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Altitudinal Distribution of Ammonia-Oxidizing Archaea and Bacteria in Alpine Grassland Soils Along the South-Facing Slope of Nyqentangula Mountains, Central Tibetan Plateau

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Nitrogen is a major limiting nutrient for the net primary production of terrestrial ecosystems, especially on sentinel alpine ecosystem. Ammonia oxidation is the first and rate-limiting step on nitrification process and is thus crucial to nitrogen cycle. To decipher climatic influence on ammonia oxidizers, their communities were characterized by qPCR and clone sequencing by targeting *amoA* genes (encoding the alpha subunit of ammonia mono-oxygenase) in soils from 7 sites over an 800 m elevation transect (4400–5200 m a.s.l.), based on "space-to-time substitution" strategy, on a steppe-meadow ecosystem located on the central Tibetan Plateau (TP). Archaeal *amoA* abundance outnumbered bacterial *amoA* abundance at lower altitude (<4800 m a.s.l.), but bacterial *amoA* abundance was greater in surface soils at higher altitude (≥4800 m a.s.l.). Archaeal *amoA* abundance decreased with altitude in surface soil, while its abundance stayed relatively stable and was mostly greater than bacterial *amoA* abundance in subsurface soils. Conversely, bacterial *amoA* abundance gradually increased with altitude at all three soil depths. Statistical analysis indicated that altitude-dependent factors, in particular pH and precipitation, had a profound effect on the abundance and community of ammonia-oxidizing bacteria, but only on the community composition of ammonia-oxidizing archaea along the altitudinal gradient. These findings imply that the shifts in the relative abundance and/or community structure of ammonia-oxidizing bacteria and archaea may result from the precipitation variation along the altitudinal gradient. Thus, we speculate that altitude-related factors (mainly precipitation variation combing changed pH), would play a vital role in affecting nitrification process on this alpine grassland ecosystem located at semi-arid area on TP.

Keywords: ammonia oxidizer, *amoA* gene, semi-arid grassland, Tibetan Plateau

Introduction

Since the Industrial Revolution, human activities have played a key role in altering the processes of ecological systems. These alterations include increases of atmospheric CO_2, CH_4, and N_2O concentrations, with resulting changes in air temperature and precipitation (IPCC 2007). On terrestrial ecosystems, how plant communities responded to climate change has been well documented (Bradford et al. 2012; Dukes et al. 2005; Smith et al. 2012; Xu et al. 2013).

However, soil microbe-mediated processes can determine the nutrient cycling that limits primary production. Thus, previous studies have suggested that microbes-mediated underground processes are central to understanding how ecosystems respond to global climate change (Singh et al. 2010; van der Heijden et al. 2008; Wardle et al. 2004). Whereas, how soil bacterial communities respond to climate change, such as warming and altered precipitation, is still largely undetermined, especially on Tibetan Plateau (TP) ecosystem.

Ammonia oxidation is thus central to the nitrogen cycle because it is the first and rate-limiting step of nitrification. During past century, ammonia oxidation had been thought to be performed exclusively by ammonia-oxidizing bacteria (AOB). Recent metagenomic studies demonstrated that the ammonia oxidation process could also be mediated by ammonia-oxidizing archaea (AOA) (Könneke et al. 2005; Treusch et al. 2005). Subsequent studies showed that AOA are ubiquitous in various extreme environments, for example, marine environments (Francis et al. 2005; Wuchter et al. 2006), terrestrial hot springs (de la Torre et al. 2008; Reigstad et al. 2008; Wang et al. 2013a), and subglacial sediments

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(Boyd et al. 2011). The ecological role of AOB and AOA has become a hot topic in recent years due to their important functions in N cycling.

Although both ammonia oxidizers (AOA and AOB) play important roles in nitrification, their accurate contribution to the nitrification process still remains elucive. Archaeal *amoA* genes outnumber that of bacteria in many environments (Lam et al. 2007; Leininger et al. 2006; Mincer et al. 2007; Nicol et al. 2008; Shen et al. 2008; Yao et al. 2011; Zhang et al. 2012), and additionally high abundance of archaeal *amoA* gene copies suggested that AOA might have imposed greater impact on nitrification process. In contrary, greater abundance of AOB *amoA* gene were demonstrated in higher altitudes (>5400 m) (Zhang et al. 2009), subglacial sediments (Boyd et al. 2011), lakes (Jiang et al. 2009) and agricultural soil (Jia and Conrad 2009). Therefore, it becomes important to understand environmental factors driving their distribution.

Some recent investigations showed that the abundance, distribution and community structures of ammonia oxidizers (AOA and AOB) are mainly influenced by pH (Nicol et al. 2008; Pereira e Silva et al. 2012), temperature (Tourna et al. 2008), salinity (Hu et al. 2010; Santoro et al. 2008), fertilization regimes (Daebeler et al. 2012; Shen et al. 2011) and ammonia concentration (Verhamme et al. 2011). However, studies on the influence of environmental driving factors over ammonia oxidizers (AOA and AOB) communities in higher altitude soils (higher than 4000 m) are still rare.

Previous studies showed that Tibetan Plateau sequestered a large amount of carbon (Piao et al. 2006; Yang et al. 2007), and additionally is one of the most sensitive areas to climatic changes (Liu and Chen 2000; Zhao et al. 2004), which is often considered sentinels of global change. Moreover, lower temperature restricts the nitrogen mineralization process, and thus leads to lower available nitrogen for plant and soil microorganisms.

Therefore, investigation on the changes of AOA and AOB communities along large altitudinal gradients via "space-for-time substitution" strategy in this region is extremely helpful for us to understand the response of nitrogen cycle to future climate change. In the present study, we selected an altitudinal gradient (4400 m to 5200 m a.s.l) on the central TP to characterize the distribution of ammonia-oxidizers (AOA and AOB). We further explored whether the altitude-dependent environmental factors might choose specific species of ammonia oxidizers along the altitudinal gradient.

