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Original article

Activity, abundance and structure of ammonia-oxidizing microorganisms in plateau soils

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Abstract

Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) can be involved in biotransformation of ammonia to nitrite in soil ecosystems. However, the distribution of AOA and AOB in plateau soils and influential factors remain largely unclear. In the present study, the activity, abundance and structure of ammonia oxidizers in different soils on the Yunnan Plateau were assessed using potential nitrification rates (PNRs), quantitative PCR assay and clone library analysis, respectively. Wide variation was found in both AOA and AOB communities in plateau soils. PNRs showed a significant positive correlation with AOB abundance. Both were determined by the ratio of organic carbon to nitrogen (C/N) and total phosphorous (TP). AOB could play a more important role in ammonia oxidation. AOB community diversity was likely affected by soil total nitrogen (TN) and total organic carbon (TOC) and was usually higher than AOA community diversity. Moreover, *Nitrososphaera*- and *Nitrosospira*-like organisms, respectively, were the dominant AOA and AOB in plateau soils. AOA community structure was likely shaped by TP and C/N, while AOB community structure was determined by pH.

Keywords: Nitrification; Acidic soil; *Nitrososphaera*; *Nitrosospira*; Environmental factor

1. Introduction

Soils usually shelter highly diverse bacterial communities which participate in a variety of biogeochemical processes [2,5,19,32]. Microbial transformation of ammonia to nitrite, as the first and rate limiting step in the nitrification process, is of fundamental importance for soil nitrogen cycling. Ammonia oxidation in soil ecosystems is known to be carried out by ammonia oxidizing archaea (AOA) and bacteria (AOB) that carry ammonia monooxygenase encoded by *amoA* gene [18,22,38,43]. The activity, abundance and structure of soil AOA and AOB can be shaped by a number of environmental variables, such as pH [7,20,21], fertility [3,21,40,41], heavy metal [13] and vegetation type [39,43,45]. However, thus far, how multiple factors collectively shape the distribution of soil AOA and AOB remains unclear.

Plateaus are known for their special environmental conditions (e.g. low atmospheric pressure, low oxygen content in air and strong ultraviolet exposure) which might have significant impact on soil microbes and can lead to a distinctive below-ground microbial community [4,10,25,26]. A few previous studies have investigated ammonia-oxidizing microorganisms in meadow, forest and farmland soils on the Tibetan Plateau (China) [34,36,47] and in grassland soils on the Inner Mongolia Plateau (China) [3], while factors regulating the distribution of AOA and AOB in alpine soils are not well

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understood. The Yunnan Plateau (southwest China) is located in the subtropical monsoon climate zone and is warmer and has much more precipitation compared to the Tibetan Plateau and the Inner Mongolia Plateau. Thus far, only two recent studies reported a change in the abundance and structure of AOA and AOB communities in agricultural soils on the Yunnan Plateau [4,43], but those studies paid no attention to nitrifier activity. Moreover, information on AOA and AOB in unmanaged soil on this plateau is still lacking. Therefore, the objective of the current study was to investigate the activity, abundance and structure of AOA and AOB in plateau soils. Environmental factors shaping ammonia-oxidizing microorganisms were also investigated.

2. Materials and methods

2.1. Site description and sampling

Eight soil samples (0–5 cm depth) near the Baoxiang River, in triplicate, were collected in August 2014 (Fig. S1). The river is located in Kunming City (on the Yunnan Plateau). Soils R1, R2 and R3, planted with *Cryptomeria fortune*, *Poa annua* L. and *Tripogon bromoides*, respectively, were obtained from a protected reservoir region. Soils V1, V2 and V3, planted with *Eriobotrya japonica*, *Salix cavaleriei* and *Zea mays*, respectively, were collected from a village region. Moreover, soils T1 (*Vitisv inifera*) and T2 (*Brassica oleracea*) were collected from the transitional zone between the reservoir region and village region. The soils in agricultural sites had been linked to a long history of ammonia nitrogen fertilization, while those in the protected reservoir region had no previous exposure to fertilization. These eight soil samples were immediately transported to the laboratory and homogenized and subsampled for further analysis. Soil pH was measured with an IQ150 pH meter (IQ Scientific Instruments, Inc.). Soil total organic carbon (TOC) and total nitrogen (TN) were determined using an Elemental Analyzer (Vario EL III, Elementar, Germany). Total phosphorus (TP) was measured using the ascorbic acid-molybdate blue method [18] after 2 h of combustion (500 °C) and 16 h of extraction with 1 M HCl. Ammonium (NH_4^+–N) and nitrate (NO_3^-–N) contents were extracted from fresh soil samples with 2 M KCl and then determined using a continuous flow Analyzer (SAN++, Skalar, Holland). The geographic features and physicochemical characteristics (pH, TOC, TN, ratio of TOC to TN (C/N), NH_4^+–N, NO_3^-–N and TP) of soil samples are shown in Table 1.

2.2. Potential nitrification rate (PNR)

The PNR of each soil was assessed using the chlorate inhibition method [3,12]. Briefly, fresh soil (5 g) was added to 50-mL centrifuge tubes containing 20 mL of 1 mM phosphate buffer solution (PBS) and 1 mM $(NH_4)_2SO_4$. Potassium chlorate (10 mg L^{-1}) was amended to the tubes to inhibit nitrite oxidation. These tubes were incubated in the dark at 25 °C for 24 h and nitrite was extracted with 5 mL of 2 M KCl for analysis. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test was used to test the difference ($P < 0.05$) in the potential nitrification rates among different soils using software SPSS 20.

