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Distribution of ammonia oxidizers in relation to vegetation characteristics in the Qilian Mountains, northwestern China

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Abstract

Nitrogen is the major limiting nutrient in cold environments, and its availability is strongly dependent on nitrification. However, microbial communities driving this process remain largely uncharacterized in alpine meadow soils in northwestern China, namely those catalyzing the rate-limiting step of ammonia oxidation. In this study, ammonia-oxidizing communities in alpine meadow soils were characterized by real-time PCR and clone sequencing by targeting on *amoA* genes, which putatively encode ammonia monooxygenase subunit A. The results demonstrated that ammonia-oxidizing archaea (AOA) outnumbered ammonia-oxidizing bacteria (AOB) in the alpine meadow soils. Most of the AOA phylotypes detected in the study region fell within typical Group I.1b of Thaumarchaeota. Interestingly, a new ammonia-oxidizing archaeal group named "*Kobresia* meadow soil group" was found. Phylogenetic analysis of AOB communities exhibited a dominance of *Nitrosospira*-like sequences affiliated to beta-Proteobacteria. Compared with other alpine environments, Qilian Mountains had a great phylogenetic diversity of ammonia oxidizers. Principal Component Analysis (PCA) analysis showed that distinct AOA/AOB phylotype groups were attributed to different meadow types, reflecting an overall distribution of ammonia-oxidizing communities associated with meadow types. Redundancy Analysis (RDA) analysis showed that Axis 1 (90.9 %) together with Axis 2 (9.1 %) explained all the variables while Axis 1 exhibited a significant explanatory power. So that vegetation coverage mostly correlated to Axis 1 was the most powerful environmental factor in the study region. Characteristics of ammonia-oxidizing communities showed a close association with vegetation coverage.

1 Introduction

The oxidation of ammonia to nitrite plays a significant role in the transformation of fixed nitrogen in the global nitrogen cycle (Junier et al., 2010). Ammonia-oxidizing microorganisms are a diverse microbial group found in most environments where ammonia

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is available: soils (Zhang et al., 2009; Jung et al., 2011; Daebeler et al., 2012; Wang et al., 2012; Alves et al., 2013), freshwater (Jiang et al., 2009; Hu et al., 2010; Huang et al., 2011; Peng et al., 2012), and marine habitats (Kalanetra et al., 2009). Despite their significant contributions to the global carbon and nitrogen cycle, many questions about the physiology, metabolism, and ecological niches remain unanswered owing to the difficulty of isolation (Kim et al., 2011). Culture-independent approaches have contributed importantly to our understanding of the diversity and distribution of these microorganisms in different environments (Junier et al., 2010). The *amoA* gene encoding the catalytic α-subunit of ammonia monooxygenase which is responsible for catalyzing the rate-limiting step in bacterial and archaeal ammonia oxidation (Francis et al., 2005), represents a very powerful molecular tool for analyzing indigenous ammonia-oxidizing communities (Rotthauwe et al., 1997).

Ammonia oxidation was previously considered to be performed largely by autotrophic AOB that form two distinct monophyletic groups within the beta- and gamma-Proteobacteria (as reviewed in Nicol and Schleper, 2006). The cultivation of several AOA as well as the discovery that archaeal *amoA* gene sequences are nearly ubiquitously distributed in the environment and outnumber their bacterial counterparts in many habitats fundamentally revised our understanding of nitrification (Hatzenpichler, 2012). To date, AOA appear not to form a monophyletic clade but rather to belong to different lineages within the Thaumarchaeota (Spang et al., 2010). Phylogenetic analysis of ammonia oxidizers revealed distinct lineages that in general reflect a certain level of ecological differentiation based on the environment (Nicol and Schleper, 2006). The relative roles of archaeal vs. bacterial ammonia oxidizers are controversial (Prosser and Nicol, 2008). The question under which conditions AOA or AOB dominate ammonia oxidation is currently attracting a lot of attention (Pester et al., 2011). More studies about the relative contributions of AOA and AOB to ammonia-oxidation are necessary.

Qilian Mountains is located in the northeastern of Qinghai-Tibetan Plateau. The alpine meadow covered about one-third of the Qinghai-Tibetan Plateau is a dominant plant community in this vast plateau, hence it is important to profile the unique geo-

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graphical flora and assess the response of the microbial communities to environmental variables (Kato et al., 2006; Yang et al., 2013; Zhang et al., 2013). Soil ammonia-oxidizing communities will play a key role in the global cycling of nitrogen underlying nutrient fluxes aroused by climate changes (Alves et al., 2013; Ke et al., 2013). Previous studies indicated that AOA could be the main drivers of nitrification, supporting the hypothesis of niche separation between AOA and AOB, with AOA being better adapted to extreme conditions (Schleper et al., 2010; Glanville et al., 2012). Leininger and colleagues (2006) demonstrated the activity of the archaea in situ and supported the numerical dominance of archaeal over bacterial ammonia oxidizers. However, their distributions and relative contributions to nitrification remain unclear in the study region. In the present study, five types of alpine meadow soils were sampled and ammonia-oxidizing communities were studied, focusing on two major issues: (1) whether the distribution of ammonia-oxidizers reflect an ecological differentiation among different alpine meadow soils? (2) Whether ammonia-oxidizing archaea are the dominant ammonia oxidizers in the alpine meadow soils in the study region?