Materials and Methods

Field Sites, Soil Sampling and Soil Physicochemical Analyses

Soils were sampled from seven sites (including 4400, 4500, 4650, 4800, 4950, 5100 and 5200 m a.s.l) located on the south-facing slope of Nyaiqentanglha Mountains (30°30′-30°32′N, 91°03′E) (Figure 1). Climatic data of these sites and soil sampling details could be found in previous study (Yuan et al. 2014) and Table 1. Soil moisture was gravimetrically determined, and pH was measured with pH meter. Total soil organic carbon (TOC) was measured with TOC analyzer (TOC-VCPH, Shimadzu, Japan), total nitrogen (TN) was determined using a modified Kjeldahl method. NO_3^--N and NH_4^+-N were extracted by 2 M KCl solution and then measured with Continuous Flow Analyzer (SAN++; Breda, Holland).

DNA Extraction and Real-Time PCR

gDNA (genomic DNA) was obtained from frozen soil with the commercial kit for soil (MP Biomedicals, Santa Ana, CA, USA). Archaeal and bacterial *amoA* gene abundance were determined by qPCR using a LightCycler 480II thermocycler (Roche, Switzerland). Amplifications of archaeal and bacterial *amoA* genes were done with primer pairs 19F/CrenamoA616r48x and *amoA*-1F/*amoA*-2R (Leininger et al. 2006; Rotthauwe et al. 1997), respectively. A 20-μl volume system consisted of 1 μl of 10-times-diluted gDNA, 0.2 μM each primer, and 10 μl of SYBRGreen Premix (Takara, Japan). Melting-curve analysis (60°C–95°C) and gel electrophoresis were used to confirm product specificity. The standard curves for quantification of unknown samples were prepared with known plasmids containing *amoA* genes.

Table 1. Climatic and vegetation characteristics in the sampling sites along the altitudinal gradient

Sites (Elevation)	M.A.T (°C)	M.M.T (°C) (8th)	5 cm soil Tm		20 cm soil Tm		M.A.P (mm)	Predominant species[a]
			Anu	Aug	Anu	Aug	2009	
4400 m	3.7	10.1	7.8	13.4	7.6	13.2	227	*Stipa capillacea, Stipa purpurea,*
4500 m	3.2	9.5	7.2	12.8	7.1	12.5	237	*Stipa capillacea, Stipa purpurea,*
4650 m	2.4	8.5	6.3	11.5	6.4	11.4	245	*Kobresia pygmaea,*
4800 m	1.6	7.7	6.9	10.5	6.1	10.2	338	*Kobresia pygmaea,*
4950 m	0.2	6.0	4.6	8.7	4.6	8.7	434	*Kobresia pygmaea,*
5100 m	−1.2	5.0	3.6	8.7	3.6	7.9	420	*Kobresia pygmaea,*
5200 m	−1.6	4.3	3.8	7.1	3.4	7.6	362	*Kobresia pygmaea,*

Note: a: data from Wang et al. (2013b).

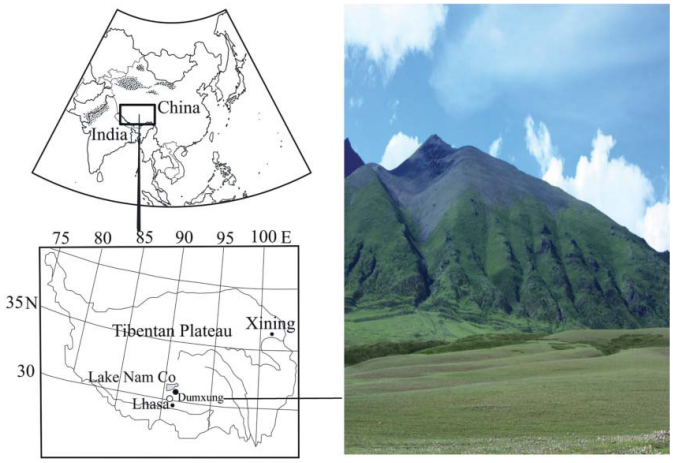

Fig. 1. The location of the altitudinal transect along south-facing slope on Nyaiqentanglha Mountain at Dumxung county. The photo was taken from an elevation of 4300 m, and the view is straight up the slope transect. (Courtesy: Zhong Wang).

Cloning and Sequencing

Clone libraries were constructed from 0–5 cm soils at three altitudes (4400, 4800, and 5100 m) and two altitudes (4400 and 4800 m) for archaeal and bacterial *amoA* gene, respectively. First, targeted *amoA* fragments of AOA and AOB were amplified with the primer pairs amoA-AF/amoA-AR and amoA-1F/amoA-2R, respectively (Francis et al. 2005; Rotthauwe et al. 1997). After gel purification, clones were generated using a pGEM-T Easy Vector Systems I (Promega, Madison, WI, USA) according to the manufacturer's instructions. Finally, 150 AOA clones and 110 AOB clones were sequenced.

For each clone library, sequences that exhibited >97% similarity to each other were assigned as an OTU, but only few representatives from each OTU were then selected for phylogenetic trees construction. The GenBank sequences most similar to clone sequences in this study and reference sequences for defining clusters were included in phylogenetic tree construction. Two phylogenetic neighbor-joining bootstrap trees based on nucleotide sequences were performed using MEGA version 4 with 1000 replicates (Tamura et al. 2007).

Statistical Analyses

The log-transformed *amoA* gene abundance data was used for statistical analyses. Bivariate correlations were carried out to link different parameters. All statistical analyses were performed with SPSS version 16.0 (IBM Inc., USA).

Nucleotide Sequence Accession Numbers

All the sequences from our study had been deposited into the GenBank database. The accession number is from KF709701 to KF709942 (KF70971-KF709849 for AOA and KF709850-KF709942 for AOB).