2.3. Molecular analyses

Soil genomic DNA was extracted using the Powersoil DNA extraction kit (Mobio Laboratories, USA). Quantitative PCR assay of archaeal and bacterial *amoA* genes was performed using the ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) based on the SYBR Green I method. The primer sets Arch-amoAF/Arch-amoAR and AmoA-1F/AmoA-2R were used for determination of the number of archaeal and bacterial *amoA* genes, respectively [14,39]. Standard curves ranging from 10^3 to 10^7 *amoA* gene copies mL^{-1} were obtained using serial dilutions of linearized plasmids. The average amplification efficiency and coefficient (r^2) for AOA and AOB *amoA* genes were 95% and 0.997, 94% and 0.998, respectively. One-way ANOVA followed by Student–Newman–Keuls test was applied to determine the difference ($P < 0.05$) in the abundance of the AOA or AOB *amoA* gene among different soils.

For construction of AOA and AOB clone libraries, the above-mentioned primer sets were also applied for amplification of archaeal and bacterial *amoA* genes [14,39]. The amplicons from each triplicate soil sample were mixed in equal amounts and then subjected to cloning. Chimera-free sequences were assigned to operational taxonomic units (OTUs) using a 97% similarity cutoff. The Shannon diversity index and rarefaction curve of each soil sample were generated using the MOTHUR program [28]. Phylogeny-based weighted UniFrac environmental clustering was applied to compare the

Table 1
Geographic and physicochemical features of soil sample.

Sample	Longitude	Latitude	Vegetation type	pH	TN (g kg^{-1})	TP (g kg^{-1})	TOC (g kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})	C/N
V1	102°51′36″	25°02′17″	*Eriobotrya japonica*	5.40 ± 0.05	1.41 ± 0.12	1.00 ± 0.06	12.81 ± 0.22	27.00 ± 0.18	4.59 ± 0.08	9.09 ± 0.13
V2	102°52′20″	25°02′41″	*Salix cavaleriei*	4.82 ± 0.31	2.8 ± 0.09	1.06 ± 0.08	18.80 ± 0.19	17.10 ± 0.27	5.75 ± 0.12	6.71 ± 0.11
V3	102°53′36″	25°02′48″	*Zea mays*	5.25 ± 0.12	1.53 ± 0.01	0.96 ± 0.08	11.63 ± 0.09	1.07 ± 0.07	4.61 ± 0.11	7.60 ± 0.06
T1	102°54′26″	25°02′52″	*Vitisv inifera*	5.01 ± 0.08	2.05 ± 0.12	0.87 ± 0.11	28.09 ± 0.15	25.30 ± 0.32	4.09 ± 0.05	13.72 ± 0.10
T2	102°54′39″	25°02′34″	*Brassica oleracea*	5.44 ± 0.13	0.71 ± 0.02	0.26 ± 0.03	15.72 ± 0.17	34.20 ± 0.27	3.20 ± 0.10	22.13 ± 0.23
R1	102°54′42″	25°01′37″	*Cryptomeriafortunei*	5.95 ± 0.14	2.84 ± 0.04	0.09 ± 0.01	41.31 ± 0.23	38.71 ± 0.23	7.75 ± 0.05	14.55 ± 0.21
R2	102°55′12″	25°01′14″	*Poa annua* L.	5.09 ± 0.20	1.80 ± 0.11	0.14 ± 0.02	33.70 ± 0.52	0.92 ± 0.02	4.11 ± 0.02	18.72 ± 0.09
R3	102°55′16″	25°01′26″	*Tripogon bromoides*	5.83 ± 0.06	1.34 ± 0.01	0.51 ± 0.04	23.85 ± 0.18	0.46 ± 0.01	3.79 ± 0.06	17.80 ± 0.05

difference in the soil AOA or AOB community using the UniFrac program [15]. Phylogenetic analysis of soil ammonia-oxidizing communities was conducted using MEGA software version 6.0, with the neighbor-joining and maximum parsimony methods [33]. Pearson's correlation analysis using SPSS 20.0 software was used to identify links between the microbial community and PNR or soil physicochemical parameters. Correlations between microbial community composition and environmental factors were also determined by redundancy analysis (RDA) using CANOCO 4.5. The abundance of *amoA* sequences in each OTU was used as species input, and environmental factors were used as environmental input. The significance test of Monte Carlo permutations was conducted to construct the best model of microbe–environment relationships. The sequences obtained in the present study were deposited in the GenBank database under accession numbers KM404790–KM404836 and KM404883–KM405198 for AOA and KM404342–KM404386 and KM404430–KM404741 for AOB, respectively.

3. Results

3.1. Potential nitrification rate

In this study, a wide variation in PNR occurred in the eight studied plateau soils, ranging from 0.02 to 0.59 μg NO_2^-–N g^{-1} dry soil h^{-1} (Fig. 1). The soils in the village region showed the highest PNR, while those in the reservoir region had the lowest PNR. PNR in soil V1 was significantly higher than that in soils V2 or V3 ($P < 0.05$), but there was no significant difference between soils V2 and V3 ($P > 0.05$). Soil T1 had a much higher PNR than soil T2 ($P < 0.05$). In addition, soil R1 illustrated significantly higher PNR than soils R2 and R3 ($P < 0.05$), while no significant difference was observed between soils R2 and R3 ($P > 0.05$). Therefore, the soil PNR might be very different even in the same sampling region.