2 Materials and methods

2.1 Soil sampling and physicochemical parameters determination

Soils were sampled from five types of alpine meadow named by their constructive species in the upper reaches of the Heihe River in northwestern China between 20 and 21 August, 2012 (Fig. 1). Species and numbers of plants were investigated in situ, as well as vegetation coverage (Wang et al., 2003; Wu, 2011). We placed three quadrats within each study site, and five soil samples were collected from each quadrat and pooled, for a total of three samples from each site. The samples were cooled on ice until they were delivered to the laboratory and further processed.

Soil pH and salt concentration were determined with a soil to water ratio of 1 : 2.5 by a pH meter (sartorius PB-10; XinShenghongYang SCI&TECH Co., Ltd, Beijing, China)

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and a conductivity meter (DDSJ-308A; SCRPF Co., Ltd, Shanghai, China). Soil NO_3^- and NH_4^- were extracted from fresh soil with 2 M KCl solution (including absorbed nitrogen) and determined by a continuous flow analyzer (FIAstar 5000 Analyzer; Foss Analytic AB Co., Ltd, Hoeganaes, Sweden). Content of soil anions (HCO_3^-, Cl^-, SO_4^{2-}) and cations (Na^+, K^+,Ca^{2+}, Mg^{2+}) were detected by an ion chromatograph (ICS 3000; DIONEX Co., Ltd, Sunnyvale, USA) while soil available P and K were tested by a modified Kelowna extraction (Qian et al., 1994). Soil physicochemical properties and vegetation characteristics are provided in Table 1.

2.2 Cloning PCR and sequencing

We performed three DNA extractions per site from the three composite samples using the PowerSoil DNA Isolation Kit (MoBio Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The three DNA extractions were used for polymerase chain reaction (PCR). The archaeal *amoA* gene fragments (635 bp) were amplified by using the PCR primers arch-*amoA*-F and arch-*amoA*-R with the reported protocol (Francis et al., 2005). The PCR primers *amoA*-1F and *amoA*-2R were used to target bacterial *amoA* gene fragments (491 bp) with the standard thermal profile (Rotthauwe et al., 1997). Clone library of *amoA* gene from each soil sample were constructed by the previously described method (Tai et al., 2013). Fifty clones of each library were delivered for sequencing.

2.3 Quantitative PCR analysis of *amoA* gene

The primers arch-*amoA*-F/arch-*amoA*-R targeted archaeal *amoA* gene and the ones *amoA*-1F/*amoA*-2R did bacterial *amoA* gene were used in quantitative PCR analysis. Q-PCR was conducted in triplicate for both the standard and the samples as previously described (Tai et al., 2013). The standard curve of archaeal *amoA* gene Q-PCR was $Y = -3.846 \times \log(X) + 34.74$, based on the average of the triplicate data; the R^2 value of the curve was 0.999, and the efficiency of Q-PCR was 82.0 %. The Q-PCR cycling

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parameters for archaeal *amoA* gene were 30 s at 95 °C, 40 cycles of 95 °C for 10 s, 63 °C for 25 s and 72 °C for 45 s, a dissociation stage of 95 °C for 30 s and 63 °C for 30 s, and a final ramp-up to 95 °C. The standard curve of bacterial *amoA* gene Q-PCR was $Y = -3.578 \times \log(X) + 33.85$, based on the average of the triplicate data; the R^2 value of the curve was 0.992, and the efficiency of Q-PCR was 90.3 %. The Q-PCR cycling parameters for bacterial *amoA* gene were 30 s at 95 °C, 40 cycles of 95 °C for 10 s, 57 °C for 25 s and 72 °C for 45 s, a dissociation stage of 95 °C for 30 s and 57 °C for 30 s, and a final ramp-up to 95 °C. Melting curve analysis was employed to confirm the specificity of the Q-PCR technique.

2.4 Statistic analyses

The diversity of vegetation in each quadrat was calculated with Shannon–Wiener's index: $H = -\sum(P_i \times \log P_i)$, where $P_i = N_i/N$, N_i is the individuals of species i, N is the total individuals of all plant species present (Wang et al., 2003).

Sequences obtained from each soil sample were assigned to operational taxonomic units (OTUs) by Mothur (V.1.31.2) software at cutoffs of 0.03 and the sequences were clustered using the furthest neighbor algorithms (Schloss et al., 2009). These OTUs have been deposited in the GenBank database (accession nos. KF754126-KF754301). According to the genotypes of the OTUs and the number of clones for each OTU based on the clone library approach, the Shannon–Wiener diversity index of the *amoA* gene from each soil sample was calculated using the formula: $H = -\sum(P_i \times \ln P_i)$, where $P_i = N_i/N$, N_i is number of clones of the ith OTU, N is the total number of clones (Duc et al., 2009).