Results

Soil Properties

Soil pH varied in the surface soil collected from different altitudes. The 4400 m and 4500 m profile had the highest pH (6.56–7.02), followed by the elevation 4650 m (6.01–6.13), and above 4800 m (5.78–5.98) except for the 10–20 cm soil at elevation 5200 m. The 4950 m topsoil had the lowest pH (5.38). Correspondingly, precipitation reached the highest value for the 4950 m topsoil (Figure S1a). NO_3^- significantly increased at higher altitude (>4950 m a.s.l) at all soil depths, whereas NH_4^+ increased with altitude until it reached the highest value in surface soil at 5100 m a.s.l but not in deeper soil (Figures S1b and S1c).

Changes of Archaeal and Bacterial amoA Gene Abundances along the Altitudinal Transect

At 0–5 cm soil, archaeal *amoA* gene copies (AOA) were lower at altitudes 4800 and 5100 m, whereas bacterial *amoA* gene copies (AOB) were significantly higher at above 4800 m than that at lower altitudes (below 4800 m) (Figure 2a). Consequently, the mean $\log_{10}$AOA:AOB *amoA* genes decreased from 2.43 at 4500 m to −2.01 at 5200 m (Figure 2d). At 5-10 cm soil, the abundance of AOA stayed relatively higher than AOB at all altitudes (Figure 2b), and thus the mean $\log_{10}$AOA:AOB *amoA* genes were greater than zero at all altitudes (Figure 2e).

Similarly, the abundance of AOA stayed relatively stable at all altitudes at 10–20 cm soil, whereas the abundance of AOB significantly increased with altitude and reached the highest value at 5100 m (Figure 2c). Consequently, the mean $\log_{10}$ ratio of AOA:AOB *amoA* gene copies decreased with altitudes (Figure 2f). Of all soil and site variables examined, pH ($r^2 = 0.52$) was, by far, the best predictors of $\log_{10}$AOA:AOB *amoA* genes with the lower levels of $\log_{10}$AOA:AOB observed in lower pH soils (Figure 3a), followed by MAP ($r^2 = 0.43$) and moisture ($r^2 = 0.33$) (Figures 3b and 3c). MAP significantly determined the pH values in soils, while moisture showed lower correlation with soil pH (Figures 3d, 3e, and 3f).

Community Compositions of AOA and AOB

The diversity of AOA and AOB communities was calculated based on 3 AOA and 2 AOB clone libraries. With ascending altitude, the slope of rarefaction curves for 3 AOA clone libraries and 2 AOB clone libraries became more and more asymptotic at higher altitude (Figures 4a and 4b). Meanwhile, Shannon index of both AOA and AOB significantly decreased with increasing altitudes (Table S1). For AOA, the relative proportions of each OTU (based on 97% similarities) significantly differed among 3 altitudes. The OTU 1, 7 and 8 existed in roughly equal proportions at altitude 4400 m, OTU 7 and 23 mainly existed at altitude 4800 m, whereas OTU 6 and 25 mainly existed at altitude 5100 m (Figure 4c).

For AOB, the relative proportion of each OTU also significantly differed between two altitudes. The OTU 2 existed