3.2. Abundance of AOA and AOB communities

In this study, the number of soil ammonia-oxidizing microorganisms was assessed using quantitative PCR targeting *amoA* genes. A wide variation in either AOA or AOB abundance was detected in the eight plateau soils (Fig. 2). The average number of archaeal and bacterial *amoA* genes ranged from 8.3×10^3 to 1.45×10^6 and 3.85×10^4 to 1.22×10^6 copies per gram of dry soil, respectively. Village soils (V1, V2 and V3) had much higher AOA abundance than the other five soils ($P < 0.05$). A significant difference in archaeal *amoA* gene copy numbers was observed among village soils ($P < 0.05$), and soil V2 had the highest AOA abundance. The AOA abundance also showed a significant difference in reservoir soils (R1, R2 and R3) ($P < 0.05$). In contrast, no significant difference in the density of the archaeal *amoA* gene was found in soils T1 and T2 ($P > 0.05$). Moreover, village soils (V1, V2 and V3) also had much higher numbers of bacterial *amoA* genes than the other five soils ($P < 0.05$). Compared to soil T2, soil T1 had a relatively higher AOB abundance ($P < 0.05$). However, no significant difference in AOB abundance was found among the three reservoir soils (R1, R2 and R3). In addition, except for soil V2, the studied plateau soils showed numerical dominance of AOB over AOA. A positive correlation was observed between PNR and AOB abundance ($R^2 = 0.85$, $P < 0.01$), while AOA abundance showed no significant correlation with PNR ($P > 0.05$) (Fig. 3).

3.3. Diversity of AOA and AOB communities

In this study, 363 archaeal and 357 bacterial *amoA* gene sequences were retrieved from the AOA and AOB clone

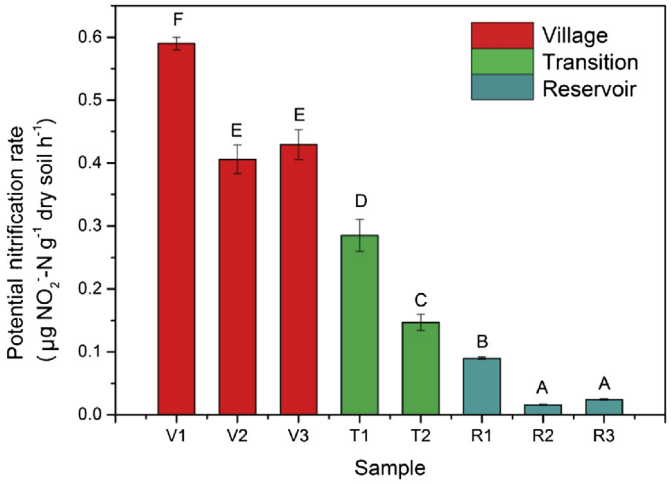

Fig. 1. Potential nitrification rates of different soils. Soils R1–R3, V1–V3 and T1 and T2 were obtained from a reservoir region, a village region and a transitional zone, respectively. Different letters above the columns indicate significant differences ($P < 0.05$).

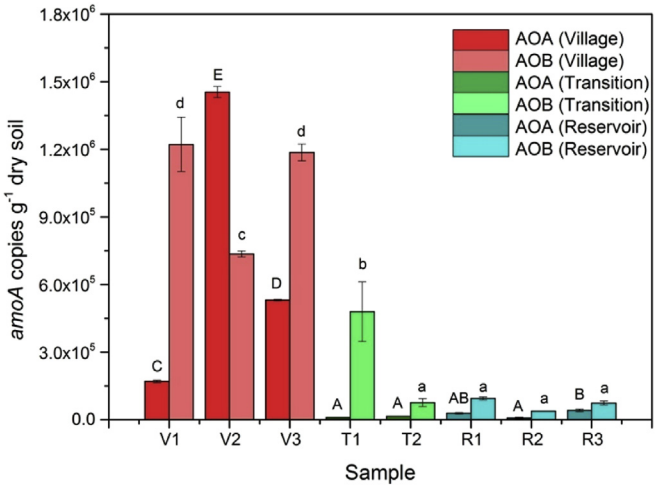

Fig. 2. Abundance of archaeal and bacterial *amoA* genes in different soils. Soils R1–R3, V1–V3 and T1 and T2 were obtained from a reservoir region, a village region and a transitional zone, respectively. Different letters above the columns indicate significant differences ($P < 0.05$).

