Fig. 4b—a), but *Nitrosotalea* (62 %) and *Nitrososphaera* subclusters 1.1 (20 %) and 7 (18 %) represented the dominant AOA taxa in the ACI (Fig. 4b—b).

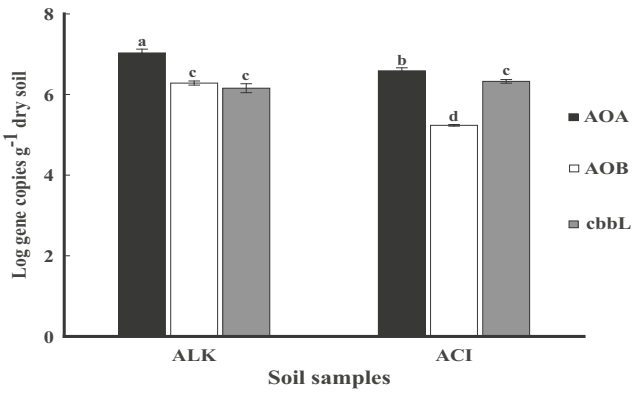

Fig. 1 Abundance of *amoA* and *cbbL* [gene encoding ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) pathway] in the ALK and ACI purple soil samples. The gene copy numbers were log-transformed. *Error bars* indicate standard deviations (n=3). *Different letters above the columns* indicate significant differences (P<0.05)

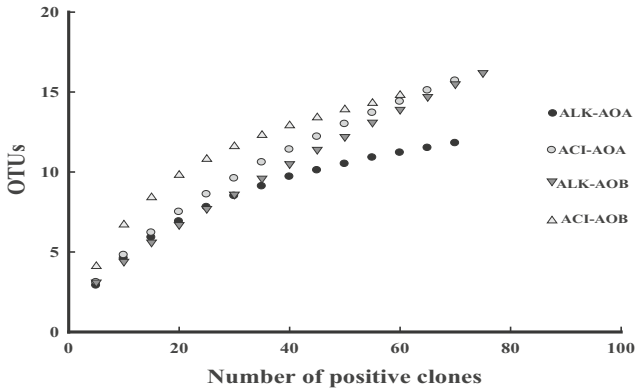

Fig. 2 Rarefaction analysis of bacterial and archaeal *amoA*- positive clones for clone libraries of acidic (*ACI*) purple soils and alkaline (*ALK*) purple soils using Analytic Rarefaction software. *AmoA* Gene encoding ammonia monooxygenase, *AOB* ammonia-oxidizing bacteria, *AOA* ammonia-oxidizing archea, *OTU* operational taxonomic unit

Discussion

It has been widely reported that the abundance of AOA is usually higher than that of AOB in a broad range of soil environments (He et al. 2007; Nicol et al. 2008; Shen et al. 2008; Ying et al. 2010; Wessen et al. 2011). The results of our study are similar to those of previous studies and indicate that the abundance of AOA was clearly higher than that of AOB both in alkaline (ALK) and acidic (ACI) purple soils. Additionally, the ratio of AOA to AOB in the ACI soil (22.79) was nearly fourfold higher than that in the ALK soil (5.65), which is also consistent with the results of other soil molecular investigations in China (Yao et al. 2011; Hu et al. 2013; Shen et al. 2014), suggesting a competitive advantage of AOA over AOB in acidic soils (Zhang et al. 2012; Hu et al. 2013). Both AOA and AOB were more abundant in ALK soil than in ACI soil, which might indicate the effect of soil pH on the abundance of soil AO. A lower abundance of AOB in acidic soils has also been reported in other studies (Nicol et al. 2008; Stopnisek et al. 2010; Andert et al. 2011; Yao et al. 2011; Isobe et al. 2012). Moreover, lower AOA abundance also was found in ACI, which might support the positive relationship between soil pH and AOA abundance (He et al. 2007; Hallin et al. 2009; Cao et al. 2012; Hu et al. 2013; Hu et al. 2014). Additionally, it was reported that most AOA might be more adapted to neutral or alkaline environments (Konneke et al. 2005; de la Torre et al. 2008; Tourna et al. 2011; Kim et al. 2012). Therefore, the results here were consistent with the physiological features of cultivated or isolated AOA strains.