All the OTUs of ammonia-oxidizing archaea (AOA) and bacteria (AOB) acquired from the study region were assigned again by Mothur as AOA and AOB phylotypes for phylogenetic analysis. Phylogenetic analysis of ammonia-oxidizing communities and redundancy analysis (RDA) on correlations between characteristics of ammonia-oxidizing communities and environmental factors were processed with the methods described by the previous study (Tai et al., 2013). Principal Component Analysis (PCA) based on

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the relative abundance of each AOA or AOB phylotype was applied to attribute different AOA/AOB phylotypes to certain meadow types, processing by using Canoco for Windows (Version 4.5) software, where the relative abundance indicates the proportion of OTUs of each phylotype in the total OTUs of all the phylotypes.

3 Results

3.1 Soil physicochemical properties

The annual precipitation and air temperature of the meteorological station located at 3180 m in the study region are 407 mm and −3.0 °C respectively. The former rises with increasing of altitude by 30.6 mm per 100 m while the latter decreases by 0.58 °C per 100 m (Zhang and Guo, 2002; Zhang and Zhao, 2008). Nutrient poor soils are usually indicated by the phenomenon that dissolved organic N comprises the majority of total dissolved N poor. However, mineralization of organic N into available inorganic forms is often limited in alpine ecosystems due to low temperature (Glanville et al., 2012). During the summer of the study region, a relative higher temperature enhances the mineralization of organic N, there will be a pulse of plant-available nutrients, so that a high NH_4^+ was observed.

3.2 Diversity and abundance of ammonia-oxidizing archaea and bacteria

Compared with AOB, the AOA communities displayed a higher abundance in all the alpine meadow soils (Fig. 2b), as well as diversity excepting the one in the *Carex* meadow soil (Fig. 2a). Diversity of AOA/AOB and abundance of AOB were highest in the *Carex* meadow soil, whereas AOA was the most abundant in the *Thermopsis* meadow soil.

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3.3 Phylogenetic analysis of ammonia-oxidizing archaeal and bacterial communities

We have detected 41 AOA phylotypes by the method of clone library from the alpine meadow soils in the study region (Fig. S1). Group I.1a (distributed in marine and other environments represented by *Nitrosopumilus maritimus*), Group I.1a-associated (represented by *Nitrosotalea devanaterra*), Group I.1b (distributed in soils and other environments represented by *Nitrososphaera gargensis*, *Nitrososphaera viennensis* and soil fosmid clone 54d9) and ThAOA group (distributed in thermophilic environments represented by *Nitrosocaldus yellowstonii*) are typical thaumarchaeotic groups associated with ammonia oxidation. Most of the AOA phylotypes detected in this study fell within Group I.1b. Interestingly, a new ammonia-oxidizing archaeal group including AOA phylotyes A1, A14, A30, A31 and A28 was found in the study region mainly attributed to the *Kobresia* meadow soil (Fig. 4).

To date, AOB were affiliated to two distinct monophyletic groups within the beta- and gamma-Proteobacteria. Ammonia-oxidizing bacterial communities in the alpine meadow soils were investigated by the method of clone libraries excepting the *Kobresia* meadow soil due to a low abundance of AOB (Fig. 2b). A total of 55 AOB phylotypes were obtained from the study region (Fig. S2). Figure 5 showed that AOB phylotypes detected in this study fell within beta-AOB group and had high similarity with *Nitrosospira* sp. and *Nitrosovibrio* sp.

3.4 Distributions of ammonia-oxidizing archaea and bacteria

PCA showed that different AOA/AOB phylotypes were distinctly attributed to different meadow types (Fig. 6). Interestingly, AOA phylotypes (A1, A14, A30 and A31) fell within the new ammonia-oxidizing archaeal group were attributed to the *Kobresia* meadow soil, while A1 was the most abundant AOA phylotype in the study region (data not shown) (Fig. 6a). These results indicated that niche differentiation of ammonia-oxidizing communities was based on the environmental choice.

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3.5 Characteristics of ammonia-oxidizing communities in relation to soil physicochemical properties

The component of environmental factors have been extracted by the method of Principal Component Analysis (Table 2). The cumulative percentage of Initial Eigenvalues of the three components reached up to 95.9 %, indicating that the extraction was efficient. Therefore, the environmental factors (soil pH, NO_3^-, HCO_3^-, Ca^{2+}, vegetation coverage and annual air temperature) with more information load were applied to make a correlation analysis with the ammonia-oxidizing community characteristics.

Figure 3 showed that Axis 1 (90.9 %) together with Axis 2 (9.1 %) explained all the variables while Axis 1 exhibited a significant explanatory power. So that vegetation coverage mostly correlated to Axis 1 was the most powerful environmental factor in the study region. Vegetation coverage had a close association with ammonia-oxidizing communities, whereas soil pH, annual air temperature and Ca^{2+} showed little effect on community characteristics, HCO_3^- and NO_3^- had no significant explanatory power.

