Soil Ecol. Lett. 2019, 1(3-4): 126–135
https://doi.org/10.1007/s42832-019-0016-8

RESEARCH ARTICLE

AOA and AOB communities respond differently to changes of soil pH under long-term fertilization

Ruibo Sun[1,2], David D. Myrold[3], Daozhong Wang[4], Xisheng Guo[4], Haiyan Chu[1,*]

1 *State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, China*

2 *Key Laboratory of Agricultural Water Resources, Center for Agricultural Resources Research, Institute of Genetic and Developmental Biology, Chinese Academy of Sciences, 286 Huaizhong Road, Shijiazhuang 050021, China*

3 *Department of Crop and Soil Science, Oregon State University, Corvallis OR 97331, Oregon, USA*

4 *Soil and Fertilizer Research Institute, Anhui Academy of Agricultural Sciences, South Nongke Road 40, Hefei 230031, China*

ARTICLE INFO

Article history:
Received December 12, 2018
Revised March 1, 2019
Accepted March 18, 2019

Keywords:
AOA
AOB
Microbial diversity
Soil pH
Long-term fertilization
High-throughput sequencing

ABSTRACT

Archaeal and bacterial ammonia-oxidizers drive the first step of nitrification, ammonia oxidation. Despite their importance, the relative contribution of soil factors influencing the abundance, diversity and community composition of ammonia oxidizing archaea (AOA) and bacteria (AOB) are seldom compared. In this study, the AOA and AOB communities in soils from a long-term fertilization experiment (which formed gradients of pH and nutrients) were measured using 454 pyrosequencing of the *amoA* gene. Results showed that both AOA and AOB communities were influenced by fertilization practice. Changes of AOA abundance, diversity and community structure were closely correlated with a single factor, soil pH, and the abundance and diversity of AOA were lower under the acidified treatments. By contrast, AOB abundance was higher in the acidified soil than in the control soil while AOB diversity was little impacted by soil acidification, and both the abundance and diversity of AOB were most highly correlated with soil carbon and available phosphorus. These results indicated that AOB diversity seemed more resistant to soil acidification than that of AOA, and also suggested that AOB have greater ecophysiological diversity and broader range of habitats than AOA in this lime concretion black soil, and the potential contribution of AOB to ammonia oxidation in acid environments should not be overlooked.

1 Introduction

Ammonia oxidation, converting ammonia to hydroxylamine, is the first step of nitrification (Martens-Habbena et al., 2009), which is catalyzed by ammonia-oxidizing microorganisms (AOMs), including bacteria (AOB) and archaea (AOA) (Nunes-Alves, 2016). Since the discovery of AOA (Könneke et al., 2005), studies on the contribution of AOA and AOB to ammonia oxidation and the ecological niches of AOA and AOB have received considerable attention.

Extensive research revealed that AOA are widely distributed in terrestrial environments and are often more dominant than AOB in terrestrial ecosystems (Leininger et al., 2006; Nicol et al., 2008; Kelly et al., 2011; Stahl and de la Torre 2012; Meng et al., 2017). It is thought that ammonia, pH, carbon sources, temperature, moisture, oxygen and other factors define distinct ecological niches of AOA and AOB (Schleper, 2010; Hatzenpichler, 2012; Zhalnina et al., 2012; Xu et al., 2017). Among these drivers, pH was a strong factor defining

* Corresponding author
E-mail address: hychu@issas.ac.cn (H.Y. Chu)

distinct ecological niches of AOA and AOB. Studies have revealed that AOB dominate microbial ammonia oxidation in neutral soils (Jia and Conrad, 2009) while AOA play an important role in acidic soils (Huang et al., 2011; Zhang et al., 2012). However, Zhang et al. (2010) found that AOA also catalyzed ammonia oxidation in neutral soil with low concentration of ammonia derived from mineralization. A large-scale study found that the variation of AOA and AOB diversity and composition across different soils were strongly correlated with soil pH, and the ratio of AOA to AOB abundance decreased with increasing pH (Hu et al., 2013). Another study also observed a sharp increase in the ratio of archaeal/bacterial *amoA* abundance below pH 4 (Yao et al., 2011). These results indicate that AOA may have a competitive advantage over AOB in acidic soils. However, only a few clusters of AOA appear to be adapted to acid soil, mainly *Nitrososphaera*, and the changes of AOA community in soils with different pH were mostly correlated to such clusters (Gubry-Rangin et al., 2017; Ying et al., 2017). Moreover, AOA richness is lower in acid soils, and greatest in soils at pH 6–8 (Gubry-Rangin et al., 2011).