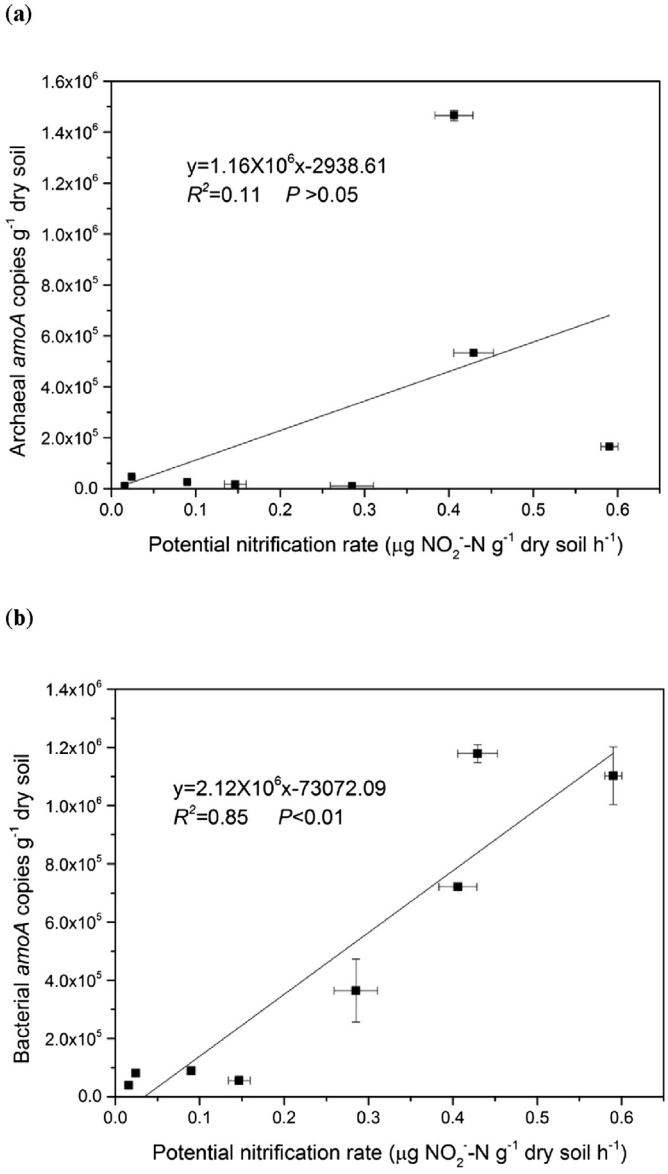

Fig. 3. Relationship between potential nitrification rate and number of archaeal (a) and bacterial (b) *amoA* gene copies.

libraries constructed with eight plateau soils. These AOA and AOB libraries included 5–18 and 6–36 OTUs, respectively (Table 2). Each AOA rarefaction curve nearly leveled off (Fig. S2). However, the absence of the appearance of a plateau in the rarefaction curve for some AOB clone libraries indicated that further sequencing could result in more OTUs. AOA community diversity showed wide variation among different soils (Shannon index = 0.9–2.48). Compared to other soils, soils V1 and R3 had much higher AOA diversity. A large difference in AOB diversity also occurred in the studied plateau soils (Shannon index = 0.99–3.49). In each sampling region, different soils illustrated the marked difference in both AOA and AOB diversity. In addition, except for soil T1, the studied plateau soils showed higher diversity of AOB than AOA.

Table 2
Richness and diversity of archaeal and bacterial *amoA* genes in different soils. Soils R1–R3, V1–V3 and T1 and T2 were obtained from a reservoir region, a village region and transitional zone, respectively.

amoA gene	Sample	Number of clones	OTUs	Shannon index
AOA	V1	47	18	2.48
	V2	46	9	1.18
	V3	52	6	1.34
	T1	44	11	1.42
	T2	45	8	1.19
	R1	49	8	0.93
	R2	33	5	0.90
	R3	47	13	2.06
AOB	V1	45	19	2.51
	V2	45	10	1.28
	V3	44	36	3.49
	T1	47	8	1.26
	T2	46	23	2.76
	R1	48	6	0.99
	R2	38	12	1.57
	R3	44	22	2.78

3.4. Comparison of AOA and AOB communities

In this study, the difference between soil ammonia-oxidizing communities was compared using phylogeny-based weighted UniFrac environmental clustering analysis. Three distinctive AOA clades were found in the eight plateau soils (Fig. 4a). Soils V1, V2 and R3 were grouped together, but

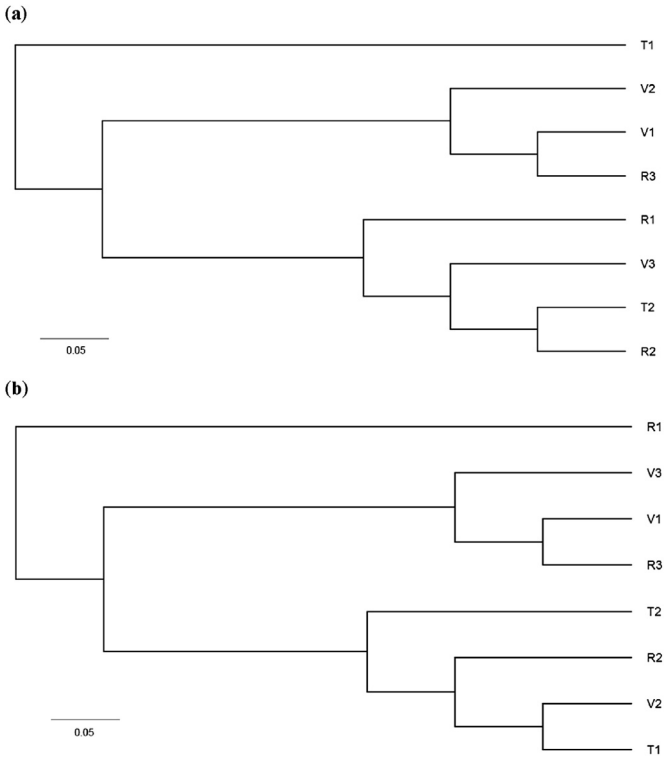

Fig. 4. Jackknife environment clusters for archaeal (a) and bacterial (b) *amoA* gene assemblages obtained from UniFrac software. Soils R1–R3, V1–V3 and T1 and T2 were obtained from a reservoir region, a village region and a transitional zone, respectively.

they were separated from other village and reservoir soils. Moreover, soil T1 was distantly separated from soil T2. These results indicated that soils in the same sampling region could have highly variable AOA community composition, while soils from different sampling regions could still have similar AOA composition.

