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Ammonia oxidizers and denitrifiers in response to reciprocal elevation translocation in an alpine meadow on the Tibetan Plateau

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Abstract
Purpose Global climate change, in particular temperature variation, is likely to alter soil microbial abundance and composition, with consequent impacts on soil biogeochemical cycling and ecosystem functioning. However, responses of belowground nitrogen transformation microorganisms to temperature changes in high-elevation terrestrial ecosystems are not well understood.
Materials and methods Here, the effects of simulated cooling and warming on the abundance and community composition of ammonia-oxidizing archaea (AOA) and bacteria (AOB), as well as the abundance of denitrifiers, were investigated using quantitative polymerase chain reaction and clone library approaches, on the basis of a 2-year reciprocal elevation translocation experiment along an elevation gradient from 3,200 to 3,800 m above sea level on the Tibetan Plateau.
Results and discussion We found that, compared with the temperature variations caused by elevation translocation, the soil origin exerted a much stronger influence on AOA abundance. There were significant effects of both soil origin and elevation translocation on AOB abundance, which was particularly decreased by elevation-enhanced (simulated cooling) and increased by elevation-decreased (simulated warming) treatments. Altered temperature affected the abundance of *nirK* rather than *nirS* and *nosZ* genes, and the latter two seemed to be associated tightly with the soil origin. Furthermore, the results showed that temperature changes had obvious influences on the community structure and diversity of AOB, but not AOA. More apparent response of AOB to warming than in other studies on grassland and forest ecosystems may be attributed to higher elevation and lower mean annual temperature in this study.
Conclusions Our findings thus suggest that, in comparison with AOA and denitrifying populations, AOB may respond more sensitively to natural temperature variation caused by elevation translocation in this alpine grassland ecosystem on the Tibetan Plateau.

Keywords Abundance · Ammonia-oxidizing microorganism · Clone library · Community structure · Denitrifying bacteria · Quantitative PCR

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1 Introduction

Climate change, particularly warming, is predicted to affect most regions of the northern hemisphere with temperature changes in the range of 2–5 °C during this century (IPCC 2007). Substantial lines of evidence reported that warming

could stimulate plant biomass production (Luo et al. 2009) and litter decomposition (Luo et al. 2010), alter community composition of plant and symbiont (Bertrand et al. 2011; Yang et al. 2013), and thus influence the metabolic balance and C sequestration of terrestrial ecosystems (Wan et al. 2009; Yvon-Durocher et al. 2010). Meanwhile, warming can increase soil respiration rates (Rustad et al. 2001) and inorganic nitrogen (N) supply (Dijkstra et al. 2010), thereby affecting soil carbon (C)/N pools (Rui et al. 2011) and N-cycling (Weedon et al. 2012), which were mainly driven by microorganisms (Ollivier et al. 2011).

As the first and rate-limiting step of nitrification, aerobic ammonia oxidation is generally believed to be controlled by ammonia-oxidizing archaea (AOA) and bacteria (AOB). Denitrification, a multiple-step process mainly mediated by denitrifying bacteria (denitrifiers), is also of significant importance in the global N-cycling. These two processes together make considerable contributions to atmospheric emissions of greenhouse gas nitrous oxide (N_2O). Therefore, AOA, AOB, and denitrifiers have been widely studied regarding their ecological distributions characterized by various environmental factors. For example, the abundance and composition of AOA and AOB have been found to respond differently to fertilization regimes (Shen et al. 2008; Wu et al. 2011; Ai et al. 2013; Zhou et al. 2014), heavy metal contamination and municipal solid waste (Mertens et al. 2009; Paranychianakis et al. 2013), and global climatic factors (Long et al. 2012b; Chen et al. 2013). Soil denitrifiers possessing different phylogenetic markers (e.g., *nirK*, *nirS*, and *nosZ*) could also have been affected distinctly by the long-term fertilization (Enwall et al. 2005; Chen et al. 2010b) and some other factors such as seasonal patterns and spatial locations, inputs of different irrigation water, and organic C (Dandie et al. 2008; Zhou et al. 2011; Clark et al. 2012).

In comparison, studies regarding the influences of simulated temperature changes on soil N-cycling microorganisms, particularly under field conditions, were less documented. There were no consistent results in the abundance and community structure responses of ammonia oxidizers (Horz et al. 2004; Malchair et al. 2010; Long et al. 2012a) and denitrifiers (Cantarel et al. 2012) to the warming. Therefore, improved understanding of the impacts of altered temperature on N-cycling microbial community structure and diversity is highly necessary to enable future prediction of warming on terrestrial N turnover. Moreover, elevated temperature tended to have a more pronounced effect in alpine and arctic ecosystems (Singh et al. 2010). Therefore, as the Earth's largest (2×10^6 km^2) and highest plateau (mean altitude 4,500 m a.s.l.), the Tibetan Plateau represents one of the largest typical alpine grasslands in the world (He et al. 2006). The Tibetan Plateau is considered as one of the most special regions sensitive to the global climate, and the global warming may have a complex impact on belowground microorganisms (Xu et al. 2009; Zhang et al. 2009; Zheng et al. 2012).

The aims of this study were to determine (1) the responses of soil N-cycling microbial abundance to temperature changes caused by elevation translocations and (2) whether the AOA and AOB community composition and diversity were influenced by cooling and warming, which resulted from the elevation up- and down-translocation, respectively. We hypothesized that the abundance of N-cycling microorganisms would be affected by temperature changes, and AOA and AOB community structure might respond differently to the elevation translocation treatment in this climate-sensitive alpine meadow.