One important reason why pH is an important factor in niche separation of AOA and AOB is that pH influences the availability of NH_3, the substrate of ammonia oxidation, which contributes to the distinct ecological niches of AOA and AOB in soil (Verhamme et al., 2011). Low-pH conditions decrease the availability of NH_3, thus favoring the AOA community, which prefer lower NH_3 concentration than AOB (Hatzenpichler, 2012; Zhalnina et al., 2012). This was supported by observations that AOB respond more strongly to nitrogen addition than AOA (Carey et al., 2016; Ouyang et al., 2016; Xiang et al., 2017). Nitrogen addition increased AOB growth rate but decreased the growth rate of AOA (Ying et al., 2017), and nitrogen fertilization increased the abundance and diversity of AOB but not AOA (Rudisill et al., 2016; Segal et al., 2017; Xiang et al., 2017).

Although studies have revealed that AOA dominate ammonia oxidation in acid soils (Gubry-Rangin et al., 2010; Huang et al., 2011), some AOB have also been isolated from acid soil (Hayatsu et al., 2017) and some groups of AOB seemed well adapted to acidic soils (De Boer et al., 1991; Hu et al., 2013), indicating the potential for ammonia oxidation of AOB in acid soil. Thus, the response of AOA and AOB to soil pH is still unsettled.

Besides pH, organic carbon was considered to be another important factor influencing AOA community. Previous studies found that a greater size of AOA community could be unrelated to ammonia oxidation. For example, the abundance of AOA increased even when nitrification activity was completely inhibited by acetylene, suggesting that AOA may be capable of heterotrophic activity (Prosser and Nicol, 2008; Jia and Conrad, 2009). Liu et al. (2018) revealed that long-term manure application increased AOA abundance in some soils. Wessén et al. (2010) found that AOA abundance was positively related to addition of labile organic carbon. These results indicated that some AOA may be mixotrophs or heterotrophs using organic carbon; however, recent studies through genomics analysis and culture assays have revealed that the AOA species isolated from marine and terrestrial habitats are strictly autotrophic in pure culture (Kerou et al., 2016; Kim et al., 2016; Rice et al., 2016). An alternative explanation is that some organic compounds, such as α-keto acids, dimethyl thiourea and catalase, could enhance the growth of AOA by detoxifying H_2O_2, which is inhibitory to AOA growth (Kim et al., 2016).

Although the impact of soil pH and soil carbon on AOA and AOB communities has been studied, the relative contribution of soil pH and soil carbon in determining AOA and AOB community composition deserves further attention. In this study, a long-term fertilization experiment with chemical fertilizers and different amounts of organic matter from animal and plant sources was chosen to compare the impact of soil pH and soil carbon content on AOA and AOB communities. As previous studies revealed that AOA dominate the process of ammonia-oxidation in acidic soils, we hypothesize that soil acidification will increase the diversity of AOA community but decrease the diversity of AOB community, and AOA and AOB communities may also be correlated with soil carbon.

2 Methods

2.1 Experimental design, soil sampling, determination of soil properties, and DNA extraction

The experimental design, methods of soil sampling, soil chemical analysis and DNA extraction were described previously (Sun et al., 2015a; Sun et al., 2016). Briefly, the experiment was set up at the Madian Agro-Ecological Station in Mengcheng county, Anhui province, China (N33°13′, E116°35′). The soil is a typical lime concretion black soil. Six treatments with four replicate plots were started in 1982, including: (i) Control (no fertilization), (ii) chemical NPK fertilizers only (NPK, 180 kg N ha^{-1} y^{-1}, 90 kg P_2O_5 ha^{-1} y^{-1} and 86 kg K_2O ha^{-1} y^{-1}), (iii) chemical NPK fertilizers added with 3 750 kg ha^{-1} y^{-1} wheat straw (NPK + LS), (iv) chemical NPK fertilizers added with 7 500 kg ha^{-1} y^{-1} wheat straw (NPK + HS), (v) chemical NPK fertilizers added with 15 000 kg ha^{-1} y^{-1} fresh pig manure (NPK + PM), and (vi) chemical NPK fertilizers added with 30 000 kg ha^{-1} y^{-1} fresh cow manure (NPK + CM). Surface soils (0–10 cm) of each plot were collected after the harvest of soybean, but before wheat seeding in October 2012. Twelve soil cores were collected in each plot along a zigzag line, and were mixed together as a single sample. Soil pH, total carbon (TC), total nitrogen (TN), nitrate (NO_3^--N), ammonium (NH_4^+-N), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), available potassium (AK), and available phosphorus (AP) were measured as previously described (Sun et al., 2015a), and these soil properties are shown in Table S1. Total DNA of each sample was extracted from 0.5 g soil using the Fast®DNA SPIN Kit (MP Biomedicals, Santa Ana, CA).