4 Discussion

Compared to other alpine environments, more phylotypes of ammonia oxidizers were found in the alpine meadow soils (Table S1). Di and colleagues (2010) found that AOB and AOA prefer different soil N conditions to grow: AOB under high ammonia (NH_3) substrate and AOA under low NH_3 substrate conditions. However, AOA were dominant ammonia oxidizers (Arctic tundra soils, Icelandic grassland soils, QTP fir forest soils and alpine meadow soils), although the NH_4^+ concentrations were higher than AOB dominated environment, such as Antarctic soils (Table S1). In the present study, some of the AOA phylotypes found in the study region were similar with soil AOA *Nitrososphaera viennensis* EN76 (Fig. 4), which was found to be adapted to considerably higher NH_4^+ concentrations (Tourna et al., 2011).

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Ammonia-oxidizing archaea are often outnumbering ammonia-oxidizing bacteria. This phenomenon was also found in the study region (Fig. 2b). Although the quantification of archaeal and bacterial *amoA* gene copies indicated that AOA outnumbered AOB in most marine and terrestrial ecosystems by a factor of 10 to 1000, suggesting a possible higher contribution to nitrification (Prosser and Nicol, 2008; Junier et al., 2010), the relative importance of AOA in nitrification, compared to AOB, is still under debate (Hatzenpichler, 2012). AOA were the only ammonia oxidizers detected in five out of 11 Arctic tundra soils and outnumbered AOB in four of the remaining six soils (Alves et al., 2013). In alpine and permafrost soils from the northern slope of the Mount Everest (Qinghai-Tibetan Plateau), where archaeal *amoA* abundance was greater than bacterial *amoA* abundance in lower altitude soils (≤ 5400 m a.s.l.), but a reversed situation was detected in higher altitude soils (≥ 5700 m a.s.l.) (Zhang et al., 2009). Compared with AOB, the AOA displayed a higher abundance in alpine fir forest soil on the eastern Qinghai-Tibetan Plateau (Wang et al., 2012). However, bacterial *amoA* was found to be more abundant than archaeal *amoA* in Antarctic soils (Jung et al., 2011). Copy numbers of archaeal and bacterial *amoA* gene were assumed to be equivalent to actual cell numbers (Glanville et al., 2012). Leininger et al. (2006) demonstrated that *amoA* genes were actively transcribed in soils and the transcription correlated well with gene abundance, that was, transcripts of archaeal *amoA* dominated in the soils.

Besides the known lineages of AOA (Group I.1a, Group I.1a-associated, Group I.1b and ThAOA group), sequence data suggested that more, as-yet-unidentified *amoA*-encoding and potentially ammonia-oxidizing groups might exist (as reviewed in Hatzenpichler, 2012). Based on phylogenetic analysis of AOA phylotypes, a new ammonia-oxidizing archaeal group named "*Kobresia* meadow soil group" was detected, which contained the most abundant AOA phylotype A1. The result indicated that the specialness of the study region might lead to new species of ammonia-oxidizing archaea. It will be fascinating to see whether all the members of Thaumarchaeota have the capability to perform ammonia oxidation. Most of the AOA phylotypes detected in this study were fell within Group I.1b represented by *Nitrososphaera gargensis*, *Nitrososphaera*

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viennensis and soil fosmid clone 54d9. Pratscher et al. (2011) gave support that members of the very abundant soil Group I.1b of Thaumarchaeota were actually involved in ammonia oxidation in soils. The enrichment of clade A represented by soil fosmid clone 54d9 provided the first direct evidence for their ammonia oxidation activity (Alves et al., 2013). Namely that the AOA phylotypes detected in this study possibly acted as ammonia oxidizers in the alpine meadow soils.

AOB are traditionally divided into two monophyletic lineages based on their 16S rRNA gene sequences. The first lineage belongs to the beta-Proteobacteria (beta-AOB) and comprises *Nitrosomonas* (including *Nitrosococcus mobilis*) and *Nitrosospira* (including *Nitrosolobus* and *Nitrosovibrio*) species. The second lineage, affiliated with the gamma-Proteobacteria (gamma-AOB), contains *Nitrosococcus oceani* and *Nitrosococcus halophilus* (Junier et al., 2010). In the present study, phylogenetic analysis of AOB communities showed a dominance of *Nitrosospira*-like sequences, while few were affiliated with the *Nitrosovibrio* genus, namely AOB in the study region fell within the beta-AOB.

PCA showed that distinct AOA/AOB phylotype groups were attributed to different meadow types and indicated that niche differentiation of ammonia-oxidizing communities was based on the environmental choice. Previous studies dealing with the amplification of archaeal *amoA* demonstrated the ubiquitous presence of AOA in marine, freshwater, and terrestrial environments showing an apparent niche adaptation to different habitats (Hatzenpichler, 2012). AOA can also be found over a wide range of pH, temperature, salinity, and phosphate concentrations with some AOA being adapted to sulfidic environments, which extends the potential range of AOA niche differentiation to a multitude of environmental factors (Schleper and Nicol, 2010). Phylogenetic analysis of AOA revealed distinct lineages that in general reflect a certain level of ecological differentiation based on the environment (Nicol and Schleper, 2006).