For AOB, three distinctive clades were observed (Fig. 4b). Soils T1 and T2 were grouped with soils V2 and R2, while soils V1, V3 and R3 fell into a cluster. Soil R1 was distantly separated from all the other seven soils. These results also suggested that the same sampling region might have much different soil AOB community composition, while different sampling regions could still have similar soil AOB composition. In addition, the similarity of either AOA or AOB community composition was observed in soils V1 and R3. Soils T2 and R2 also showed similar AOA or AOB community composition.

3.5. Phylogeny of AOA and AOB

In this study, for each AOA or AOB OTU containing at least two *amoA* gene sequence members, a representative sequence was selected for further phylogenetic analysis. All of the archaeal *amoA* gene sequences fell into five AOA clusters (Fig. 5). They were mainly distributed in clusters a and d (Fig. S3a). AOA species affiliated with cluster a predominated in soils V1, V2, V3 and T1, while soils T2, R1 and R2 were mainly composed of cluster-d-like AOA organisms. The AOA sequences from soil R3 were more evenly distributed in clusters a, d and e. These results further confirmed a wide variation in AOA community structure. Moreover, cluster a was the second largest AOA group and contained 149 archaeal *amoA* gene sequences that could be grouped with uncultured sequences from a variety of soil and sediment ecosystems. Cluster b was composed of four AOA sequences from soils T1 and V2. They were related to the uncultured ones from grassland soil and marine sediment. Cluster c was the smallest AOA group and included only two archaeal *amoA* gene sequences from soil R3. The AOA sequences in cluster c were affiliated with an uncultured one from the soil in the Eastern Mediterranean Basin. Cluster d was the largest AOA group and contained 156 archaeal *amoA* gene sequences. These AOA sequences could be affiliated with two cultivated soil AOA species (*Nitrososphaera* sp.JG1 and *Nitrososphaera viennensis* EN76) [11,35] and many uncultured ones from various soil and sediment ecosystems. In addition, cluster e was a nine-member group and had only AOA sequences from soil R3. They were closely related to an uncultured archaeal *amoA* gene sequence from estuary sediment.

The bacterial *amoA* gene sequences obtained could be divided into four AOB clusters (Fig. 6). These AOB sequences were mainly distributed in cluster II (Fig. S3b). Soils V1 and R3 mainly included cluster I-like AOB species, while cluster II-like AOB microorganisms were dominant in other soils. Moreover, cluster IV-like AOB species were at high proportions in soils V3, T1 and R2. These results further

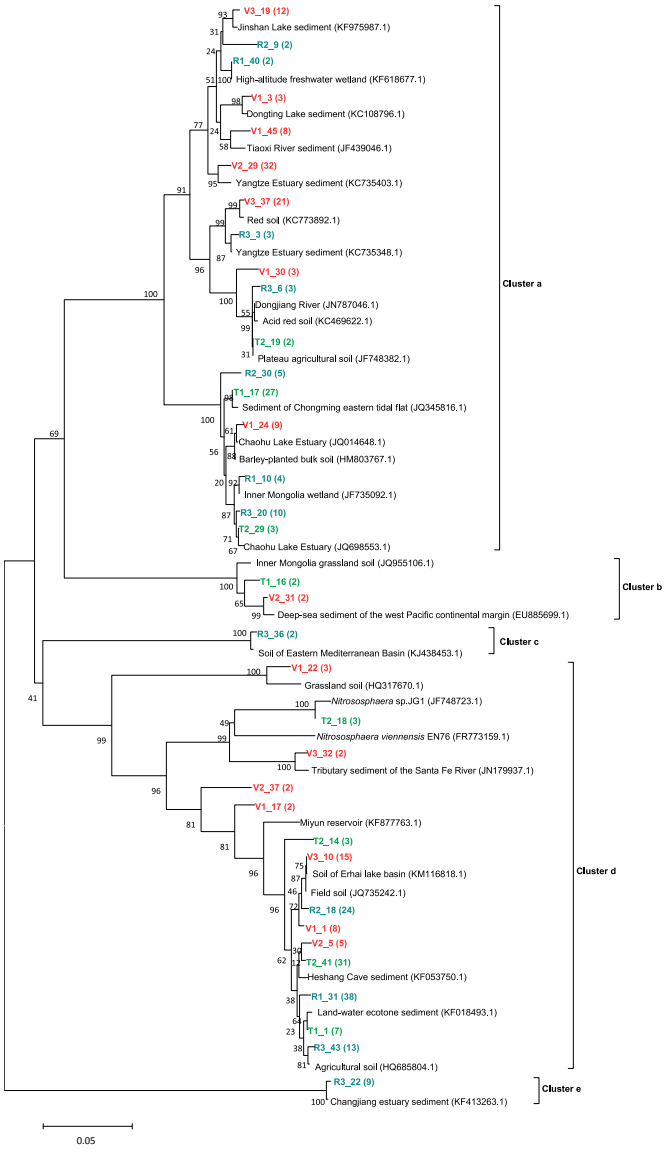

Fig. 5. Phylogenetic tree of the representative archaeal *amoA* sequences and their reference sequences from GenBank. The obtained archaeal sequences beginning with 'R1–R3', 'V1–V3' and 'T1' and 'T2' were referred to sequences retrieved from soils R1–R3, V1–V3 and T1 and T2, respectively. The bold number in parentheses represents the number of sequences in the same OTU in a given clone library. Numbers at the nodes indicate levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The bar represents 5% sequence divergence.