2.2 Quantitative real-time PCR and 454 pyrosequecing for *amoA* gene

Primer sets CrenamoA-23F (5′-ATGGTCTGGCTWAGACG-3′)/CrenamoA-616R (5′-GCCATCCATCTGTATGTCCA3′) (Tourna et al., 2008) and amoA-1F (5′-GGGGTTTCTACTGG TGGT-3′)/amoA-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′) (Cytryn et al., 2012) were used to amplify archaeal and bacterial *amoA* genes respectively. The qPCR procedure was described in our previous study (Sun et al., 2015a). Amplifications for 454 pyrosequecing were done in a 50 μL mixture containing 1 μL (20 ng) genomic DNA, 25μL Premix Taq DNA polymerase, 0.5 μL forward and reverse primers (20 mM) respectively and 23 μL double distilled water under the following protocol: initial denaturation at 95°C for 3 min, 30 cycles of denaturation at 95°C for 30 s, annealing for 1min (50°C for archaeal *amoA* gene and 56°C for bacterial *amoA* gene), and extension at 72°C for 1 min, then a final extension at 72°C for 10 min. PCR products were sequenced on Roche 454 GS-FLX + System (Roche Diagnostics Corporation, Branford, CT, USA). The raw data are available in the European Nucleotide Archive under the accession number PRJEB21824.

2.3 Analysis of pyrosequencing data

Raw sequences were first denoised using the Mothur package (version 1.35.1) (Schloss et al., 2009). The sequences were translated to amino acids using TranslatorX (Abascal et al., 2010); sequences that could not be translated correctly or had termination codons were removed. Finally, the clean data were processed using the QIIME pipeline (version 1.9.1) (Caporaso et al., 2010). Archaeal and bacterial *amoA* sequences were clustered into OTUs (operational taxonomic units) using UCLUST (Edgar, 2010) at 85% and 90% similarity respectively (Pester et al., 2012; Ouyang et al., 2016). The longest sequence within each OTU was picked as the representative sequence. Taxonomy of the OTUs were assigned using the BLASTn tool with the nucleotide database of NCBI (National Center for Biotechnology Information). Singletons and non-*amoA* OTUs were discarded. For down-stream analysis, the data were subsampled to 1000 sequences per sample for AOA and 2000 sequences per sample for AOB.

2.4 Statistical analyses

Non-metric multidimensional scaling (NMDS) was performed in R software using the vegan package based on Bray-Curtis distance to compare beta diversity of AOA and AOB communities in different treatments. A hierarchical clustering tree was generated by unweighted pair group method with arithmetic mean (UPGMA) (Milligan, 1985) in QIIME and edited with the Molecular Evolutionary Genetics Analysis (MEGA) package (version 7.0.18) (Kumar et al., 2016). The Mann–Whitney U test was used to compare diversity indices between treatments. A multivariate regression tree created by MVPARTwrap package in R was used to identify the species-environment relationships (De'Ath, 2002). Correlations between environmental factors and *amoA* communities were estimated using the Mantel test in R software (vegan package).

3 Results

3.1 Changes of AOA and AOB composition under different fertilization regimes

After removing low-quality reads in the raw data generated by 454 pyrosequencing, 241 898 archaeal *amoA* and 302 425 bacterial *amoA* sequences remained. Samples with low sequences were excluded for further analysis, but at least three replicates remained for each treatment. The AOA and AOB communities were each dominated by about a dozen OTUs (Fig. 1A, B). BLAST results revealed that the dominant AOA were mostly closely related to *Nitrososphaera*, *Nitroso-talea* and some unclassified species Archaeon G61 and Thaumarchaeota archaeon MY3. The dominant AOB were within the *Nitrosospira* and *Nitrosolobus* genera (Table S2).

Fertilization resulted in obvious shifts of ammonia-oxidizing communities. AOTU108, AOTU267, AOTU296 and AOTU343 were the dominant AOA species in the Control and NPK + PM treatment soils, but the application of NPK + CM increased the relative abundance of AOTU108. In the NPK, NPK + LS and NPK + HS treatments, AOTU294 became the dominant taxon, accounting for about 70% of the total community on average (Fig. 1A). For AOB communities, BOTU129 had the highest relative abundance in Control and NPK + CM soils. It was also present in the NPK + PM treatment, but at lower relative abundance than BOTU186. The relative abundance of BOTU186 was greatest in the fertilized soils without manure amendment (NPK, NPK + LS and NPK + HS treatments) (Fig. 1B).

The AOA communities of the six treatments were divided into two major branches of a hierarchical clustering tree, one that included NPK, NPK + LS and NPK + HS treatments, with the other three treatments, Control, NPK + PM and NPK + CM, grouped into the other branch (Fig. 1A). By contrast with AOA communities, AOB communities in control and NPK + PM treatments were clustered into the same branch, and the other four treatments clustered into the other branch (Fig. 1B). Similar results were shown in 3D NMDS plots (Fig. 1C, D). AOA communities in NPK, NPK + LS and NPK + HS were grouped closely, while Control, NPK + PM and NPK + CM were grouped closely. AOB communities in NPK, NPK + LS and NPK + HS also clustered together, separated from the control and NPK + CM treatments. The NPK + CM treatment was separated from the two groups, but closer to the NPK, NPK + LS and NPK + HS treatments than control and PK + CM treatments.