Ammonia-oxidizing archaea were negatively correlated with increasing elevation (Zhang et al., 2009). Soil pH is a major determinant of microbial ecosystem processes and potentially a major driver of evolution, adaptation, and diversity of ammonia oxidiz-

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ers, which control soil nitrification (Gubry-Rangin et al., 2011). However, altitude and soil pH showed little effect on characteristics of ammonia-oxidizing communities in the study region. Alves et al. (2013) demonstrated that AOA reflected an overall distribution associated with tundra type in Arctic soils. Consistently, community characteristics of the ammonia oxidizers identified in the alpine meadow soils showed a close association with vegetation coverage, moreover, their distributions were obviously affected by meadow types.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/5123/2014/bgd-11-5123-2014-supplement.pdf.

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Table 1. Geography, soil physicochemical properties, vegetation and climatic characteristics of sampling sites.

		Site1	Site2	Site3	Site4	Site5
Sample site locations	Latitude (N)/ Longitude (E)	(1) 38°32′05″/ 99°27′37″ (2) 38°32′12″/ 99°27′53″ (3) 38°32′19″/ 99°28′09″	(1) 38°34′5.42″/ 99°28′58.24″ (2) 38°34′5.7″/ 99°29′4″ (3) 38°34′6.07″/ 99°29′7.78″	(1) 38°35′30.58″/ 99°28′58.62″ (2) 38°35′29″/ 99°29′8″ (3) 38°35′27.30″/ 99°29′15.32″	(1) 38°36′12.74″/ 99°28′34.32″ (2) 38°36′12″/ 99°28′40″ (3) 38°36′11.55″/ 99°28′44.75″	(1) 38°36′37″/ 99°28′21″ (2) 38°36′35″/ 99°28′29″ (3) 38°36′32″/ 99°28′38″
	Altitude (m)	3400	3600	3800	4000	4200
Soil physicochemical properties	pH	$7.85 \pm 0.02^{a*}$	7.53 ± 0.01^{b}	7.37 ± 0.01^{c}	7.26 ± 0.01^{d}	6.87 ± 0.00^{e}
	NO_3^-_N (mg kg^{-1})	0.063 ± 0.004^{b}	0.163 ± 0.012^{b}	2.828 ± 0.043^{a}	0.591 ± 0.002^{a}	1.375 ± 0.077^{a}
	NH_4^+_N (mg kg^{-1})	89.4 ± 0.1^{b}	91.8 ± 0.7^{a}	86.2 ± 0.2^{c}	91.8 ± 0.4^{a}	89.8 ± 0.2^{b}
	Available P (mg kg^{-1})	0.64 ± 0.00^{b}	5.55 ± 1.74^{a}	5.76 ± 1.20^{a}	4.34 ± 1.51^{ab}	5.39 ± 1.20^{a}
	Available K (mg kg^{-1})	104.5 ± 0.0^{b}	203.55 ± 58.3^{a}	212.6 ± 27.7^{a}	95.05 ± 21.7^{b}	107 ± 0.9^{b}
	Salt (%)	1.067 ± 0.004^{ab}	1.057 ± 0.002^{b}	1.017 ± 0.002^{c}	1.077 ± 0.003^{a}	1.055 ± 0.008^{b}
	HCO_3^- (%)	0.007 ± 0.0000^{ab}	0.008 ± 0.0005^{a}	0.006 ± 0.0006^{b}	$0.005 \pm 0.0008\ b^{c}$	0.004 ± 0.0004^{c}
	Cl^- (%)	0.003 ± 0.0000^{ab}	0.004 ± 0.0001^{a}	0.004 ± 0.0001^{ab}	0.003 ± 0.0006^{bc}	0.002 ± 0.0001^{c}
	SO_4^{2-} (%)	0.0003 ± 0.0000^{b}	0.0001 ± 0.0001^{b}	0.0047 ± 0.0008^{a}	0.0002 ± 0.0000^{b}	0.0004 ± 0.0000^{b}
	Na^+ (%)	0.004 ± 0.0000^{ab}	0.003 ± 0.0006^{b}	0.006 ± 0.0019^{a}	0.002 ± 0.0003^{b}	0.003 ± 0.0004^{ab}
	K^+ (%)	0.0005 ± 0.0000^{b}	0.0029 ± 0.0011^{a}	0.0022 ± 0.0001^{a}	0.0005 ± 0.0000^{b}	0.0005 ± 0.0000^{b}
	Ca^{2+} (%)	0.028 ± 0.0000^{a}	0.021 ± 0.0006^{b}	0.02 ± 0.0000^{b}	0.016 ± 0.0035^{b}	0.01 ± 0.0012^{c}
	Mg^{2+} (%)	0.012 ± 0.0000^{ab}	0.017 ± 0.0041^{a}	0.012 ± 0.0014^{ab}	0.008 ± 0.0007^{b}	0.011 ± 0.0007^{ab}
Vegetation information	Vegetation coverage (%)	76.5 ± 0.9^{a}	74.5 ± 2.0^{a}	80.0 ± 6.4^{a}	62.0 ± 15.6^{a}	32.5 ± 10.1^{b}
	Vegetation diversity	0.966 ± 0.001^{c}	1.836 ± 0.076^{ab}	1.498 ± 0.133^{b}	2.099 ± 0.018^{a}	1.934 ± 0.252^{a}
	Meadow type	*Kobresia* (*K. humilis*)	*Thermopsis* (*T. lenceolata*)	*Carex* (*C. atrofusca*)	*Wormwood* + *Carex sieversiana*)	*Wormwood* (*Artemisia*
Climatic characteristics	Annual precipitation (mm)	474	536	597	658	719
	Annual air temperature (°C)	−4.3	−5.4	−6.6	−7.8	−8.9