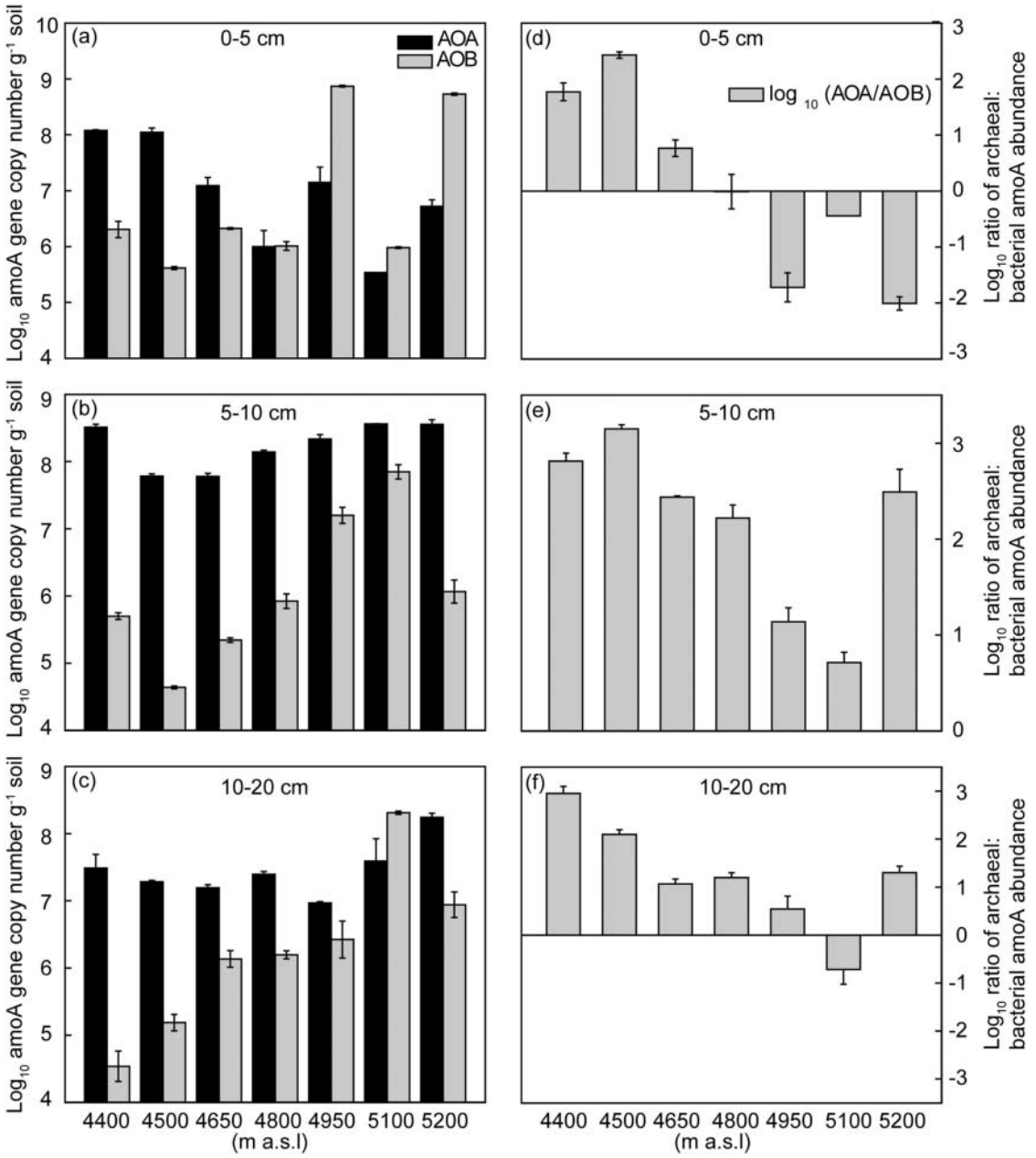

Fig. 2. Abundance and the ratios of the archaeal and bacterial *amoA* along the altitudinal transect of Nyqentangula Mountains on the Tibetan Plateau at different soil depths. (a-c) The abundance of archaeal and bacterial *amoA* genes at 0–5 cm, 5–10 cm and 10–20 cm soil; (d-f) $\log_{10}$ ratio of archaeal: bacterial *amoA* abundance at 0–5 cm, 5–10 cm and 10–20 cm soil.

exclusively at altitude 4400 m, whereas OTU 5 mainly existed at altitude 4800 m. Figure 5 and Figure 6 showed the phylogenetic affiliations of AOA and AOB *amoA* genes, respectively. The representatives of obtained AOA sequences and their related NCBI sequences clustered into three soil/sediment lineages (Figure 5).

Soil and sediment lineages 1 and 2 included all AOA clones obtained from three elevations, and these sequences were closely related to *amoA* sequences obtained from many soil environments (Hansel et al. 2008; He et al. 2007; Shen et al. 2008) and sediments (Jiang et al. 2009). However, sequences belonging to soil and sediment 2 sequences (66.7%–72.3%) were much more than that belonging to cluster 1 (6.25%–28.3%) in three altitudinal soils.

All obtained AOB sequences were affiliated to *Nitrosospira* clusters 3a.1, clusters 11/12, and *Nitrosomonas* clusters 6 (Figure 6). The relative abundances of sequences affiliated to same OTUs (based on 97% similarity) in two clone libraries were calculated (Figure 4d). AOB community was dominated by OTU 2 belonging to cluster 3a.1, constituting 86.5% of clone library at altitude 4400 m (Figure 6). Although OTU 5 mainly existed at altitude 4800 m and OTU 2 was

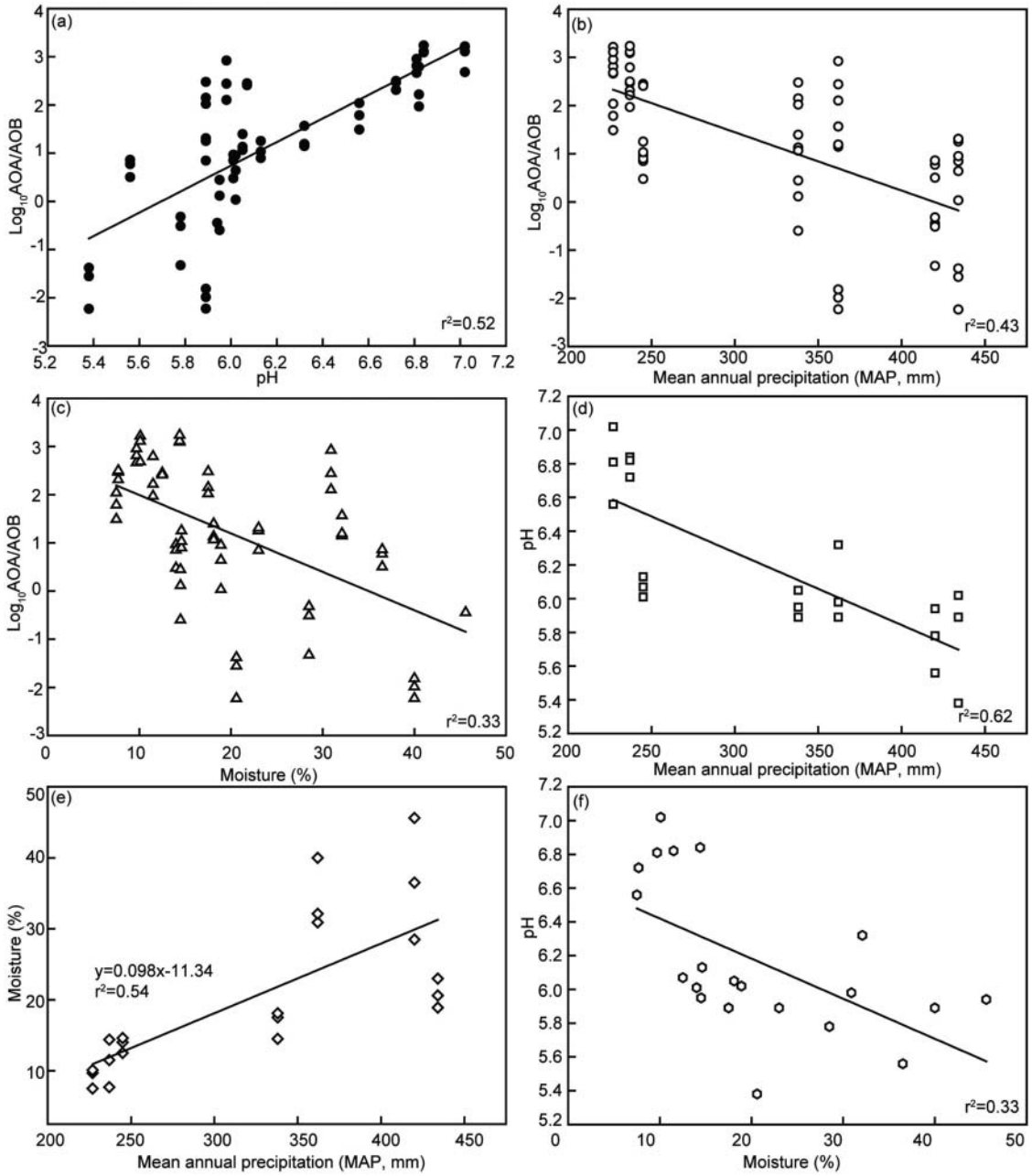

Fig. 3. Relationship between $\log_{10}$ ratio of AOA/AOB and environmental factors and among environmental factors. (a) pH and $\log_{10}$ ratio of AOA/AOB; (b) MAP and $\log_{10}$ ratio of AOA/AOB; (c) moisture and $\log_{10}$ ratio of AOA/AOB; (d) MAP and pH; (e) MAP and soil moisture; (f) Soil moisture and pH.