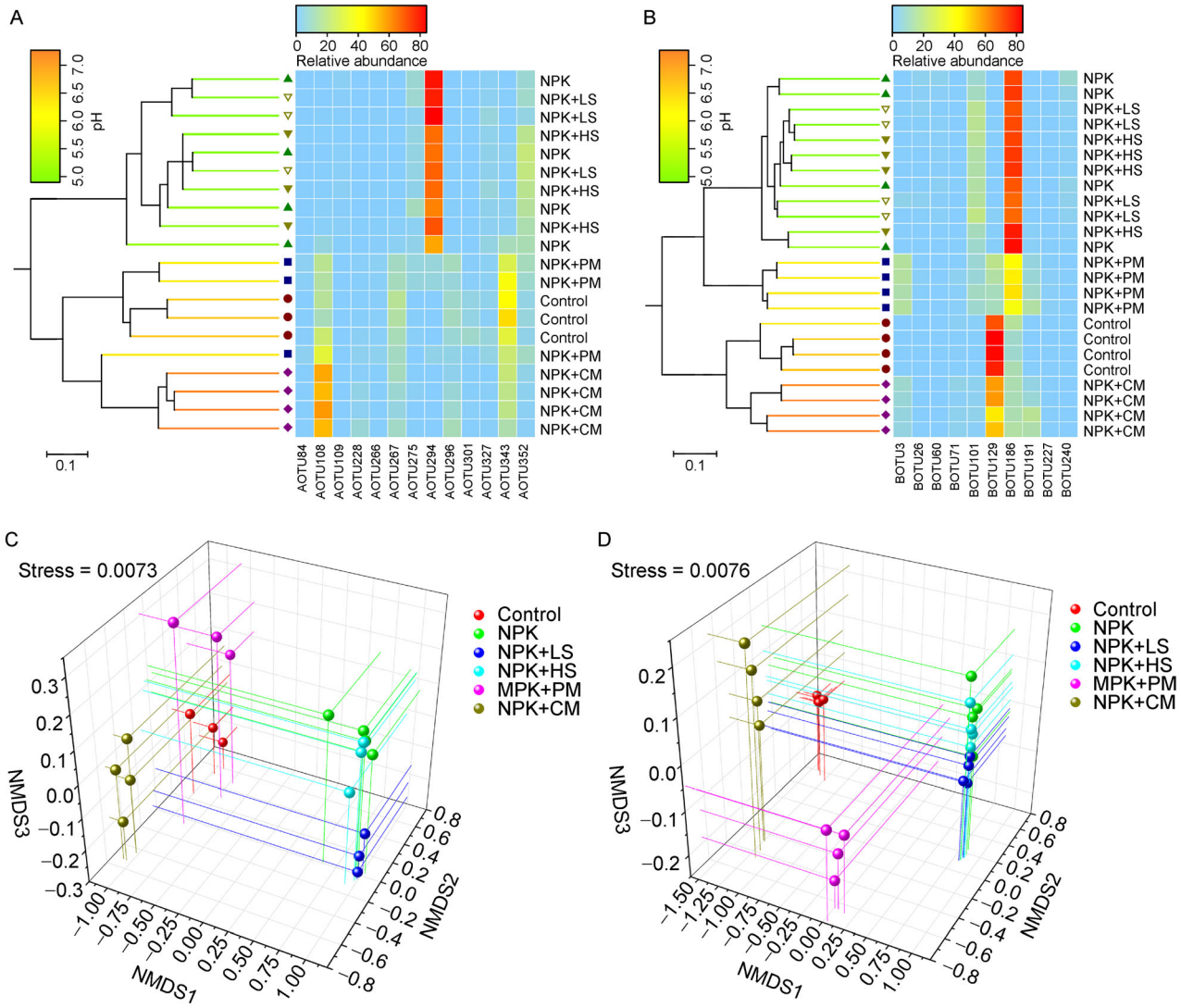

Fig. 1 Heatmaps demonstrating the dominant OTUs of AOA (A) and AOB (B) in different treatments, and NMDS plots showing the dissimilarity of AOA (C) and AOB (D) communities in different treatments. Control, non-fertilization; NPK: NPK fertilization; NPK + LS: NPK fertilizers with low-level wheat straw; NPK + HS: NPK with high-level wheat straw; NPK + PM: NPK with pig manure; NPK + CM: NPK with cow manure.