It is unclear whether AO are obligate chemolithoautotrophs or mixotrophs, but it is known that the genomes of both AOA and AOB contain genes encoding enzymes involved in carbon fixation through the modified 3-hydroxypropionate/4-hydroxybutyrate pathway and the RuBisCO pathway (Kusian and Bowien 1997; Blainey et al. 2011). Therefore, in our study, we looked at the functional gene (*cbbL* of total chemoautotrophic bacteria) for carbon fixation. The result showed that copy number of *cbbL* in the purple soil samples was approximately 10^6 g^{-1} dry soil, which was in the range of 10^6–10^9 reported by other studies (Selesi et al. 2007; Videmsek et al. 2009; Yuan et al. 2013; Xiao et al. 2014). Xiao et al. (2014) found that the *cbbL* gene copy number was in the range of 10^6–10^9 g^{-1} dry soil in five Chinese paddy soils (pH ranged from 4.09 to 6.29) and that pH might be one of the important factors affecting the abundance and diversity of *cbbL*. Our results showed that the ALK and ACI purple soils did not differ in terms of *cbbL* abundance, but that the ratios of AOA and AOB, respectively, to *cbbL* in the ACI soil were obviously lower than those in the ALK soil. This result possibly indicates that pH might not only affect the abundance of soil AO, but also their roles within the whole community of chemoautotrophic microorganisms via competition for oxygen, CO_2, and other necessary elements or substrates. Therefore, as an important part of determining how chemoautotrophic microorganisms drive the key step in N-cycling, the relationship between AO and total chemoautotrophic microorganisms should be further investigated as such knowledge may facilitate a better understanding of the roles of AO in the coupling of soil carbon and nitrogen.

Soil pH has long been considered as an important factor affecting the community composition of AO (Nicol et al. 2008; Gubry-Rangin et al. 2011). In this study, substantial variance was detected in the composition of AOA and AOB between the ACI and ALK purple soil samples, which might suggest a selective effect of soil pH on soil AO. For AOB, *Nitrosospira* was dominant both in the ALK and ACI soils, which is consistent with the results of a previous study on the structure of the AOB community in neutral purple soil (Zhou et al. 2014). However, the prevalence of *Nitrosospira* differed dramatically in the ALK and ACI soils, with clusters 3a.1 (34 %) and 3a.2 (54 %) being the dominant taxa in the ALK soil and clusters 10 (53 %) and 9 (24 %) representing almost 77 % of the AOB in the ACI soil. Similar results have been reported in other kinds of Chinese acidic soils (Ying et al. 2010; Chen et al. 2011; Jiang et al. 2014). For example, in a Chinese acidic soil (Ferric Acrisols in Jiangxi Provence, pH 4.62), 454 pyrosequencing revealed that clusters 9 and 10 were the dominant taxa and *Nitrosomonas* was not detected (Jiang et al. 2014). The composition of the AOA community in ALK and ACI soils was also completely different, with *Nitrososphaera* subclusters 9–11 and *Nitrosotalea* being the dominant AOA in the ALK and ACI soils, respectively. *Nitrososphaera* has been found to be the dominant AOA in many soil environments (Pester et al. 2012), and Zhou et al. (2014) also found it to be the dominant taxa in a neutral purple soil subjected to long-term fertilization. Our results indicate that *Nitrosotalea* was the dominant AOA in the ACI purple soil, and similar results have also been found in other acidic