2 Materials and methods

2.1 Site description and experimental design

The experimental site is located at the Haibei Alpine Meadow Ecosystem Research Station (HAMERS) of the Chinese Academy of Sciences (37° 37′ N, 101° 12′ E), northeast of the Tibetan Plateau in a large valley surrounded by the Qilian Mountains. The station experiences a typical plateau continental climate with a mean annual temperature of −2 °C and a mean annual precipitation of approximately 500 mm; >80 % of which falls during the summer monsoon season. Mean elevation of the valley bottom is 3,200 m (Duan et al. 2013).

A two-direction vegetation transplant experiment was established to investigate the effects of simulated cooling and/or warming caused by elevation translocation on the aboveground plant community and belowground soil biota. In brief, the intact soil monoliths (100×100 cm wide×40 cm deep) with attached vegetation from each altitude (3,200, 3,400, 3,600, and 3,800 m a.s.l.) were reciprocally transferred across the altitudinal gradient just after the soils had thawed in early May 2007. Among them, three monoliths from each altitude were removed and then put back at the same elevation to serve as the controls. Plastic film was used to isolate all plots from the surrounding soil. In the current study, we selected two elevations (3,200 and 3,800 m a.s.l.) and each had two treatments including the same elevation moved vegetation and up- and down-moved vegetation from 3,200 to 3,800 m a.s.l. and 3,800 to 3,200 m a.s.l. Therefore, there were four treatments: (1) CK_{3200} (control at 3,200 m), the intact vegetation moved parallelly within 3,200 m a.s.l.; (2) elevation enhanced (EE), the intact vegetation elevated from 3,200 to 3,800 m a.s.l., represented a simulated cooling; (3) CK_{3800} (control at 3,800 m), the intact vegetation moved parallelly within 3,800 m a.s.l.; and (4) elevation decreased (ED), the intact vegetation decreased from 3,800 to 3,200 m a.s.l., represented a simulated warming. Each treatment contained three replicates, which were completely randomized block

distributions in the field. Here, we attempted to test and compare the effect of soil origin and elevation translocation on the N-cycling microbial abundance, on the basis of this full factorial design (2×2) consisting two factors (i.e., 3,200 and 3,800 m a.s.l.) each with two levels (i.e., parallelly moved and elevation up- or down-translocated).

2.2 Soil sampling and chemical analysis

Three cores (2.0 cm in diameter; 10 cm in depth) were collected randomly from each replicate plot in early August 2009 and were mixed as a composite sample. Thus, a total of 12 soil samples (4 treatments×3 replicates) were obtained. Soil samples were immediately packed on ice after collection and transported to the laboratory. Soil samples were sieved (<2 mm) to remove fine roots and visible organic debris and stored at −80 °C for DNA extraction and at 4 °C for analyzing soil characteristics.

Soil total organic carbon (TOC) was determined using the $K_2Cr_2O_7$ oxidation method. Soil total nitrogen (TN) was assayed using the Kjeldahl method. The basic soil properties were shown in Table 1.

2.3 DNA extraction and quantitative PCR

Soil total DNA was extracted from 0.25 g of soil using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The quality of the extracted DNA was determined by electrophoresis on a 1.0 % agarose gel. After testing the possible inhibitory effects of humic substances through polymerase chain reaction (PCR) method, the 10-fold diluted DNA (ca. 5–10 ng μL^{-1}) was found to be optimal as the PCR template.

Quantitative PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, Hercules, CA, USA). Amplification was conducted by using the SYBR® Premix Ex Taq™ as described by the supplier (TaKaRa, Dalian, China). The primers and PCR thermal cycling conditions used in quantitative PCR were described previously (showed in Table S1 by Tian et al. 2013). The amplification mixtures contained 12.5 μL of 2× SYBR® Premix, 1 μL of bovine serum albumin (25 mg mL^{-1}), 0.5 μL of each primer (10 μmol L^{-1}), 1 μL of 10-fold diluted DNA as template, and additional 9.5 μL of deionized water to a final volume of 25 μL (Tian et al. 2013).

To establish the external standard curves for quantitative PCR, the general PCR products for each gene were gel-purified and ligated into the *p*GEM-T Easy Vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* JM109 (TaKaRa, Dalian, China). After reamplification with the vector-specific primers of T7 (5′-TAA TAC GAC TCA CTA TAG GG-3′) and SP6 (5′-ATT TAG GTG ACA CTA TAG-3′), the selected positive clones were sequenced. The plasmids were extracted from the correct insert clones for each target gene and then were used as standards for quantitative analyses. The plasmid DNA concentrations were determined through a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the target gene copies were calculated from the plasmid DNA concentration. Tenfold serial dilutions of the known copies of the plasmid DNA were then subjected to quantitative PCR in triplicate to generate an external standard curve. All R^2 values of the standard curves were higher than 0.99. Data analysis was conducted using the iCycler software (version 1.0.1384.0 CR) (Bio-Rad, Hercules, CA, USA).

2.4 Construction of AOA and AOB clone libraries

The primer sets of Arch-amoAF/Arch-amoAR and amoA1F/amoA2R were used to amplify AOA and AOB *amoA* gene fragments, respectively (showed in Table S1 by Tian et al. 2013). PCR amplification was performed in 50 μL of mixtures including 5 μL of 10× PCR buffer (plus Mg^{2+} as 15 mM), 40 nmol of dNTPs, 20 pmol of each primer, 2.5 U of *Taq* DNA polymerase (TaKaRa, Dalian, China), and 1 μL of 10-fold diluted DNA as template. The PCR thermal cycling

Table 1 Selected properties of soil samples collected from alpine vegetation plots at two elevations with or without elevation up- and down-translocation

Treatments	Mean annual soil temperature (°C)	Moisture (%)	TOC (g kg^{-1})	TN (g kg^{-1})	TOC/TN
CK_{3200}	3.96	22.4±4.8 a[a]	43.20±0.06 c	3.71±0.03 c	11.63±0.07 a
EE	0.36	31.2±2.3 a	38.47±0.17 d	3.31±0.02 d	11.63±0.05 a
CK_{3800}	0.36	31.7±2.2 a	44.97±0.13 b	3.97±0.01 b	11.33±0.02 b
ED	3.96	24.1±1.4 a	45.77±0.15 a	4.27±0.01 a	10.71±0.01 c