3.2 Abundance and diversity of AOA and AOB communities in different treatments

As shown in our previous study (Sun et al., 2015a), compared with the control, the abundance of archaeal *amoA* was significantly lower in NPK, NPK + LS and NPK + HS, not significantly changed in NK + PM, and higher in NPK + CM. By contrast, bacterial *amoA* abundance was increased by all the five fertilization regimes, and NPK + PM contained highest abundance of bacterial *amoA*, followed by NPK + CM and NPK, NPK + LS, NPK + HS (Table 1). OTU richness of AOA was significantly higher in the control, NPK + HS, NPK + PM and NPK + CM than those in the NPK and NPK + LS treatments (Table 1). A slightly different pattern was observed with the Shannon index of AOA diversity, with control and NPK + PM>NPK + CM>NPK, NPK + LS and NPK + HS. Compared to AOA, the richness of AOB communities varied less among treatments, with only the NPK + CM being significantly higher. The Shannon index of AOB community was more similar to that of the AOA community except that the control had the lowest diversity.

3.3 Correlations between ammonia-oxidizing communities and soil properties

Spearman correlations between ammonia-oxidizing communities and soil properties were determined by the Mantel test (Table 2). Results showed that both AOA and AOB communities were most highly correlated with soil pH, although several other soil variables also had significant correlations.

Table 1 Abundance and diversity of AOA and AOB communities in different treatments. Gene abundance data sourced from previous study (Sun et al., 2015a).

Treatment	AOA			AOB		
	Gene abundance[†]	OTU	Shannon	Gene abundance	OTU	Shannon
Control	43.1±7.80^{b‡}	20±2^{a}	2.60±0.19^{a}	0.87±0.17^{c}	13±2^{b}	0.82±0.23^{c}
NPK	12.6±1.59^{c}	14±4^{b}	1.67±0.41^{c}	2.98±0.76^{b}	11±3^{b}	0.93±0.34^{bc}
NPK + LS	9.64±4.54^{c}	11±0^{b}	1.21±0.19^{c}	2.69±1.12^{b}	16±2^{b}	1.25±0.13^{b}
NPK + HS	13.3±2.28^{c}	21±2^{a}	1.76±0.08^{c}	2.92±1.05^{b}	13±3^{b}	1.07±0.21^{bc}
NPK + PM	51.9±11.1^{b}	24±5^{a}	2.96±0.08^{a}	16.9±5.67^{a}	16±1^{b}	2.29±0.04^{a}
NPK + CM	88.0±8.35^{a}	18±5^{a}	2.10±0.13^{b}	11.0±0.39^{a}	19±2^{a}	2.12±0.15^{a}

Values are means±standard deviation. [†]Gene abundance was $\times 10^8$ copy number per dry soil. [‡]Different letters indicate significant differences between different treatments ($P < 0.05$). Control, non-fertilization; NPK: NPK fertilization; NPK + LS: NPK fertilizers with low-level wheat straw; NPK + HS: NPK with high-level wheat straw; NPK + PM: NPK with pig manure; NPK + CM: NPK with cow manure.

Table 2 Spearman's correlations (*r*) between AOA or AOB community (Bray-Curtis distance) and soil properties determined by the Mantel test.

	AOA		AOB	
	r	*P*	*r*	*P*
pH	0.962	0.001	0.817	0.001
TC	0.532	0.001	0.529	0.001
AP	0.506	0.001	0.392	0.004
TN	0.408	0.001	0.383	0.002
NH_4^+-N	0.403	0.001	0.379	0.001
AK	0.353	0.001	0.329	0.002
DOC	0.176	0.022	0.284	0.006
NO_3^--N	0.096	0.111	0.269	0.006
DON	0.027	0.311	0.062	0.188

TC: total carbon; AP: available phosphorus; TN: total nitrogen; NH_4^+-N: ammonium nitrogen; AK: available potassium; DOC: dissolved organic carbon; NO_3^--N: nitrate nitrogen; DON: dissolved organic nitrogen.

Most of the variation of AOA and AOB communities could be explained by the multivariate regression tree (MRT) analysis, and soil pH contributed the most explanatory power (Fig. 2). AOA and AOB communities were first split into two major groups by soil pH. One group of AOA communities contained NPK, NPK + LS and NPK + HS with soil pH < 5.69, the other group contained Control, NPK + PM and NPK + CM (Fig. 2A). MRT analysis split AOB communities into two branches, one with pH < 6.26, and Control and NPK + CM grouped into the other branch with soil pH $\geqslant$ 6.26 (Fig. 2B).