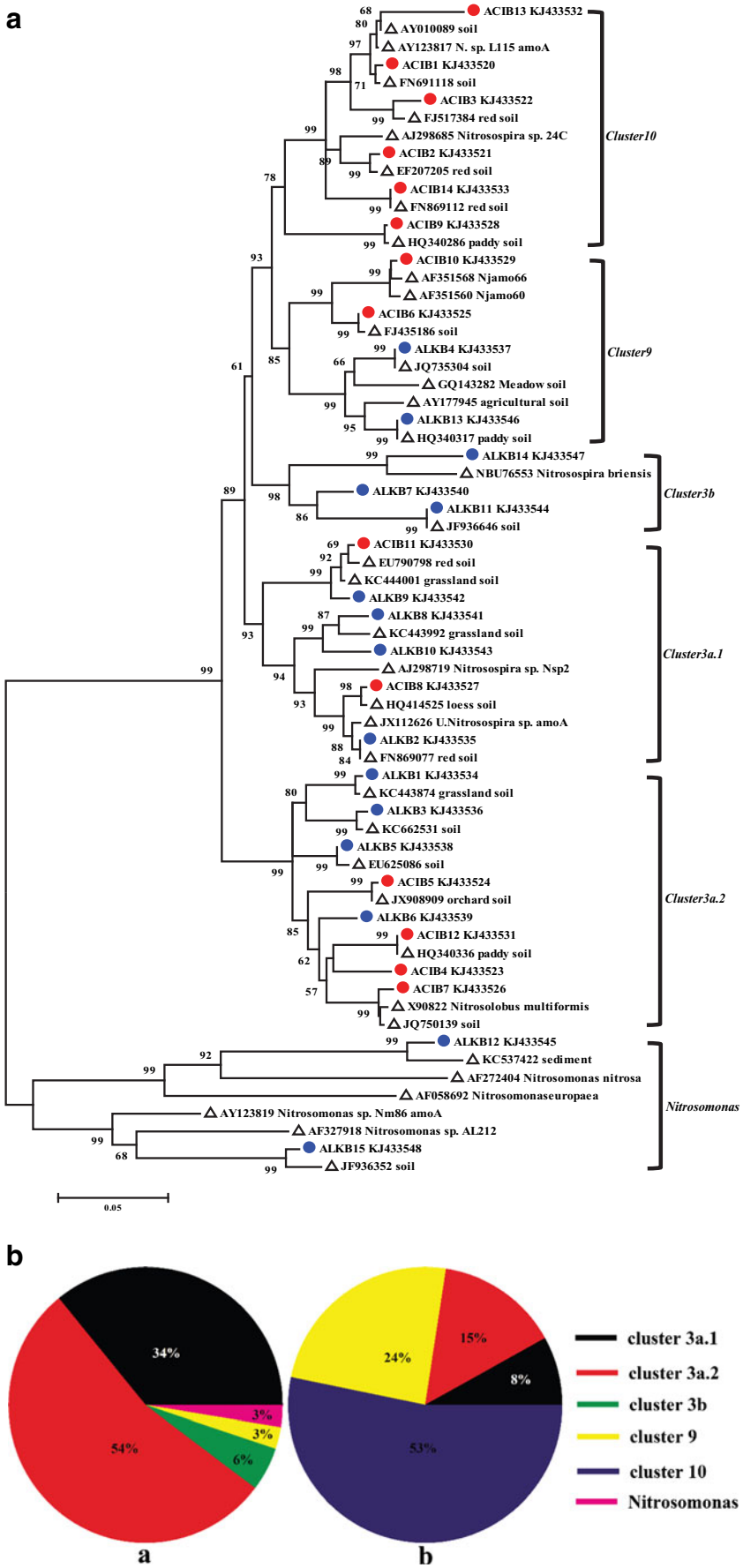

Fig. 3 **a** Neighbor-joining phylogenetic tree of AOB *amoA* sequences retrieved from the ACI and ALK libraries. *Filled circles* (*red* ACI, *blue* ALK) Sequence numbers from this study, *open black triangles* reference sequences. Bootstrap values (>50) are indicated at *branch points*. *Scale bar* represents 5 % estimated sequence divergence. **b** Composition analysis of the bacterial *amoA* in the ALK (*a*) and ACI (*b*) libraries