* Values sharing a letter within rows are not significantly different ($P > 0.05$).

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Table 2. The three components of environmental factors extracted with the method of Principal Component Analysis.

Component	1	2	3
Initial Eigenvalues	48.9	31.2	15.8
% of Variance			
Altitude	−0.946	0.317	0.050
pH	**0.907***	−0.380	−0.130
NO_3^-	−0.144	**0.941**	−0.300
NH_4^+	−0.265	−0.731	0.617
Available P	−0.375	0.719	0.585
Available K	0.554	0.703	0.444
Salt	−0.273	−0.941	0.132
HCO_3^-	**0.911**	−0.174	0.372
Cl^-	0.764	0.384	0.376
SO_4^{2-}	0.265	0.891	−0.321
Na^+	0.539	0.707	−0.442
K^+	0.554	0.514	0.653
Ca^{2+}	**0.925**	−0.299	−0.218
Mg^{2+}	0.612	0.106	0.598
Vegetation Coverage	**0.911**	0.088	0.002
Vegetation Diversity	−0.728	0.136	0.617
Precipitation	−0.945	0.319	0.053
Temperature	**0.948**	−0.312	−0.040

* The numbers in bold indicates that the corresponding environmental factors have more information load.

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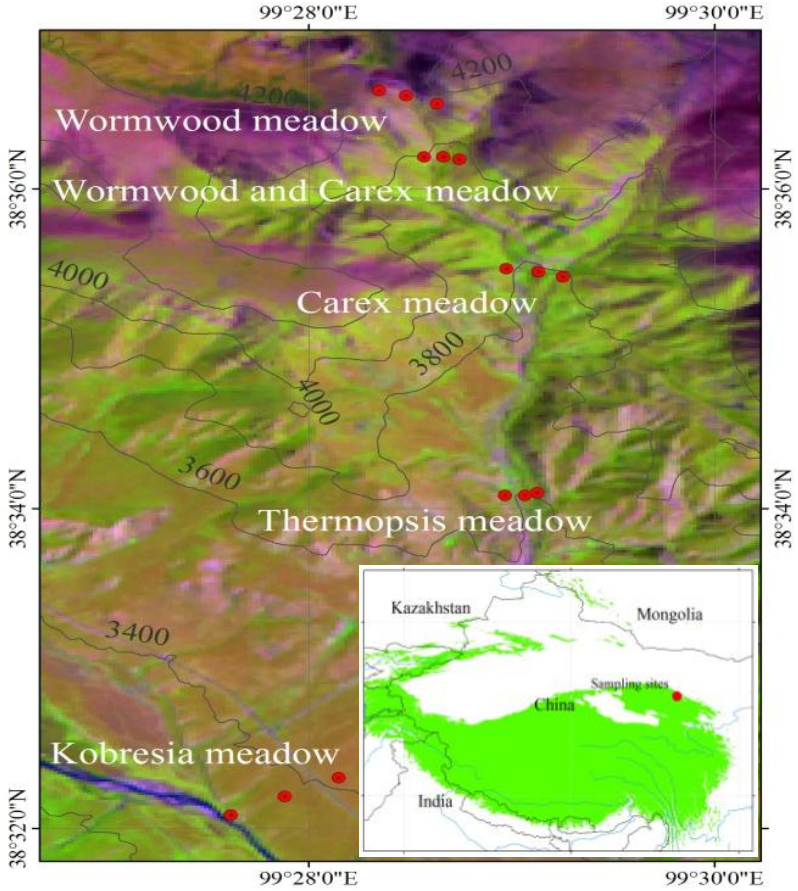

Fig. 1. The study region and the sampling sites (each site was named by meadow type with its constructive species).