CK_{3200}: the intact vegetation (soil monolith) was moved parallelly within 3,200 m a.s.l.; *EE* (elevation enhanced): moved from elevation of 3,200 up to 3,800 m a.s.l.; *CK_{3800}*: moved parallelly within 3,800 m a.s.l.; *ED* (elevation decreased): moved from elevation of 3,800 down to 3,200 m a.s.l.; *TOC* total organic carbon; *TN* total nitrogen

[a] Mean±SE, $n=3$; The values within the same column followed by the different letters indicate significant difference at $P<0.05$ level

conditions were as follows: an initial 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 53 °C (55 °C) for AOA (AOB) and 1 min at 72 °C, followed by a final extension of 10 min at 72 °C. The PCR products from three replicate plots of each treatment were pooled into a composite sample.

All eight (four for AOA and four for AOB) PCR composite products were gel-purified using Agarose Gel DNA Purification Kit (TaKaRa, Dalian, China) and were ligated into the *p*GEM-T Easy Vector (Promega, Madison, WI, USA) for blue and white clone screening. For each library, ≥50 white colonies were picked and subsequently PCR reamplified using primers of T7 and SP6. The amplicons which contained the correct gene fragment were sequenced. The evaluation of the eight clone libraries was performed using the software aRarefactWin version 1.3. The results obtained from rarefaction analysis were used to construct the cumulative curves.

2.5 Sequence analyses

The obtained *amoA* gene sequences were manually proofread, corrected, and edited to start and end with the AOA- and AOB-specific primers using the MEGA 4.0 (Tamura et al. 2007). The good *amoA* sequences were further analyzed with the program DOTUR (Schloss and Handelsman 2005), and the number of operational taxonomic units (OTUs) achieved by DOTUR was calculated at a 97 % level of similarity. One representative sequence of each OTU was used for referenced sequence search in the GenBank database via the BLAST program. Phylogenetic analyses were performed within MEGA 4.0, and the neighbor-joining trees containing the representative OTU sequences and referenced sequences were constructed using *p*-distance with 1,000 replicates to produce bootstrap values. Representative AOA and AOB *amoA* gene sequences obtained in this study were submitted to European Nucleotide Archive with the accession numbers HG380616−HG380652 and HG380653−HG380678, respectively.

2.6 Data analysis

On the basis of OTU data, the coverage (C) for each clone library was calculated using the equation $C=[1-(n_1/N)]\times 100$, in which n_1 is the number of OTU containing only one *amoA* gene sequence, and N is the number of corrected sequences analyzed (Singleton et al. 2001). The Shannon diversity index (H) of each clone library was calculated using the following formula: $H=-\sum_{i=1}^{S} pi\ln pi=-\sum_{i=1}^{S}(Ni/N)\ln(Ni/N)$, where N_i is the abundance (clone number) of the ith OTU, N is the total abundance of all OTUs within that clone library, and S is the number of OTUs. The similarities of AOA and AOB community compositions among the four treatments were calculated based on the Bray-Curtis cluster analysis and were clustered using the BioDiversity Pro software (McAleece et al. 1997). A two-way analysis of variance (ANOVA) was used to analyze the effects of the soil origin and elevation translocation on the microbial abundance, followed by the multiple comparisons of group means among treatments which were carried out within AOA and/or AOB by the Student-Newman-Keuls tests. All statistical analyses were performed using the SPSS Statistics version 20.

3 Results

3.1 AOA and AOB abundance

The two-way ANOVA analysis revealed that the soil origin had significant effects on AOA abundances ($P=0.004$, Table 2), indicating that AOA abundances in two original 3,200-m treatments (CK_{3200} and EE) were different from those in treatments which originated from 3,800 m (CK_{3800} and ED). This finding was further corroborated by T test results that AOA abundances were dependent upon the soil original location (data not shown). No significant effect of elevation translocation on the AOA abundance was found, with the exception that there is a lower abundance of AOA *amoA* gene in EE compared with CK_{3200} (Fig. 1). However, the elevation translocation had a strong effect on the abundance of AOB ($P<0.001$, Table 2). The treatment EE significantly decreased the AOB abundance (Fig. 1). By contrast, the AOB abundance was increased by the ED treatment, compared with CK_{3800} (Fig. 1). Moreover, the AOB abundance was concurrently affected by the soil origin ($P=0.017$, Table 2). There was no interactive effect of soil origin and elevation translocation on the abundances of AOA and AOB.

3.2 Abundance of soil denitrifiers

In this study, the nitrite reductase genes were dominated by *nirS* followed by *nosZ* and *nirK* types with the exception in the ED treatment (Fig. 2). A significant elevation translocation effect was found on the abundance of *nirK* gene ($P<0.001$, Table 2), whereas the abundances of *nirS* and *nosZ* genes were closely related with the soil origin ($P=0.004$ and 0.045, respectively, Table 2). There were significant interactive effects of soil origin and elevation translocation on *nirK* and *nosZ* genes copies, but not on *nirS* gene (Table 2). Significantly higher *nirK* gene copies were detected in the original elevation of 3,200 m (CK_{3200}) than 3,800 m (CK_{3800}), and the EE treatment decreased the abundances of *nirK* gene (Fig. 2). The abundance of *nirS* gene in 3,200 m (CK_{3200}) tended to

Table 2 Summary of two-way ANOVA analysis on the effects of the soil origin and elevation translocation on the abundance of N-cycling functional genes

Effect		AOA *amoA*		AOB *amoA*		*nirK*		*nirS*		*nosZ*	
	df_1, df_2	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil origin (O)	1, 4	16.42	**0.004***	8.964	**0.017**	1.889	0.207	15.59	**0.004**	5.651	**0.045**
Elevation translocation (T)	1, 4	5.132	0.053	26.10	**<0.001**	30.24	**<0.001**	1.492	0.257	1.812	0.215
O×T	1, 8	5.159	0.053	0.010	0.924	38.31	**<0.001**	0.251	0.630	5.986	**0.040**

Significant *P* values (<0.05) are shown in bold

outnumber that in 3,800 m (CK_{3800}), whereas the elevation translocation had no effect on it. Similarly, no significant difference in the abundance of *nosZ* gene was caused by the elevation translocation (Fig. 2).