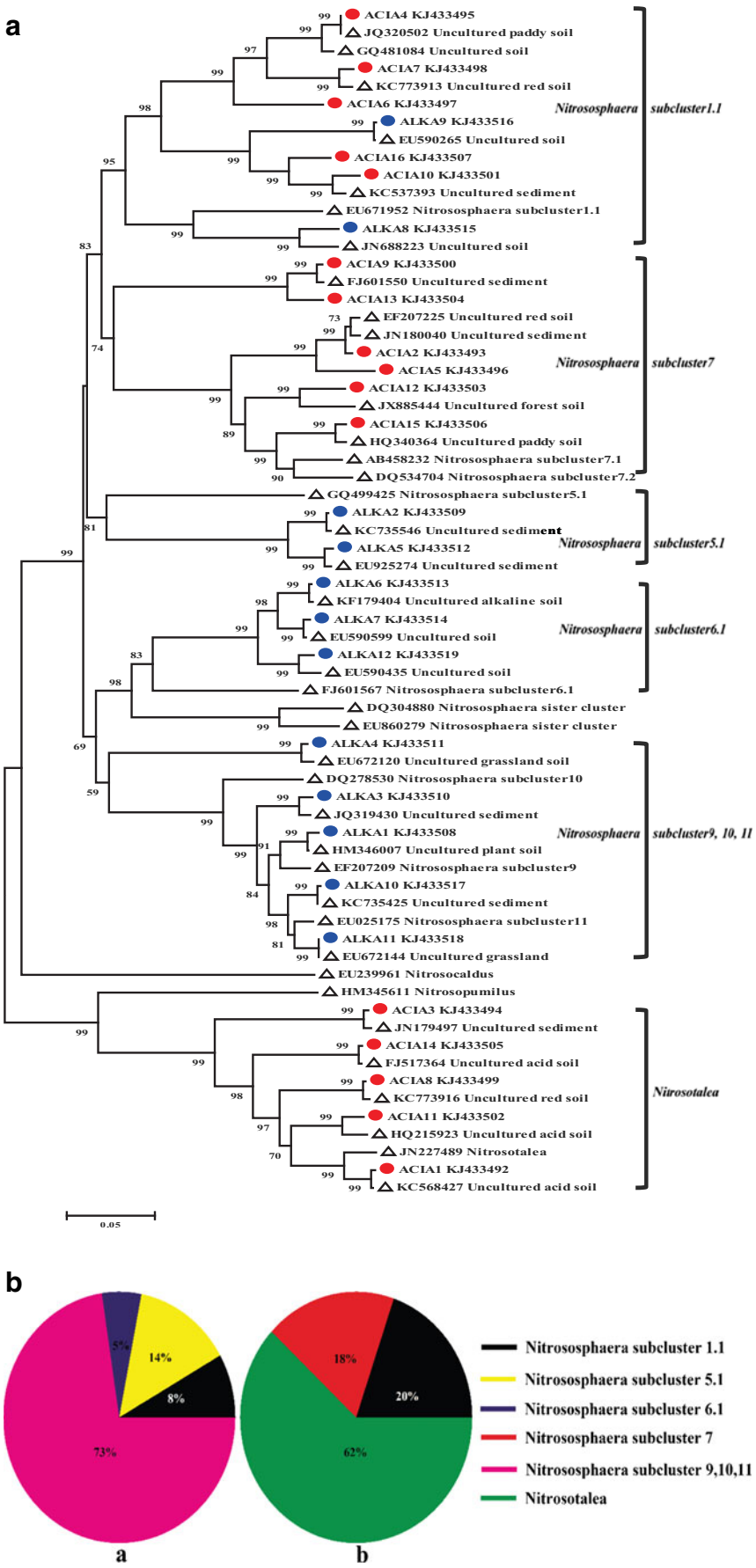

Fig. 4 **a** Neighbor-joining phylogenetic tree of AOA *amoA* sequences retrieved from the ACI and ALK libraries. *Filled circles* (*red* ACI, *blue* ALK) Sequence numbers from this study, *open black triangles* reference sequences. Bootstrap values (>50) are indicated at *branch points*. *Scale bar* represents 5 % estimated sequence divergence. **b** The composition analysis of the archaeal *amoA* gene in the ALK (*a*) and ACI (*b*) libraries

soils in China (Hu et al. 2014; Jiang et al. 2014). For example, Hu et al. (2014) investigated 32 different dry farmlands across China and found that *Nitrosotalea* (>60 %) were dominant in those soils with pH values of <6.0. Therefore, it would appear that *Nitrosotalea* might be an important AO in acidic soil environments. The adaptation of *Nitrosotalea* to low pH and its ability to grow at extremely low concentrations of ammonia has been demonstrated (Lehtovirta-Morley et al. 2011; Zhalnina et al. 2012), suggesting that it has evolved to tolerate acidic conditions that result in very low ammonia concentrations.

In conclusion, the results of this comparative study on AO in acidic and alkaline purple soils reveal that soil pH has a strong effect on the composition of the AO community. The higher ratio of AOA to AOB observed in the acidic purple soil indicates the potential advantage of AOA over AOB in acidic soil environments, and the difference in the structure of the AO community in purple soils with different pH values reveals a pH-based separation and selectivity of soil AO in purple soils. Additionally, the lower ratios of AOA and AOB *amoA* to the *cbbL* gene in the acidic purple soil illustrates the potential links among soil pH, AO, and total chemoautotrophic bacteria. Further study is necessary to investigate the relationship between the functions (ammonia oxidation and CO_2 fixation) of AO and chemoautotrophic microorganisms in soil.

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