absent at this altitude. Sequences falling within cluster 6 and cluster 11/12 were exclusively from altitude 4800 m, but with low proportions (7.0% and 2.3%, respectively) (Figure 6).

Discussion

At lower altitudes (4400 m–4650 m), our results demonstrated that archaeal *amoA* gene copies were significantly higher than bacterial *amoA* gene copies from 0–5 cm soil, which was in line with previous studies showing greater proportion of AOA than AOB in different ecosystems (Leininger et al. 2006; Shen et al. 2008). Nevertheless, an inverse distribution of relative abundance of AOA and AOB was found at higher altitudes (Figure 2d). This phenomenon was similar to previous research about Mount Everest soil (Zhang et al. 2009), while the soil pH between two cases was contrastingly different, suggesting that altitude-related factors do affect the AOA and AOB regardless of the difference of indigenous soil pH range. Therefore, our work is of significance to understand the influence of climatic change factors on the ammonia oxidizers under contrasting pH conditions,

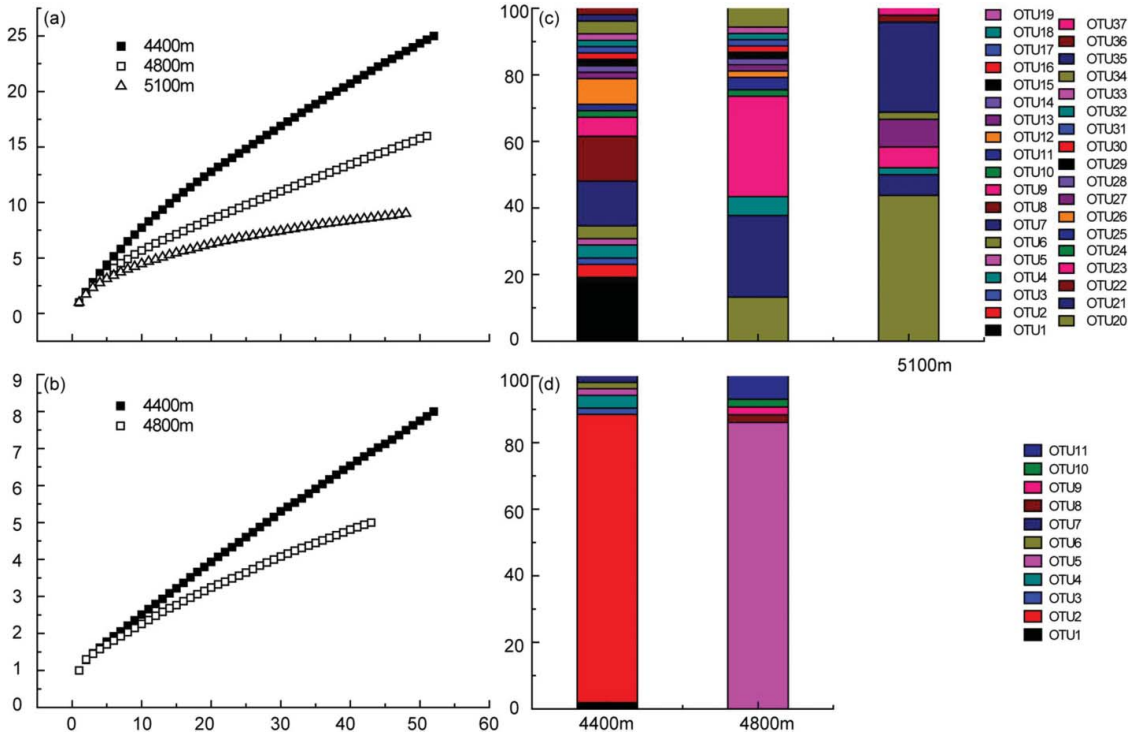

Fig. 4. Rarefaction curves and the relative abundance (%) of different OTUs of ammonia-oxidizing archaea (a and c) and bacteria (b and d).

especially in the hotspot regions relating to climate change study.

The AOA abundance showed no significant correlation with altitude, while AOB abundance significantly correlated to altitude and altitude-related environmental factors, such as pH, MAP, MAT, TOC, TON and so on, suggesting that the altitudinal distribution of AOB in these alpine meadow soils was primarily determined by their abiotic environment and substrates availability for microbial mineralization. Previous studies had demonstrated that warming at higher altitudes was more obviously than at low altitudes (Diaz et al. 2003) and precipitation also significantly changed along the certain altitudinal gradient (Yuan et al. 2014), thus we could infer that climate change (such as warming or precipitation variation) might exert different influences on soil microbial populations for the low and high altitudes based on the "space-for-time substitution" strategy along the certain altitudinal gradient in the future climatic scenarios.

Soil pH is a complex parameter, which could affect substrate availability for microbes, and directly influences the activity of microbial communities and microbe-mediated biogeochemical processes (Kemmitt et al. 2006). Correlation analysis showed that the $\log_{10}$ ratio of AOA:AOB had most significant correlation with soil pH at all soil depths (Table 2). Thus, pH probably has greater impact on the niches differentiation of AOA and AOB along the altitudinal transect at our study sites. This was consistent with previous studies in various environments (Delgado-Baquerizo et al. 2013; Liu et al. 2013; Nicol et al. 2008).