confirmed a large shift in AOB community structure. Cluster I was a 66-member AOB group and the bacterial *amoA* gene sequences in this cluster cloud be affiliated with three cultivated *Nitrosospira* species (TCH711, ATCC 25196 and NIJS18) [27]. Cluster II was the largest AOB group and contained 160 bacterial *amoA* gene sequences. These AOB sequences could be grouped with uncultured ones from various ecosystems, including soil, wetlands, river and lake. Cluster III was the smallest AOB group and included 11 bacterial *amoA* gene sequences. The AOB sequences in cluster III were related to two cultivated *Nitrosospira* species (Nsp10

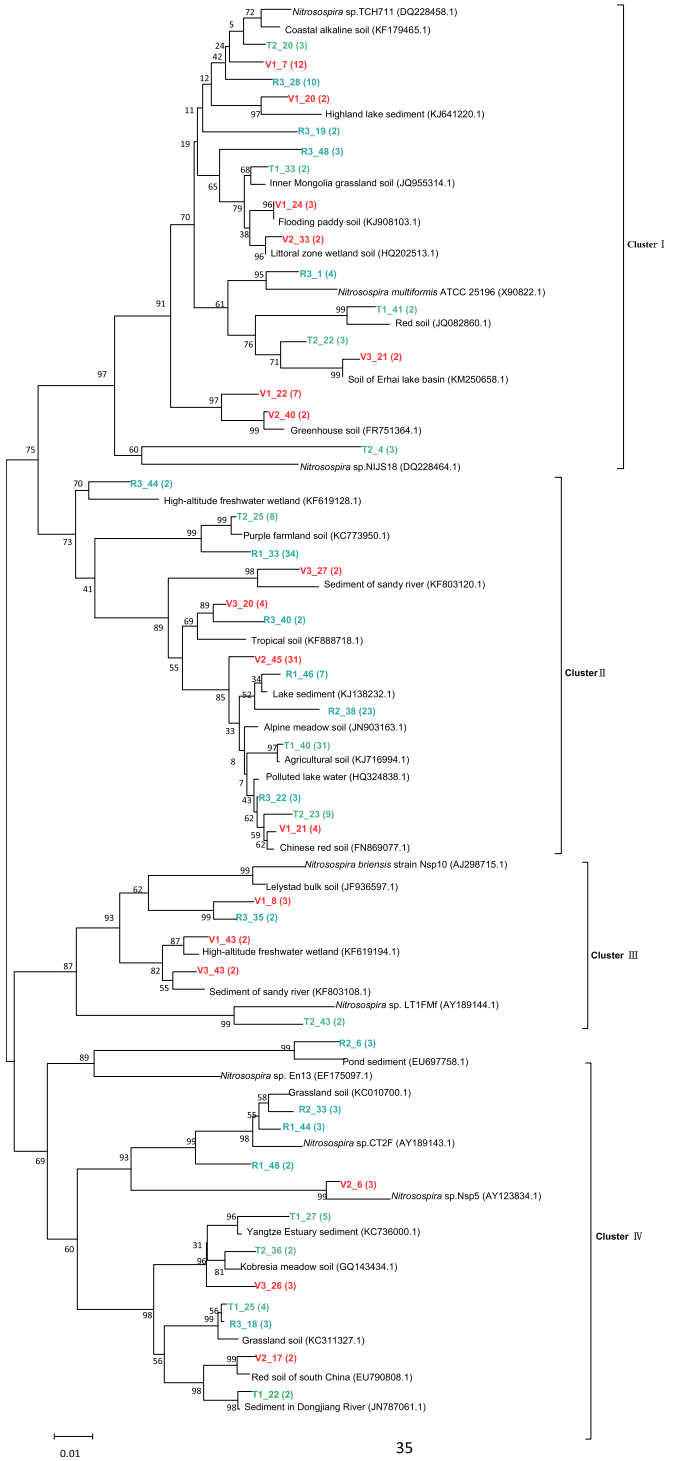

Fig. 6. Phylogenetic tree of the representative bacterial *amoA* sequences and their reference sequences from GenBank. The obtained bacterial sequences beginning with 'R1–R3', 'V1–V3' and 'T1' and 'T2' were referred to sequences retrieved from soils R1–R3, V1–V3 and T1 and T2, respectively. The bold number in parentheses represents numbers of sequences in the same OTU in a given clone library. Numbers at nodes indicate levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The bar represents 1% sequence divergence.

and LT1FMf) [1,17]. In addition, cluster IV was composed of 39 AOB sequences that could also be affiliated with several cultivated *Nitrosospira* species (En13, CT2F and Nsp5) [17,24].