The dominant OTUs of AOA and AOB were also significantly correlated with soil pH (Fig. 3). Five of the top eight AOA OTUs were most highly correlated with soil pH, whereas two were most correlated with ammonium nitrogen (Fig. 3A). The top eight AOB OTUs were significantly correlated with soil pH, but one of them was most highly correlated with DOC and two of them had the highest correlation with soil total carbon (Fig. 3). The relationship between soil pH and AOA/AOB community showed that AOA and AOB community changed differently along a pH gradient (Fig. 4). AOA community changed dramatically within pH range of 5.0 to 6.0, and changed little between pH 6.0 to 7.5 (Fig. 4A). By contrast, the greatest change in the AOB community occurred within the pH range of 6.0 to 6.5 (Fig. 4B). The correlation between the abundance, diversity of AOA/AOB and soil properties was assessed by the Spearman correlation coefficient (Table 3). Gene abundance and Shannon index of AOA was most positively correlated with soil pH, while OTU richness of AOA had no significant correlation with any soil properties measured. However, the gene abundance, OTU richness and Shannon index of AOB had significantly positive correlation with available phosphorus (AP), available potassium (AK), soil total nitrogen and carbon content (TN, TC).

4 Discussion

By catalyzing the first step of nitrification, ammonia oxidation, the role of AOA and AOB in ecosystems has attracted extensive attention. In this study, we found that long-term fertilization changed both AOA and AOB community composition, and that both AOA and AOB community were significantly correlated with soil pH, although they responded differently to changes of soil pH. Soil acidification caused by chemical fertilizer application significantly decreased AOA but not AOB diversity. These results were contrary to the

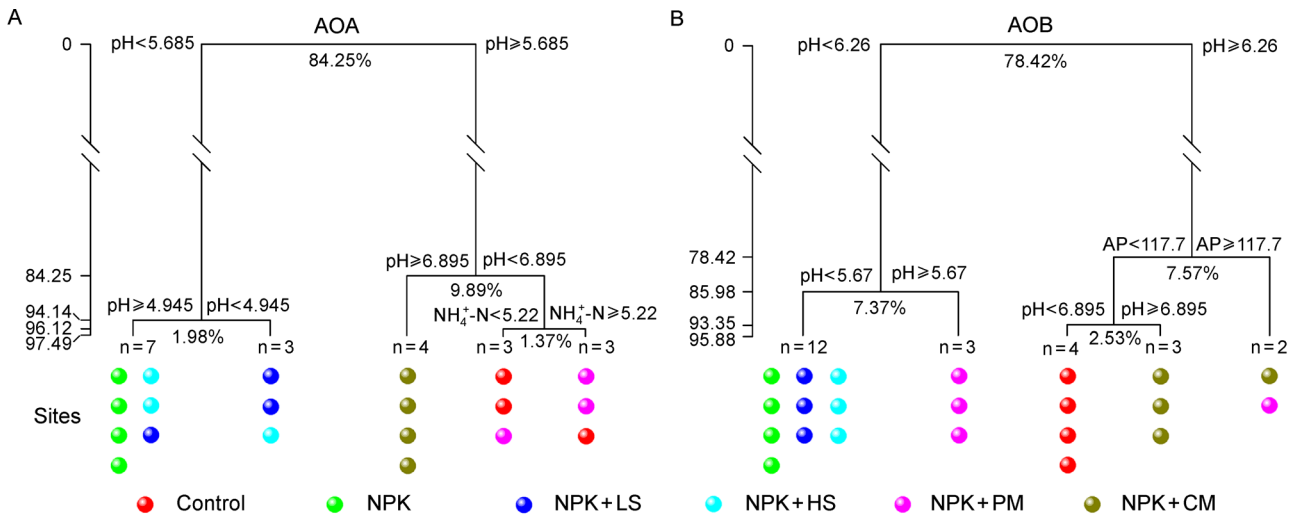

Fig. 2 Multivariate regression tree analysis of AOA (A) and AOB (B) communities under different fertilization regimes. Numbers under the crosses of each split indicate percentages of variance explained by the split. For abbreviations, see Fig. 1.