3.3 Phylogenetic analyses of the AOA and AOB *amoA* genes

A total of 198 and 190 sequences of AOA and AOB *amoA* genes were obtained, which were grouped into 37 OTUs and 26 OTUs, respectively, based on a 97 % threshold of sequence similarity. The ranks of the sequence numbers of AOA and AOB OTUs were showed in the Fig. S1. The top five abundant OTUs comprised more than 56 and 70 % of all sequences obtained in the AOA and AOB libraries, respectively. For AOA, the 37 representative OTU sequences, 35 reference sequences, and 7 *amoA*-related sequences of the marine lineage as an out-group downloaded from GenBank were included in the neighbor-joining (NJ) analysis (Fig. 3). Similarly, for the identification of AOB sequences, a NJ phylogenetic tree was constructed on the basis of 26 representative OTU sequences and 41 reference sequences downloaded from the GenBank (Fig. 4). As shown in Fig. 5a, all the 37 AOA OTUs belonged to 10 soil sub-lineages including the soil 1 (9), soil 2 (7), soil 3 (1), soil 4 (5), soil 6 (1), soil 8 (2), soil 9 (1), soil 10 (4), soil HB1 (3), and soil HB2 (4). For AOB, a total of 26 OTUs were classified into 7 lineages of the cluster 1 (5), cluster 3a.1 (6), cluster 3a.2 (3), cluster 3b (1), cluster 4 (6), cluster 7 (4), and cluster 8 (1) (Fig. 5b).

3.4 Community composition and diversity of AOA and AOB

There were 19, 17, 11, and 12 AOA OTUs retrieved from the treatments of CK_{3200}, EE, CK_{3800}, and ED, respectively (Table 3). Of these obtained OTUs from each treatment, there were eight, four, five, and five unique OTUs in the CK_{3200}, EE, CK_{3800}, and ED, respectively (Fig. 6); they together accounted for 15.2 % of the total AOA sequences (i.e., 30 out of 198 sequences). The only common OTU (i.e., OTU2) belonged to the soil 8 lineage and accounted for 10.6 % of the total sequences (21 out of 198 sequences). AOA community structures were found to be clustered firstly between two treatments which shared the same soil origin (Fig. 6a). Most

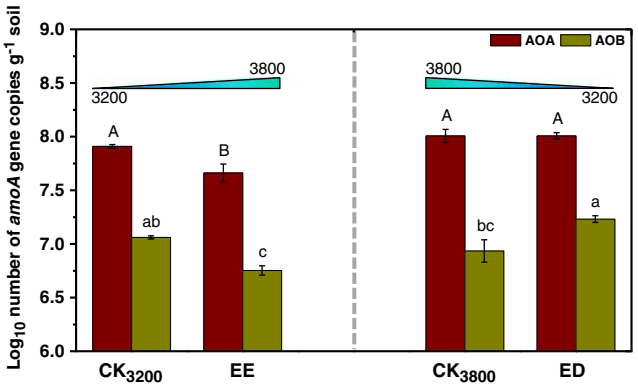

Fig. 1 The abundance of the AOA and AOB *amoA* gene under different elevations with or without translocation. All bars represent mean±SE (n=3). The *different letters above the bars* indicate significant difference at P<0.05 level. CK_{3200}: the intact vegetation was moved parallelly within 3,200 m a.s.l.. *EE* (elevation enhanced): Moved from elevation of 3,200 up to 3,800 m a.s.l.. CK_{3800}: Moved parallelly within 3,800 m a.s.l.. *ED* (elevation decreased): Moved from elevation of 3,800 down to 3,200 m a.s.l.

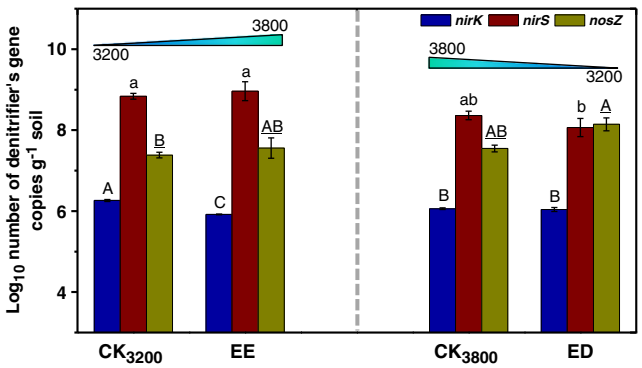

Fig. 2 The abundance of denitrification related genes of *nirK*, *nirS*, and *nosZ* in soil under different elevations with or without translocation. All *bars* represent mean±SE (n=3). The *different letters above the bars* indicate significant difference at P<0.05 level. CK_{3200}: the intact vegetation was moved parallelly within 3,200 m a.s.l.. EE (elevation enhanced): Moved from elevation of 3,200 up to 3,800 m a.s.l.. CK_{3800}: Moved parallelly within 3,800 m a.s.l.. ED (elevation decreased): Moved from elevation of 3,800 down to 3,200 m a.s.l.