3.6. Drivers of AOA and AOB communities

Pearson's correlation analysis indicated that AOB abundance showed a highly significant positive correlation with PNR ($P < 0.01$) (Table 3). Both of them were negatively correlated with soil C/N ($P < 0.05$ or 0.01), but showed significant positive correlations with soil TP ($P < 0.01$). However, no significant correlation was found between AOA abundance and PNR, or the determined soil physicochemical properties ($P > 0.05$). In addition, AOB Shannon diversity was negatively correlated with soils TN and TOC ($P < 0.05$), while environmental factors showed no significant correlation with AOA Shannon diversity ($P > 0.05$).

The environmental factors in the first two RDA axes, respectively, explained 49.7% and 31.4% of the total variance in AOA OTU composition (Fig. 7a). Soil TP ($F = 4.571$, $P = 0.004$, 499 Monte Carlo permutations) and C/N ($F = 2.976$, $P = 0.028$, 499 Monte Carlo permutations) were found to significantly contribute to the AOA assemblage–environment relationship, while other environmental factors did not pass the Monte Carlo significance test ($P > 0.05$). Moreover, environmental factors in the first two RDA axes, respectively, accounted for 53.9% and 36.5% of total variance in AOB OTU composition (Fig. 7b). Only pH ($F = 4.921$, $P = 0.002$, 499 Monte Carlo permutations) showed a significant contribution to the AOB assemblage–environment relationship.

4. Discussion

4.1. Soil potential nitrification rate

It has been well documented that soil PNR can be affected by pH [7,21,31], organic carbon [6,21] and nitrogen [3,6,30,40,48]. In this study, soil PNR showed no significant correlations with soil pH, and the levels of nitrate and ammonia nitrogen and organic carbon ($P > 0.05$), while C/N was a possible determinant of soil PNR. Thus far, the impact of phosphorus on soil PNR remains poorly understood. Zhou et al. suggested that phosphorus addition could further increase soil ammonia-oxidizing activity in nitrogen-amended microcosm [48]. In this study, TP was also found to be a possible stimulator of PNR in plateau soils. Therefore, the joint effect of C/N and TP might lead to distinctive differences in soil PNR in the three different sampling regions (reservoir region, village region and transitional zone). However, even in the same sampling region, a significant difference in PNR was found in soils with different vegetation types. This suggested that vegetation type might be an important local environmental constraint affecting soil PNR. Ying et al. also revealed that plantation type had a profound impact on soil PNR [45].

Table 3
Pearson's correlation analysis of AOA and AOB communities with PNR or soil physic-chemical properties.

Parameter	pH	TN	TP	TOC	NO_3^-–N	NH_4^+–N	C/N	PNR
PNR	−0.427	0.031	**0.867**[b]	−0.692	0.134	0.047	**−0.821**[a]	1
AOA abundance	−0.563	0.468	0.605	−0.369	−0.165	0.273	−0.701	0.483
AOB abundance	−0.361	0.009	**0.845**[b]	−0.701	−0.147	0.055	**−0.873**[b]	**0.934**[b]
AOA Shannon diversity	0.208	−0.416	0.499	−0.518	−0.084	−0.323	−0.232	0.470
AOB Shannon diversity	0.183	**−0.796**[a]	0.224	**−0.753**[a]	−0.402	−0.568	0.004	0.228

[a] Correlation is significant at the 0.05 level.
[b] Correlation is significant at the 0.01 level.

4.2. Soil AOA and AOB abundance

pH has been widely accepted as a critical factor regulating distribution of soil AOA and AOB communities [23]. AOA can have a strong capacity to adapt to low pH soils [46]. Numerous previous studies indicated that AOA outnumbered AOB in acidic soils [7,8,20,44,45]. Moreover, AOA also showed numerical dominance over AOB in soils on the other plateaus [3,4,36,47]. However, our previous study also found the dominance of AOA over AOB in both low and neutral pH agricultural soils on the Yunnan Plateau [43]. In this study, the pH value of eight plateau soils ranged between 4.82 and 5.95 and the archaeal and bacterial *amoA* genes showed wide variation in these plateau acidic soils, ranging from 8.3×10^3 to 1.45×10^6 and 3.85×10^4 to 1.22×10^6 copies per gram of dry soil, respectively. The observed number of AOA and AOB *amoA* genes in these soils was at a similar level to that of agricultural soils on the Yunnan Plateau [43]. Moreover, AOB showed a higher abundance than AOA in most of the studied acidic plateau soils. AOB abundance showed a significant positive correlation with PNR, while no links were found between AOA abundance and PNR. This was in agreement with the result reported in a previous study [3]. In addition, both AOB abundance and PNR were negatively correlated with soil C/N, but positively with soil TP. These results suggested that AOB might play a major role in ammonia oxidation in acidic soils on the Yunnan Plateau.