Thus, our results indicate that the relative abundance of both groups might be directly determined by pH patterns along the altitudinal transect on alpine meadow ecosystem. Furthermore, as a complex parameter, pH could reflect the function of parent material, time of weathering, vegetation and climate. Since the soil comes from same parent material with similar time of weathering, the decreasing soil pH could probably result from the vegetation difference and precipitation increment caused heavier leaching of basic cations at higher elevations along the altitudinal transect. Indeed, correlation and regression analysis showed that pH had a close relationship with precipitation (Figure 3d), but relatively lower correlation with soil moisture (Figure 3f).

Arid, semi-arid or seasonally arid area represent one third of the earth's total cover, and water shortage is perhaps the most common environmental stress that constraints soil microorganisms. Among these altitude-related environmental factors, precipitation had been clearly demonstrated to have a significant effect on the abundance and community composition of AOB and caused significant population shifts in northern grassland at Inner Mongolia (Chen et al. 2013).

Similarly, as the typical alpine meadow on TP, AOB abundance also significantly increased with precipitation increment, while AOA abundance not. This is in agreement with previous studies which had demonstrated that AOB abundance was significantly higher in wetter conditions, whereas AOA abundance stayed relatively stable in wetter conditions (Avarhami and Bohannan 2007; Chen et al. 2013; Gleeson et al. 2008, 2010; Singh and Kashyap 2006). Abovementioned results suggested that precipitation could significantly influence AOB abundance and their community structure, whereas AOA could be more resilient to precipitation variation in these semi-arid regions.

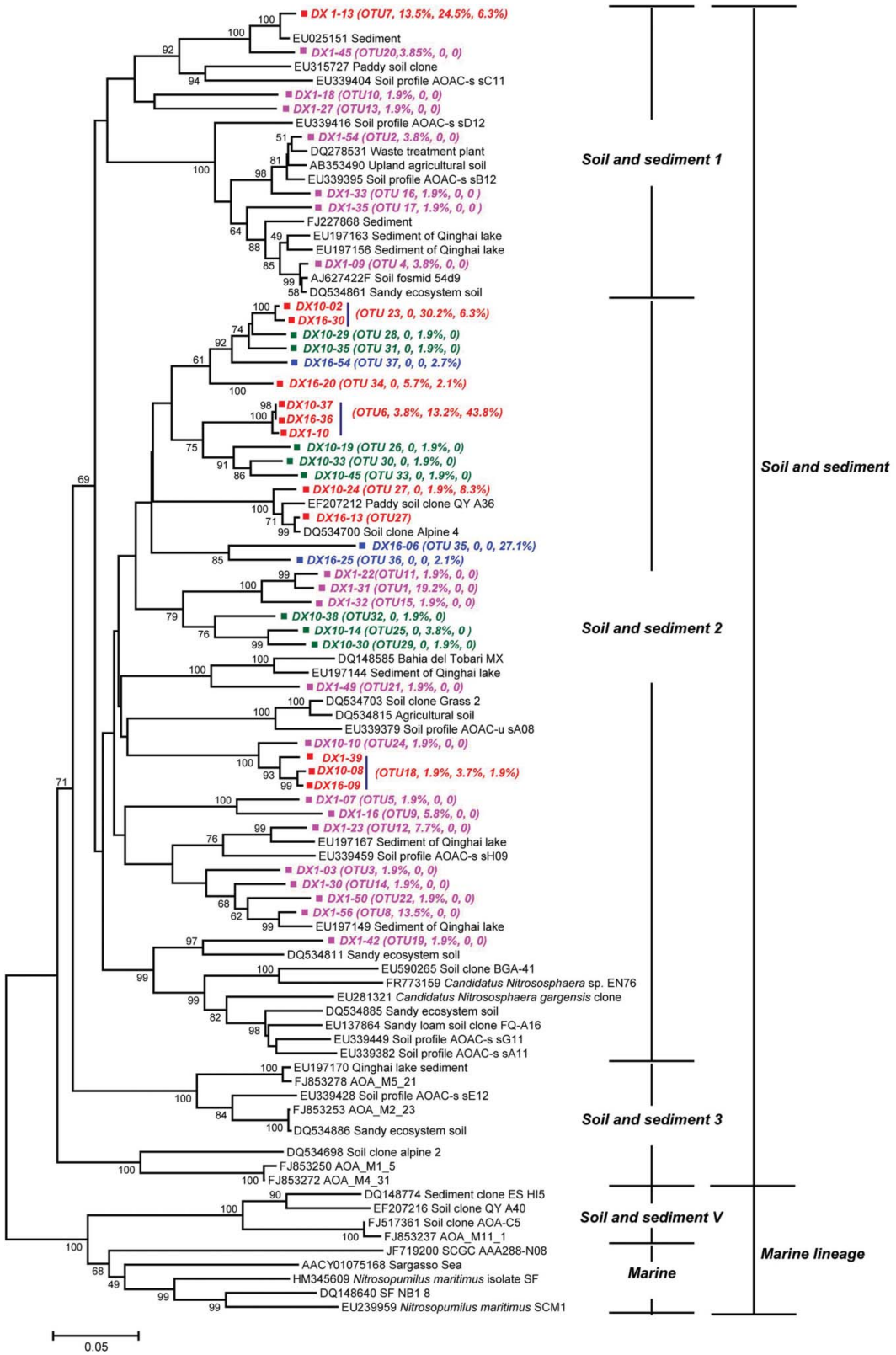

Fig. 5. Phylogenetic tree of archaeal *amoA* sequences (635 bp fragment) retrieved from 3 altitudes along the south-facing slope of Nyaiqentanglha Mountains on the Tibetan Plateau. Clones from this study are shown in bold italics with the name DX, followed by OTU number, then by the relative abundances of this OTU in 4400 m, 4800 m and 5100 m, respectively. Bootstrap values (>50) are indicated at branch points. Red: all altitudes; Pink: only in 4400 m; Dark green: only in 4800 m; Blue: only in 5100 m.