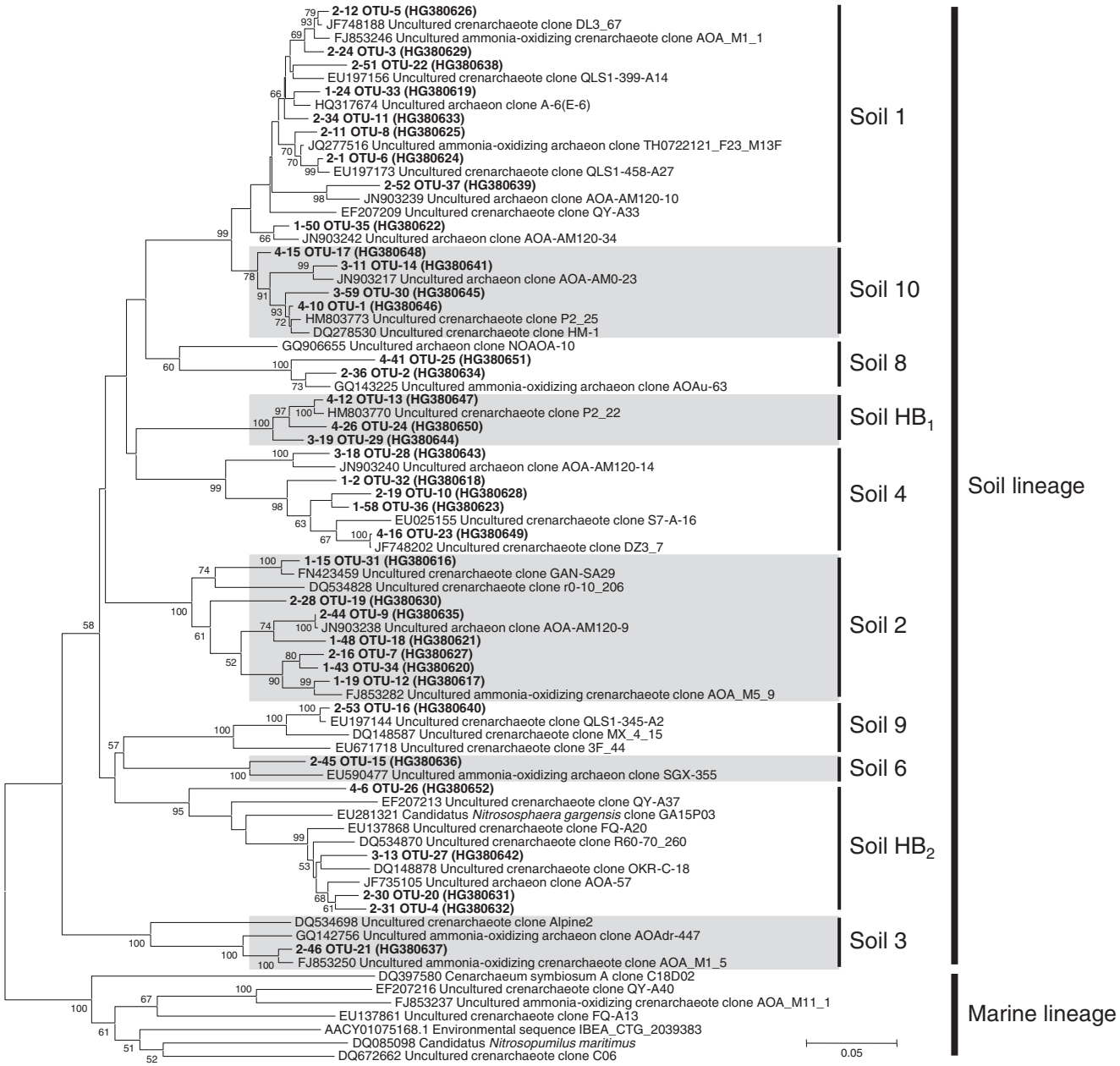

Fig. 3 A neighbor-joining tree based on the AOA *amoA* gene sequences. Bootstrap values were calculated on the basis of 1,000 data resampling. *Bold*: 37 OTUs were obtained in our study and accession numbers are given in *parentheses*. The marine lineage was used as an out-group. The *scale bar* represents 5 % sequence divergence

AOA sequences in the treatments of CK_{3200} (80.0 %) and EE (79.6 %) were grouped into the soil 1 and soil 2 lineages (Fig. 5a). However, AOA communities in CK_{3800} and ED treatments were dominated by the soil 10, soil HB2, and soil 5 lineages which accounted for 88.0 and 73.5 % of total sequences in CK_{3800} and ED treatments, respectively (Fig. 5a). In addition, the lineages of the soil 4 and soil 8 were commonly found among all the four treatments (Fig. 5a).

For the AOB community composition, the treatments of CK_{3200}, EE, CK_{3800}, and ED contained 13, 10, 10, and 14 OTUs (Table 3), of which four, three, three, and six unique OTUs were found, respectively (Fig. 6b). These 16 unique OTUs totally accounted for 13.2 % of the total AOB sequences. Three common OTUs (OTU nos. 1, 4, and 6) that belonged to the cluster 3a.1, cluster 4, and cluster 7 jointly accounted for 45.3 % of AOB sequences (86 out of 190 sequences). Similarly, the treatments from the different soil origins were distinguished in the AOB community composition by cluster analyses (Fig. 6b). The majority of AOB sequences in the treatments of CK_{3200} (73.4 %) and EE