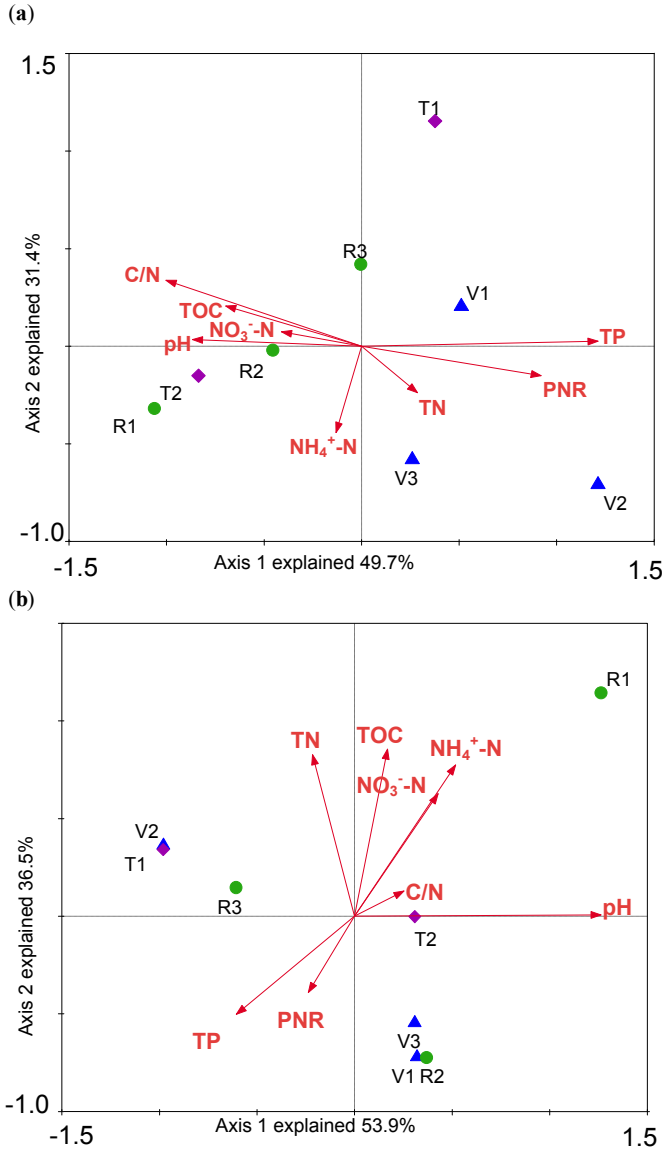

Fig. 7. RDA ordination plot for the first two principal dimensions of the relationship between AOA (a) and AOB (b) OTU composition and environmental factors.

4.3. Soil AOA and AOB diversity

Compared to AOB, AOA usually had relatively high community diversity in soil ecosystems [16,29,30,39,42]. Our previous study also found higher AOA diversity than AOB in different agricultural soils on the Yunnan Plateau [43]. However, in this study, higher diversity of AOB than AOA was found in most of the plateau soils (7 out of 8). Further efforts will be necessary to identify the difference between soil AOA and AOB community diversity. Thus far, influential factors regulating AOA and AOB diversity in plateau soil remains unclear. Only a recent study suggested that plateau soil AOA community diversity might be affected by TN and TOC [4]. In contrast, environmental factors governing AOA diversity was not found in the present study, while AOB diversity was negatively correlated with soil TN and TOC. The impact of carbon-/nitrogen-related soil nutrients on AOB diversity was also reported in other soil ecosystems [3,6,9].

4.4. Soil AOA and AOB community composition

Very limited information exists on AOA and AOB community composition and influential factors in plateau soils. Wang et al. suggested that organic nitrogen and carbon were

the determinants of AOA community composition in plateau forest soils [36]. Chen et al. indicated that nitrogen was a key factor in shaping AOB composition in plateau grassland soils, but showed very little impact on AOA community structure [3]. In this study, UniFrac environmental clustering analysis showed wide variation in the community composition of both AOA and AOB in plateau soils, which was not clearly separated by geographic distance. The result of RDA suggested that soil AOA community structure was shaped by TP and C/N, but pH affected AOB community structure. Therefore, environmental factors regulating the community structure of soil ammonia-oxidizing microorganisms might differ greatly in different plateaus.

Nitrososphaera-affiliated AOA species usually exist in acidic soils [7,8,44,45]. *Nitrososphaera*-like AOA showed low abundance in meadow soils on the Tibetan Plateau [47]. Our previous study indicated that *Nitrososphaera*-like microorganisms were the predominant AOA species in garlic soil on the Yunnan Plateau, but became a minor AOA member or was not even detected in rice and cabbage soils [43]. However, in this study, *Nitrososphaera*-like organisms were the dominant AOA species in most of the studied plateau acidic soils. Therefore, vegetation type might have direct or indirect effects on the abundance of *Nitrososphaera*-like organisms in plateau soils.

Nitrosospira-affiliated AOB species were found to be dominant in low-pH soils [7,45]. Moreover, *Nitrosospira*-like organisms predominated in meadow soils on the Tibetan Plateau [47]. The high relative abundance or dominance of *Nitrosospira* was also found in agricultural soils on the Yunnan Plateau [43]. *Nitrosospira* favor low-ammonia environments [37]. In this study, the studied soils contained relatively low levels of ammonia nitrogen (3.2–7.75 mg kg^{-1}), and *Nitrosospira*-like AOB species were detected in each plateau acidic soil. *Nitrosospira* predominated in two soils (V1 and R3) and showed high relative abundance in soils V3, T1 and T2. Therefore, *Nitrosospira*-like organisms might provide an important contribution to ammonia oxidation in plateau acidic soils.

In conclusion, the activity, abundance, diversity and composition of AOA and AOB communities showed wide variation in plateau acidic soils. AOB might play a major role in ammonia oxidation. The AOB community usually had higher diversity than AOA. *Nitrososphaera*- and *Nitrosospira*-like organisms were the dominant AOA and AOB in plateau acidic soils. Moreover, the correlations between AOA and AOB community and environmental factors varied with the activity (or abundance) and structure.

Conflict of interest

No conflict of interest was declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2015.07.012.

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