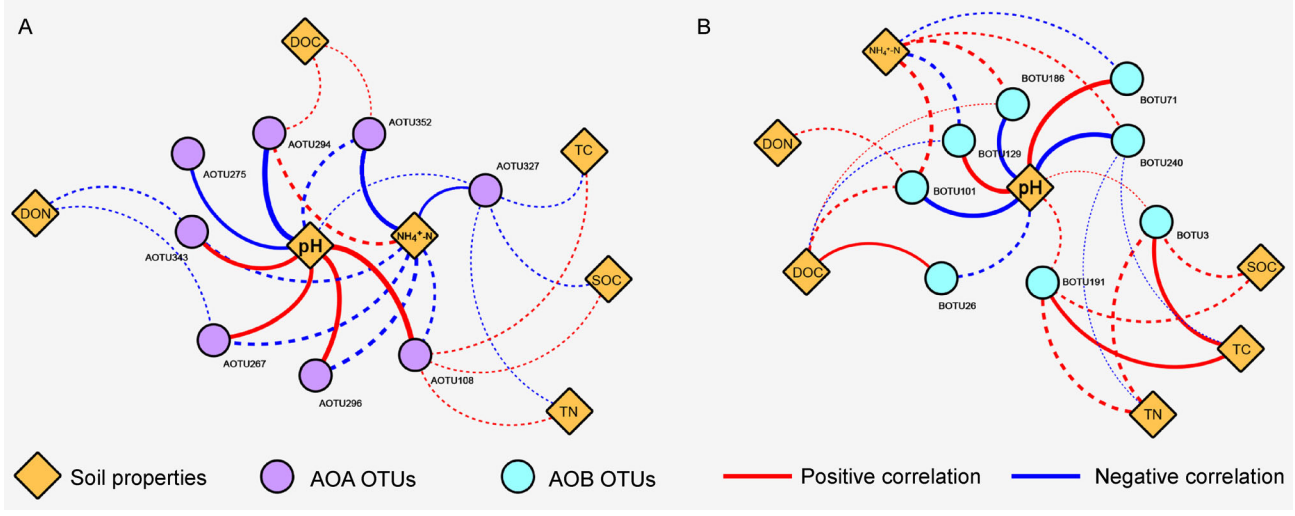

Fig. 3 Spearman's correlations between dominant OTUs (A, AOA; B, AOB) and soil properties. If an OTU had a significant correlation with a soil property, they were connected by an edge. Solid lines indicate highest correlation coefficient between OTUs and soil properties. Dashed lines indicate significant but not the highest correlations. The thicker the lines, the higher the correlation.

hypothesis we put forward, but provided a new sense about the role of AOA and AOB in ecosystems. It is thought that AOA rather than AOB dominate the ammonia oxidation process in acid soils (Gubry-Rangin et al., 2010; Huang et al., 2011), but our previous results with this field experiment revealed that although chemical fertilization significantly lowered soil pH, AOB abundance was increased in the acidified soil (Sun et al., 2015a). Conversely, AOA abundance largely decreased, and it was positively correlated with soil pH (Tables 1 and 3) (Sun et al., 2015a). In the current study, we also found that some AOB OTUs were significantly negatively correlated with soil pH (Fig. 3B), indicating their adaptation to an acidic environment. Acid-tolerant AOB strains have been isolated from soils, and their ammonia-oxidizing activity in acidic soils was confirmed, indicating that AOB can play an important role in the nitrification of acidic soils (De Boer et al., 1991; Brierley and Wood, 2001; Hayatsu et al., 2017). Similarly, the relative abundance of some AOA OTUs were higher in soil with higher pH (Fig. 1A), and they were significantly positively correlated with soil pH (Fig. 3A), indicating that some taxa of AOA may also well adapt to alkaline environment. Pan et al. (2018) have revealed that some lineage of AOA functionally predominated over AOB in some alkaline soil.

Although AOA and AOB communities were significantly

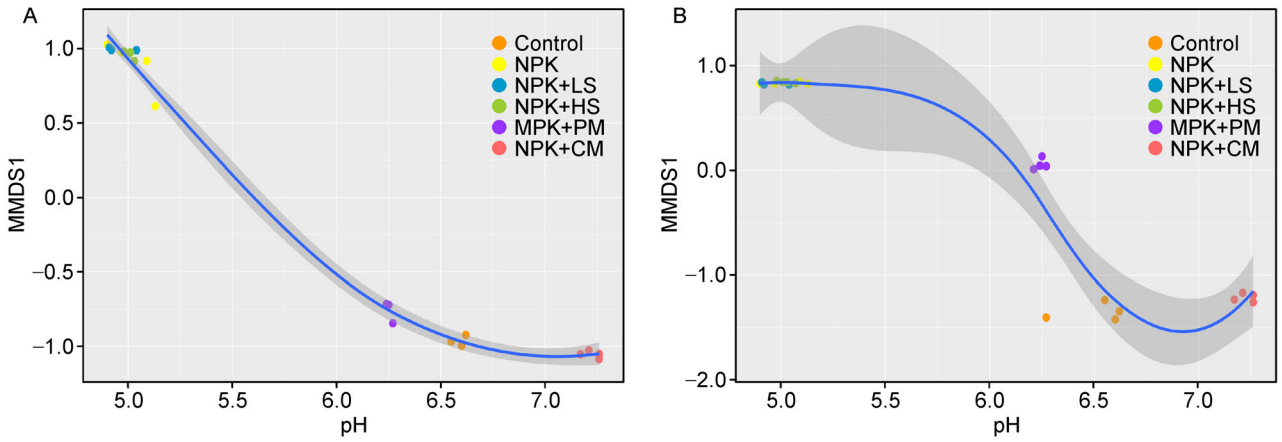

Fig. 4 Correlation of AOA (A) and AOB (B) community structure (indicated by the first axis of NMDS) and soil pH. For abbreviations, see Fig. 1.