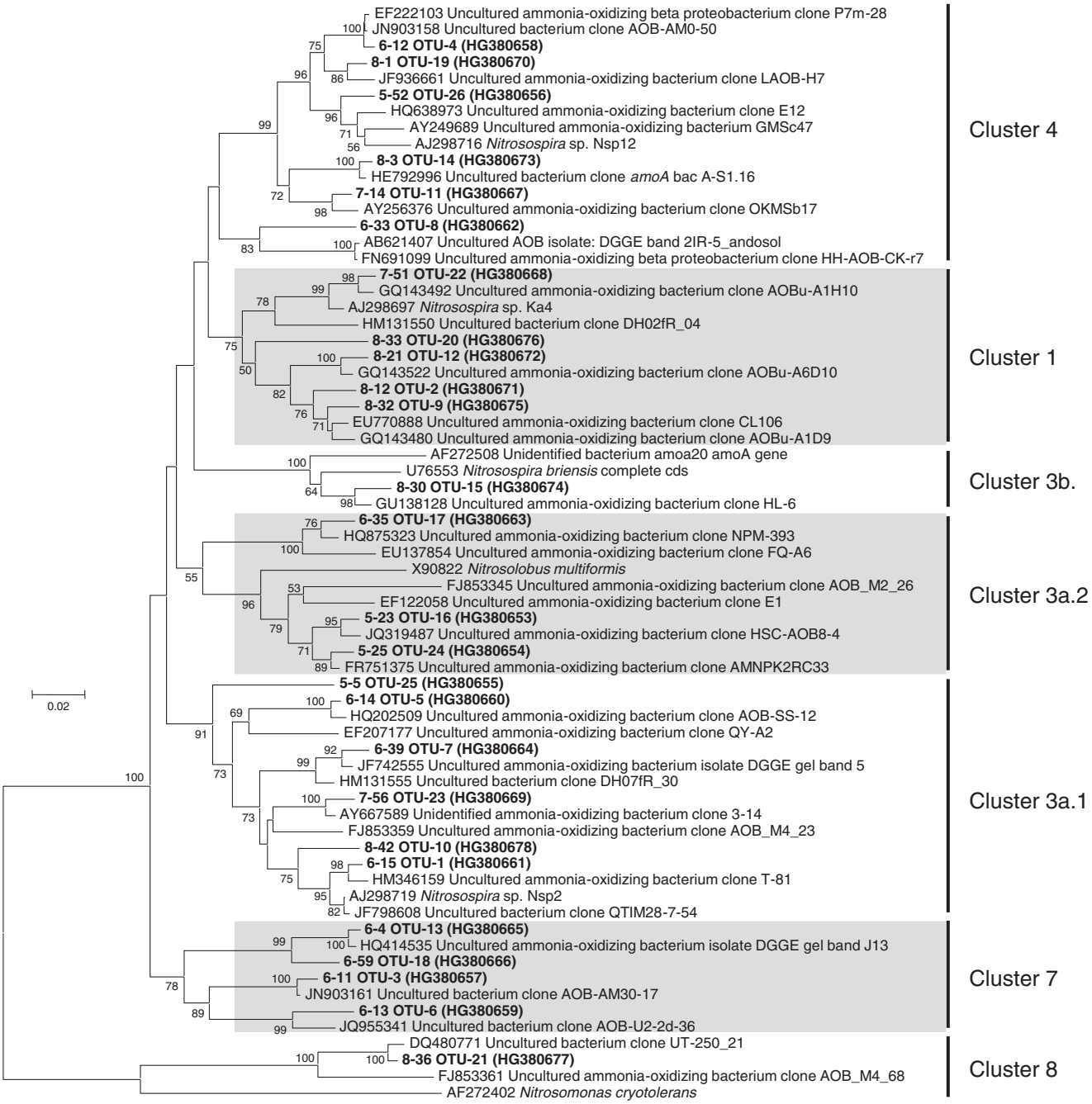

Fig. 4 A neighbor-joining tree based on the AOB *amoA* gene sequences. Bootstrap values were calculated on the basis of 1,000 data resampling. *Bold*: 26 OTUs were obtained in this study and accession numbers are given in *parentheses*. The *scale bar* represents 2 % sequence divergence

(86.1 %) were grouped into the cluster 3a.1 and cluster 7 (Fig. 5b). However, the lineages of cluster 1 and cluster 3a.1 consisted of 70.8 and 70.0 % of total sequences in the CK_{3800} and ED treatments, respectively (Fig. 5b). The AOB sequences affiliating within cluster 3a.1, cluster 4, and cluster 7 were shared among all the four treatments (Fig. 5b).

The clone libraries of AOA and AOB constructed from the four treatments were evaluated by the rarefaction analysis (Fig. S2). Basically, the rarefaction curves tended to reach the saturation platform, and the mean coverage values of clone libraries were approximately 82–90 % for AOA and 88–95 % for AOB, respectively (Table 3). There were clearly higher AOA OTU richness and Shannon indices in the treatments of CK_{3200} and EE than those in the CK_{3800} and ED (Table 3). There was no obvious change in the AOA richness and diversity indices resulting from the elevation changes. However, elevation translocation altered the AOB communities, and less