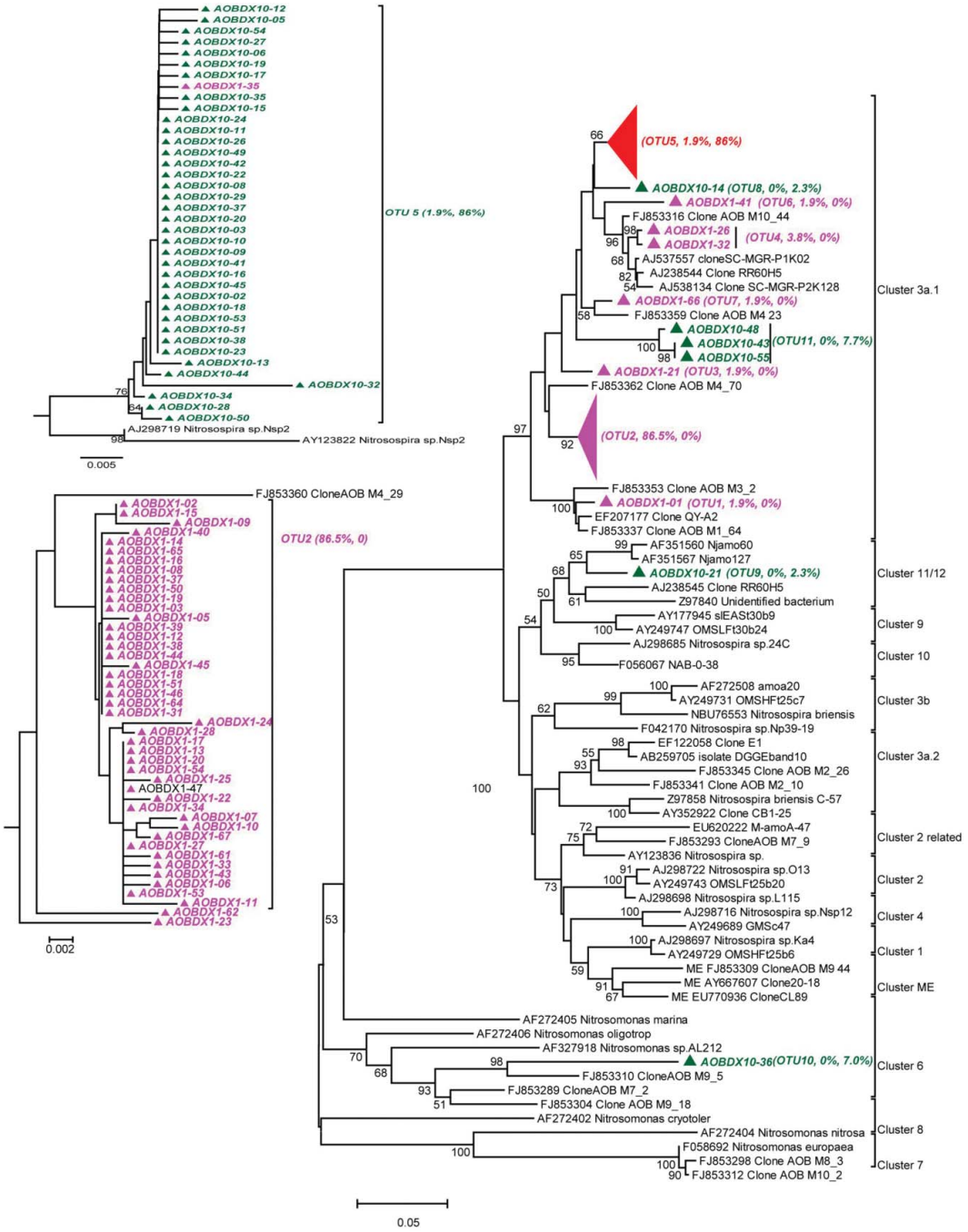

Fig. 6. Phylogenetic tree of bacterial *amoA* sequences (491 bp fragment) retrieved from soils at 4400 m and 4800 m along the south-facing slope of Nyaiqentanglha Mountains on the Tibetan Plateau. Bootstrap values (> 50) are indicated at branch points. Red: all altitudes; Pink: only in 4400 m; Dark green: only in 4800 m.

However, one microcosm study demonstrated that higher soil water content did not affect AOB abundance, and instead AOA slightly increased under wetter conditions (Bustamante et al. 2012). Ammonia oxidizers (AOB and AOA) on different ecosystems probably behave different. Another issue is that abovementioned studies just focus on the *amoA* gene at the genetic level. Therefore, the question of how the ammonia oxidizers behave on the transcriptional level and during the nitrification processes under different water regimes still requires to be addressed in future research, such as the application of the stable isotope probing, and *amoA* analyses on the transcriptional level.

Microorganisms could evolve various strategies to deal with moisture stresses, depending on their acclimation and adaptation abilities, such as the shifts of resource allocation (Schimel et al. 2007). Therefore, the distinguishing responses of ammonia oxidizer (AOA and AOB) to precipitation could be explained by their different genetic and biochemical adaptations. It has been suggested that "specialized" organisms, such as nitrifiers belonging to G^--bacteria may be more

Table 2. Correlation analyses among the abundance of AOA/AOB, altitude, pH, organic matter, total nitrogen content, NH4+, NO3− and C/N