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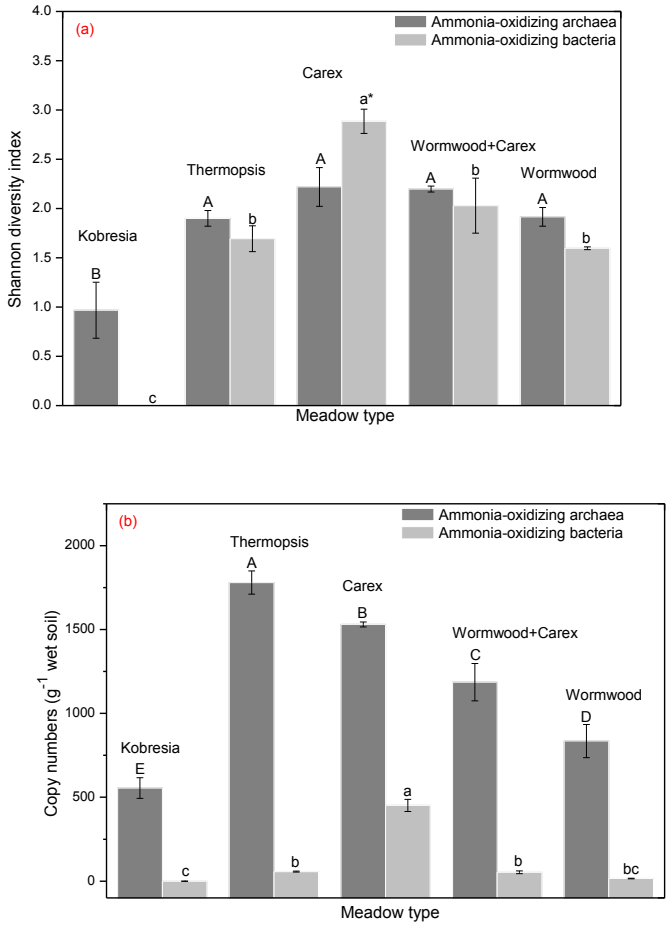

Fig. 2. Diversity **(a)** and abundance **(b)** of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in different alpine meadow soils (* the letters represent confidence levels above 95 %).

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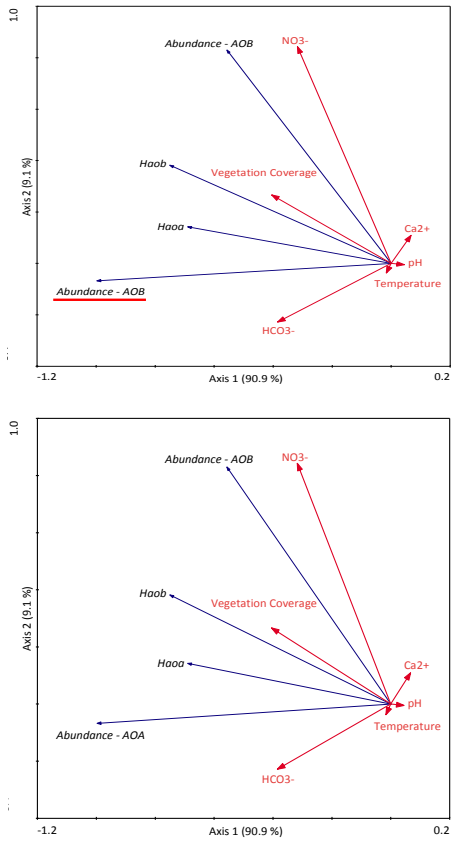

Fig. 3. Correlation analysis with the method of RDA between characteristics of ammonia-oxidizing communities and environmental factors. The red arrows showed environmental factors while the blue ones did characteristics of ammonia-oxidizing communities. Cosine of the included angle between two arrows indicated their correlation coefficient.

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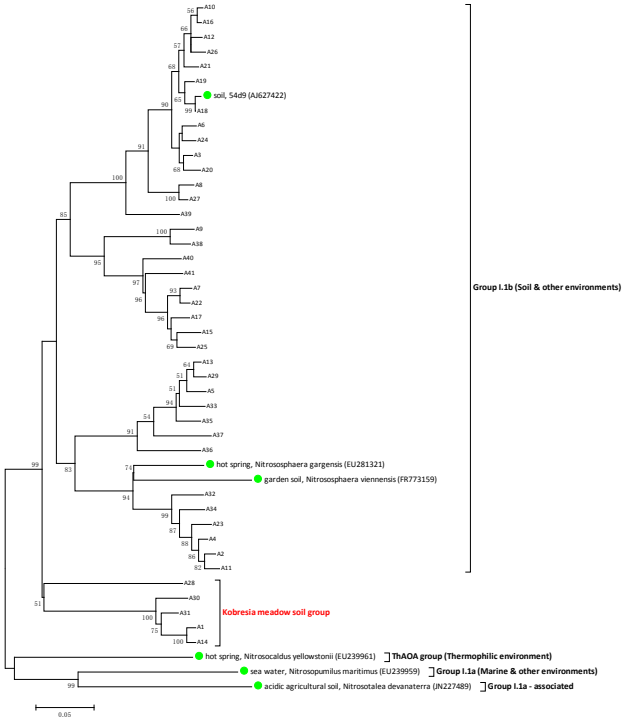

Fig. 4. Phylogenetic analysis of AOA by Neighbor-joining method with Jukes–Cantor model (bootstrap = 1000). AOA phylotypes detected in the study region were coded by A + No. ThAOA indicated thermophilic AOA group. The green filled circles marked the representatives of each thaumarchaeotic group. Names of each thaumarchaeotic group were in bold. The new thaumarchaeotic group found in this study was marked by red color. The scale bars represented an estimated 0.05 changes per nucleotide position.