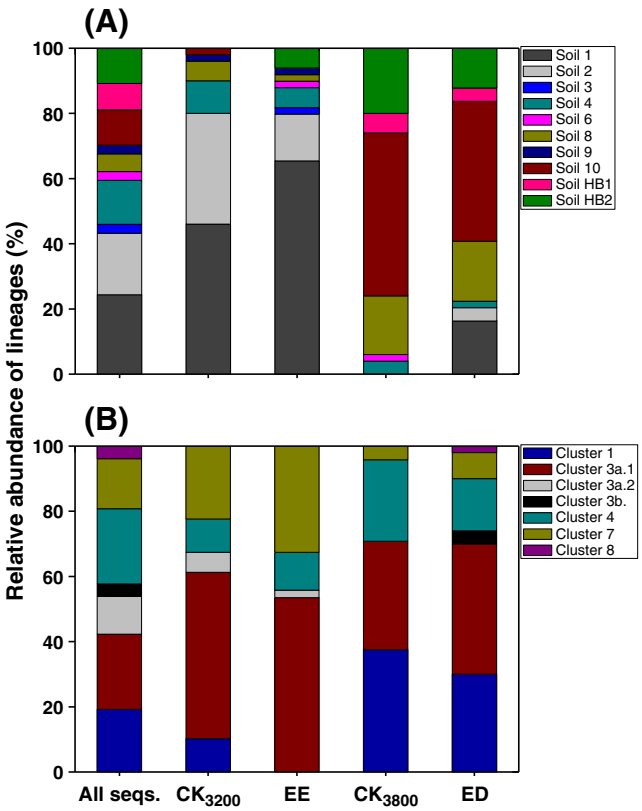

Fig. 5 Relative abundances of lineages of AOA (**a**) and AOB (**b**) in all clone libraries combined and in soils under different elevations with or without translocation. Relative abundances were estimated from the proportional abundances of classified sequences on basis of the results of AOA and AOB neighbor-joining trees. *CK_{3200}*: The intact vegetation was moved parallelly within 3,200 m a.s.l.. *EE* (elevation enhanced): Moved from elevation of 3,200 up to 3,800 m a.s.l.. *CK_{3800}*: Moved parallelly within 3,800 m a.s.l.. *ED* (elevation decreased): Moved from elevation of 3,800 down to 3,200 m a.s.l.

OTUs were observed in the EE than in the CK_{3200}, whereas more OTUs and higher diversity indices were clearly observed in the ED than those in the CK_{3800} (Table 3 and Fig. S2b).

Table 3 The diversity indices of AOA and AOB based on clone libraries data from alpine vegetation plots at two elevations with or without elevation translocation

Treatments	Coverage values (%)		Richness (OTUs)		Shannon (*H*)	
	AOA	AOB	AOA	AOB	AOA	AOB
CK_{3200}	82.0	87.8	19	13	1.160	0.898
EE	81.6	95.3	17	10	1.091	0.856
CK_{3800}	88.0	91.7	11	10	0.759	0.784
ED	89.8	92.0	12	14	0.843	0.955

CK_{3200}: the intact vegetation (soil monolith) was moved parallelly within 3,200 m a.s.l.; *EE* (elevation enhanced): moved from elevation of 3,200 up to 3,800 m a.s.l.; *CK_{3800}*: moved parallelly within 3,800 m a.s.l.; *ED* (elevation decreased): moved from elevation of 3,800 down to 3,200 m a.s.l.

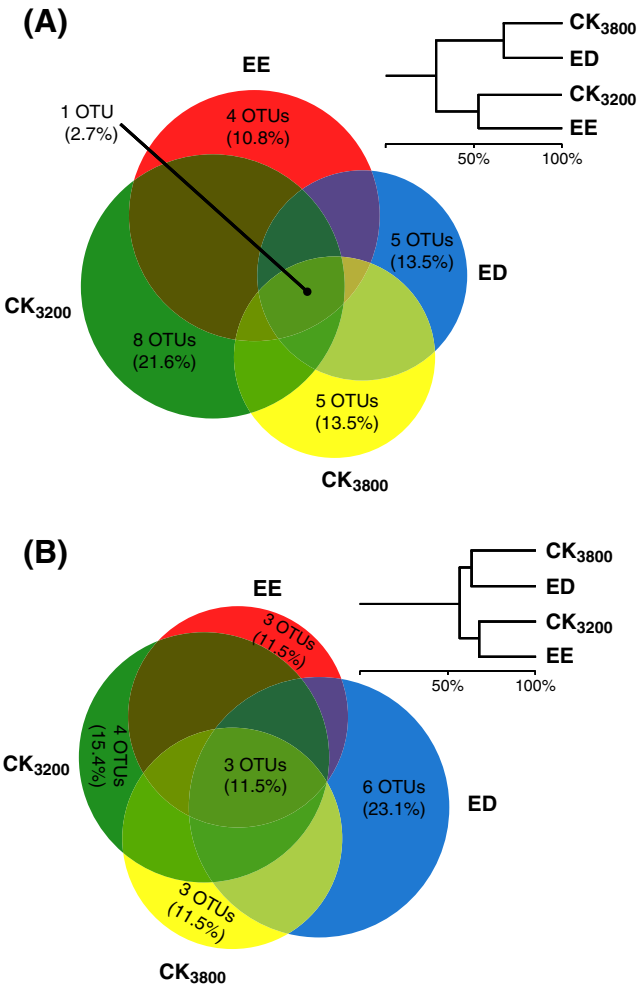

Fig. 6 Venn diagrams comparing the OTU memberships detected from AOA (**a**) and AOB (**b**) clone libraries of four treatments with or without elevation translocation. The relative total percentage of OTUs is given in *parentheses*. The size of each sample component (denoted by *different color*) is based on the total number of OTUs for that sample relative to the other samples. *CK_{3200}*: The intact vegetation was moved parallelly within 3,200 m a.s.l.. *EE* (elevation enhanced): Moved from elevation of 3,200 up to 3,800 m a.s.l.. *CK_{3800}*: Moved parallelly within 3,800 m a.s.l.. *ED* (elevation decreased): Moved from elevation of 3,800 down to 3,200 m a.s.l.

4 Discussion

4.1 Effects of temperature alteration on the abundances of AOA and AOB

Compared with AOB, greater abundance of AOA *amoA* gene was detected in the alpine grassland soil within both elevations of 3,200 and 3,800 m a.s.l. (Fig. 1). This finding was consistent with two previous studies reporting numerous dominance of AOA over AOB in alpine meadow soils (Zhang et al. 2009; Tian et al. 2013). Although warming could cause neutral effect on the AOA *amoA* gene abundance in a Sweden boreal forest soil (Long et al. 2012a), to our knowledge, however, no study has specifically addressed the effect of

cooling and/or warming on AOA in alpine meadow soils. Our results showed that the abundance of AOA was less influenced by temperature changes (caused by elevation translocation) compared with the soil origins (Table 2). Indeed, different soil properties existed in different original soil, and the soil origin seemed to have a greater role than temperature or other environmental disturbances in determining the AOA abundance and distribution (Chen et al. 2010a; Daebeler et al. 2012).