	0-5 cm			5–10 cm			10–20 cm			All		
Kendall's tau_b)	log_{10}AOB	log_{10}AOA	ABratio	log_{10}AOB	log_{10}AOA	ABratio	log_{10}AOB	log_{10}AOA	ABratio	log_{10}AOB	log_{10}AOA	ABratio
Altitude	ns[a]	−0.521**	−0.720**	ns	0.430**	ns	0.777**	ns	−0.574**	0.468**	ns	−0.435**
MAT	ns	0.521**	0.720**	ns	−0.430**	ns	−0.760**	ns	0.597**	−0.459**	ns	0.434**
MAP	ns	−0.470**	−0.679**	0.498**	ns	−0.437**	0.669**	ns	−0.688**	0.487**	ns	−0.491**
Moisture	ns	−0.612**	−0.628**	0.347*	0.490**	ns	0.760**	ns	−0.597**	0.405**	ns	−0.387**
pH	−0.504**	0.369*	**0.862****	−0.495**	ns	**0.494****	−0.579**	ns	**0.898****	−0.496**	ns	**0.585****
NH_4^+	ns	−0.733**	−0.446**	ns	ns	−0.326*	0.528**	ns	−0.447**	0.270**	ns	−0.399**
NO_3^-	ns	−0.420*	−0.537**	0.438**	0.834**	ns	0.508**	0.728**	ns	0.360**	ns	−0.253**
TOC	ns	−0.612**	−0.628**	0.468**	0.349*	−0.387*	0.709**	0.366*	−0.507**	0.334**	ns	−0.474**
TONp	ns	−0.580**	−0.597**	ns	ns	ns	0.619**	ns	−0.597**	0.295**	ns	−0.421**
C/N	ns	−0.703**	−0.537**	ns	ns	−0.397*	0.438**	0.437**	ns	0.312**	−.181*	−0.490**

Note: a: ns means no significance; *, $p < 0.05$; **, $p < 0.01$.

sensitive to water stress than G^+-bacteria (Schimel et al. 2007). Moreover, archaeal membranes are more resistant to ions penetration than that of bacteria, which could provide an energetic barrier for resistance to water stress for archaea (Valentine 2007). In this respect, AOB might be more sensitive than AOA and thus could be used as an indicator reflecting the influence of global climate change (precipitation variation) on alpine grassland ecosystems.

Clone library analysis demonstrated that both AOA and AOB community structures varied significantly between lower and higher altitudes, suggesting that altitude-dependent environmental factors (such as precipitation) significantly influenced ammonia-oxidizer compositions in the study area. This is similar to previous studies which showed the increase of water availability significantly changed ammonia oxidizer community structures (AOA and AOB) (Gleeson et al. 2010).

All AOA sequences are affiliated to soil and sediment clusters, indicating that the uniqueness of alpine AOA communities from marine environments. The relative abundances of AOA OTUs within clone libraries changed with altitudes. This is contrary to previous studies on alpine ecosystem (Zhang et al. 2009) and temperate grassland ecosystem (Chen et al. 2013). However, pronounced AOA community changes in response to water addition were observed in microcosms of a Chilean semiarid soil with 350 mm annual averaging rainfall (Bustamante et al. 2012) and two temperate forest soils (Szukics et al. 2012). Previous study had indicated that precipitation has imposed a great influence over the bacterial community at the same sites (Yuan et al. 2014). Considering the wide range of precipitation (227 mm–434 mm) along the altitudinal transect in this study and all abovementioned studies, difference of soil AOA community structure between lower and higher altitude might also be attributed to the changes of precipitation along the altitudinal transect.

Our results suggested that two unique AOB clusters existed in the soils along the altitudinal transect: one cluster was adapted to drier and higher oxic conditions, whereas another was habituated to higher water content and lower oxic conditions. The proportions of the AOB-OTU2 cluster belonging to *Nitrosospira* 3a.1 was higher at lower altitude 4400 m with drier and higher oxic conditions, whereas the proportions of AOB-OTU5 and AOB-OTU9 belonging to *Nitrosospira* 3a.1 and *Nitrosomonas* cluster 6 were higher at altitude 4800 m with wetter and lower oxic conditions.

Meanwhile, plants showed higher aboveground biomass and coverage in higher altitudes with higher precipitation at the same study site (Wang et al. 2013b), and thus soils probably provided an optimal habitat with altitude-dependent physicochemical gradients including higher nutrients that changed AOB community structures. Similarly, one previous study also demonstrated precipitation increment caused significant shifts of divergent *Nitrosospira* clusters on temperate grassland ecosystem (Chen et al. 2013).

Angel et al. (2010) observed that water content significantly correlated with bacterial community structure. On the contrary, in two Rothwald virgin forest acidic soils, AOB communities were not affected by water content (Szukics et al. 2012). These controversial results might be due to different precipitation ranges and soil types. But, currently the capacity of our clone libraries is lower and species coverage hasn't reached the plateau status, thus the potential community structure of AOB along altitudinal gradient in semi-arid soils requires further investigation in future researches, such as application of pyro-sequencing or microchip analysis (Abell et al. 2012; Gubry-Rangin et al. 2011; Pester et al. 2012).

In conclusion, changes in the abundance and community compositions of AOB were observed in surface soils between lower and higher altitudes along the altitudinal gradient, with higher abundances above 4800 m, suggesting that altitude-dependent factors, in particular pH and precipitation, had a profound effect on the abundance and community of AOB. AOA abundance stayed relatively stable in surface and subsurface soils along the altitudinal gradient. Bacterial *amoA* abundance outnumbered archaeal *amoA* abundance in surface soil with higher precipitation at higher altitude.

Combing the distinct change in the ratio of bacterial and archaeal ammonia oxidizers in response to altitude-related climatic factors (such as precipitation), this study also suggested that AOB might be highly responsive to precipitation than AOA in the semi-arid area. Thus, although AOA showed higher abundance over AOB in lower altitude and deeper soils along vertical soil profiles, AOB may play a vital role in ammonia oxidization at higher altitudes. However, the accurate contribution of AOB and AOA at higher ($\geq$4800 m) and lower altitude to nitrification remains undetermined, and the ecological function of the shifts in the relative abundance of AOB and AOA in these soils also requires further study. Therefore, studies concerning the effect of altitude-dependent precipitation increment on potential nitrification rates and changes of ammonia oxidizer community structures requires to be investigated through applications of other techniques, such as stable isotope probing, pyrosequencing or microchip analysis in future researches.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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