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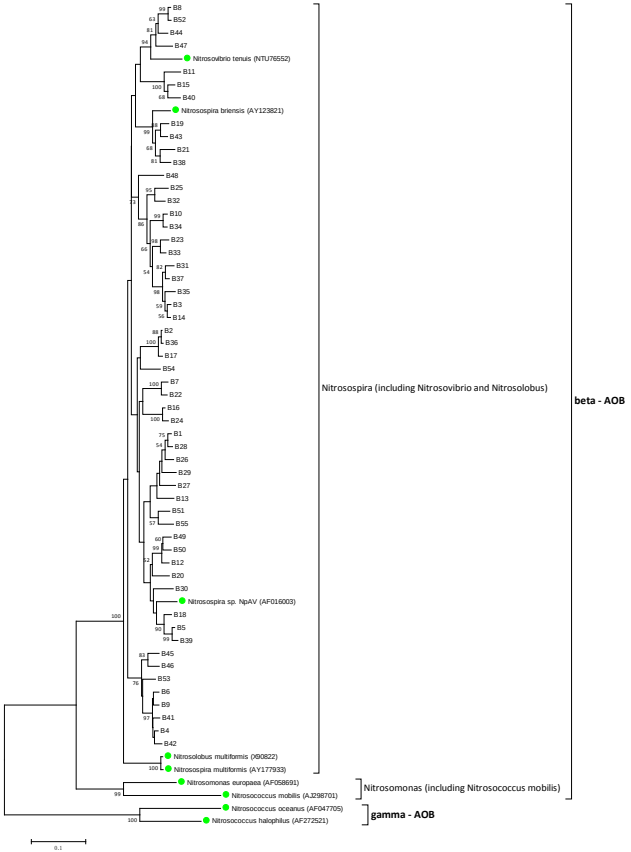

Fig. 5. Phylogenetic analysis of AOB by Neighbor-joining method with Jukes–Cantor model (bootstrap = 1000). AOB phylotypes detected in the study region were coded by B + No. The green filled circles marked the representatives of each AOB cluster. Names of the two AOB phylogenetic groups were in bold. The scale bars represented an estimated 0.1 changes per nucleotide position.

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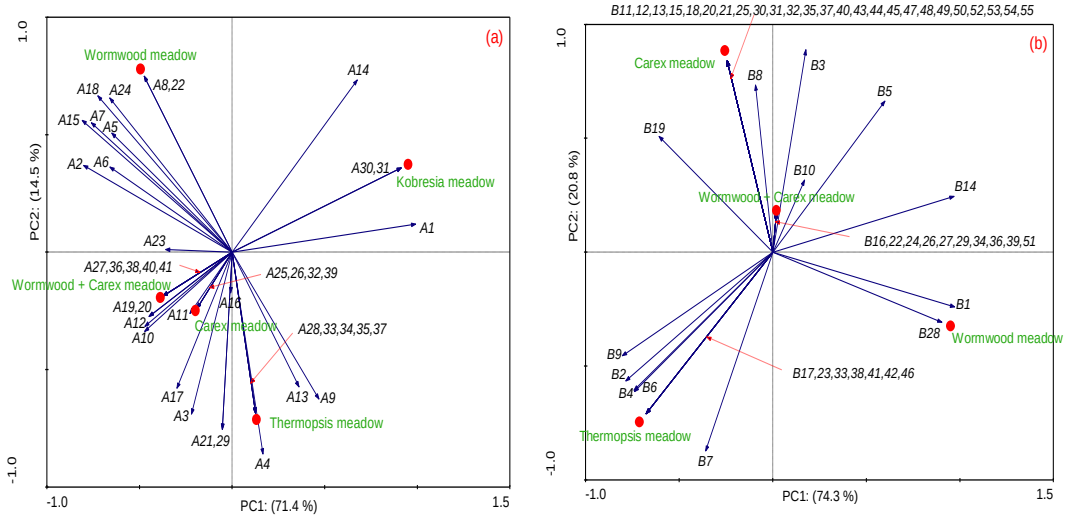

Fig. 6. Attribution of AOA **(a)** and AOB **(b)** phylotypes to meadow types by PCA. Red filled circles represented the sampling sites and the words in green were names of meadow types. The blue arrows indicated phylotypes of ammonia oxidizers. The perpendicular distance between a red filled circle and one blue arrow indicated the attribution of one phylotype to the sampling site, a shorter distance showed that the phylotype was more abundant in the closer sampling site while a value of zero demonstrated that the phylotype was only attributed to the sampling site.

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