The AOB *amoA* gene abundance was found to be strongly affected by the altitude alteration (Zhang et al. 2009), which supported that AOB abundance was also significantly shaped by the soil origin in the current study (Table 2). Since elevation translocations resulted in changed temperature as cooling (up-moved) or warming (down-moved), AOB abundance was found to be decreased by cooling and increased by warming in this study. Similarly, AOB abundance increased in response to elevated temperature in a grassland (Horz et al. 2004) and in a pristine forest soil (Szukics et al. 2010), whereas AOB abundance was not affected by warming alone in a recent study in a Sweden boreal forest soil (Long et al. 2012a). For this alpine meadow, the increased temperature can result in greater litter decomposition (Duan et al. 2013) and provide more nutrient and substrate for microbial mineralization, thereby influencing the AOB abundance (Zhang et al. 2009). In fact, AOB abundance was found to be positively correlated to soil TOC (P=0.004) and TN (P=0.003) in this study (data not shown).

4.2 Effects of elevation translocation on the abundance of soil denitrifiers

Elevation translocation affected the abundance of *nirK* gene rather than *nirS* and *nosZ* genes, indicating that *nirK*-related denitrifiers seemed to be more sensitive to the temperature shifts. This result was in agreement with a previous report in which the abundance of *nirK* gene changed with temperature alterations, but *nirS*-type denitrifiers tended to be less sensitive to temperature in Antarctic soils (Jung et al. 2011) and Canadian agricultural soils (Wertz et al. 2013). However, in a more recent climate change experiment conducted in an upland grassland soil, Cantarel et al. (2012) suggested that abundance of *nosZ* rather than *nirK* gene showed significant responses to warming. The distinct characteristics of research sites among Cantarel et al. (2012), our present study, and above-mentioned Antarctic soils, such as the mean annual temperature (8.7 versus −2 °C), may attribute to these contrasting results. Furthermore, our findings revealed that the abundances of *nirS* and *nosZ* genes were correlated with the soil origin (Table 2), suggesting the site factors could be speculated as the key determinants of soil denitrifiers in alpine meadow studied here.

4.3 Elevation translocation effects on community compositions of ammonia oxidizers

The AOA lineages of soil 1 and soil 2 dominated in the treatments of CK_{3200} and EE, whereas the lineages of soil 10, soil HB2, and soil 8 were the dominant members within the CK_{3800} and ED treatments. Thus, AOA community compositions were grouped due to the original elevation (3,200 versus 3,800 m a.s.l.) of soils (Fig. 6), indicating that soil origin had strong effects on the community of ammonia oxidizers. Furthermore, site factors were previously considered as the main determinants of microbial community structure in a high arctic lowland in Canada (Walker et al. 2008), and other findings apparently showed that archaeal community composition and diversity are highly responsive to elevation gradients (Singh et al. 2012). Given that distinct C, N, and C/N were observed between the treatments in elevation of 3,200 and 3,800 m a.s.l. (Table 1), our results were in line with previous studies in which soil C and N were associated with the distribution and community succession of AOA (Zhang et al. 2009; Abell et al. 2010; Yao et al. 2011). Therefore, although there was different proportion of specific AOA lineage (e.g., lineage of Soil 2, Fig. 5a) detected in CK_{3200} and EE, the overall AOA community diversity was subjected to the original location.

On the other hand, similar to AOA, the AOB community composition was also shaped by the soil origin of each treatment (Fig. 6b), indicating a niche differentiation of bacterial ammonia oxidizers along the elevation gradient. Since lower mean annual soil temperature was observed in 3,800 than in 3,200 m a.s.l. (Table 1), our study thus supports that soils exposed to dissimilar mean annual temperatures tended to have dissimilar AOB communities (Fierer et al. 2009). More importantly, compared with the controls (CK_{3200} and CK_{3800}), cooling (EE) and warming (ED) potentially decreased and increased the AOB diversity, respectively (Table 3). For instance, the lineage of cluster 1 disappeared from the CK_{3200} to the EE treatments, whereas the lineages of cluster 3b and cluster 8 were only observed in the ED, not in CK_{3800} (Fig. 5b). Indeed, temperature was found to be a key factor influencing the population structure and diversity of AOB in agricultural soils (Avrahami et al. 2003). Moreover, the different sequence predominance was detected between lower altitude soils and high-altitude Tibetan soils, suggesting an AOB community clearly in response to temperature, which was one of the most important altitude-dependent factors (Zhang et al. 2009; Xu et al. 2010). However, no significant warming effect on AOB community structure and diversity was observed in the European boreal forest soil (Long et al. 2012a) as well as the grassland soil (Malchair et al. 2010). It could be mainly interpreted that significantly lower mean annual temperature in this study (−2 °C) than the two previous studies (2.3 and

9.6 °C) might alter AOB community more apparently, since AOB was found to be sensitive to low temperature by Sims et al. (2012). However, whether the different responses of AOB to the warming would be dependent on the discrepancies among different ecosystems and/or on the indirect changes in aboveground plant community by warming requires more studies.

5 Conclusions

In summary, our results show that AOA and AOB abundances were associated with the soil origin, and there were significant effects of temperature alteration on the AOB abundance, which were increased by warming and decreased by cooling. Temperature changes influenced the abundance of *nirK* rather than *nirS* and *nosZ*, and the latter two seemed to be related tightly to soil origin. We further found that AOA community composition was more shaped by the soil origin compared with the elevation translocation which, however, resulted in obvious impacts on the community structure and diversity of AOB. Our results thus highlight that AOB responded more sensitively to the warming and/or cooling in this climatic sensitive Tibetan alpine meadow ecosystem.

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