Table 3 Spearman's correlation between AOA or AOB abundance, diversity and soil properties.

	Diversity	pH	TC	TN	NO_3^--N	NH_4^+-N	DOC	DON	AP	AK
AOA	Gene abundance	**0.695****	0.460*	0.398	0.480*	−0.232	0.211	−0.371	0.467*	0.503*
	OTU richness	0.441	0.169	0.143	−0.356	−0.242	0.039	−0.218	0.207	0.325
	Shannon index	**0.706****	0.148	0.099	−0.325	−0.537*	−0.104	−0.437	0.199	0.223
AOB	Gene abundance	−0.007	0.821**	0.795**	0.785**	−0.200	0.634**	−0.032	**0.840****	0.737**
	OTU richness	0.364	0.548**	0.558**	0.209	−0.128	0.085	0.418**	**0.614****	0.611**
	Shannon index	0.333	0.713**>	0.694**	0.251	−0.168	0.396	0.333	**0.833****	0.657**

TC: total carbon; TN: total nitrogen; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; AP: available phosphorus; AK: available potassium. Significance level: **, $P < 0.01$; *, $P < 0.05$. Bold fonts represent the highest correlation.

correlated with soil pH, they had different responses, with the greatest changes occurring at different pH ranges (Fig. 4). The diversity of AOA was significantly decreased in the acidic soils, whereas AOB diversity was not significantly impacted by soil acidification (Table 1). These results indicated that, although AOA were more abundant than AOB in soil, a higher proportion of AOB species could survive in acid soil. Consistent with a prior study with these soils (Sun et al., 2015a), we found that the AOB community was also impacted by soil carbon and phosphorus (Fig. 3B and Table 3). Prior research has shown that organic materials impact both AOA and AOB communities (Wessén et al., 2010; Wang et al., 2014; Wang et al., 2015; Liu et al., 2018). And there is some evidence for assimilation of organic compounds by ammonia oxidizers and the potential of heterotrophic and mixotrophic growth of ammonia-oxidizers (Hallam et al., 2006; Prosser and Nicol, 2012); however, recent studies confirmed the strictly autotrophic growth of AOA in pure culture (Kerou et al., 2016; Kim et al., 2016; Rice et al., 2016). Extrapolations of pure culture studies to natural systems are fraught, however, because of the complex interrelationships between different types of microbes (Verhagen et al., 1995; Geets et al., 2006; Keluskar et al., 2013). For example, *Nitrosomonas* sp. RA excreted organics to support the survival of heterotrophs, and the heterotrophs produced siderophores to complement *Nitrosomonas* sp. RA's high iron requirement in iron-limited condition (Keluskar et al., 2013). Another study also found effects of organic carbon sources on the make-up of the heterotroph community and AOB (Racz et al., 2010). Thus, organic carbon may impact AOB community indirectly by changing the interactions between heterotrophs. Phosphorus (P) is another important factor influencing AOA and AOB communities (Erguder et al., 2009; Zheng et al., 2014). Studies have found that the abundance of AOB had strong and positive correlation with the concentrations of available P (Tang et al., 2016), and AOB showed higher growth than AOA under P addition (Norman and Barrett, 2014), suggesting AOB prefer environments with high nutrient level. This was consistent with the result in this study that the abundance of AOB had higher correlation with AP than that of AOA (Table 3), and it may be a reason for that higher diversity of AOB was observed in soil with high content of AP. For another, greater nitrogen mineralization was observed in the P-amended soil, which increased availability of substrate favoring ammonia

oxidation by AOB (Norman and Barrett, 2014), thus indirectly impacted AOA and AOB communities. Here we found that the community composition and diversity of AOA was impacted by a single factor, soil pH, whereas the AOB community was significantly correlated with soil carbon and phosphorus in addition to soil pH, and the abundance and diversity of AOB was little impacted by soil acidification. These results represent the great role of pH in shaping AOA community, and the potentially greater ecophysiological diversity and broader range of habitats of AOB.

It is noteworthy that higher AOB diversity was observed in the manure-amended treatments than Control and other fertilized treatments, while AOA diversity in manure-amended treatments was similar with Control treatment (Table 2). This may indicate exogenous species input of AOB from manures, as we have found previously (Sun et al., 2015b; Sun et al., 2016). Thus, the high diversity of AOB in manure-amended treatments may be related to the exogenous species input. Another noteworthy aspect is that most of the AOA OTUs were not well-annotated (Table S2), with low identity against known AOA species. This indicates that there is still significant undiscovered diversity in the AOA.

In summary, long-term fertilization significantly changed both AOA and AOB communities in this lime concretion black soil. Although both were correlated with soil pH, the impact of soil pH on AOA and AOB differed. AOA diversity was positively correlated with soil pH, whereas AOB diversity was little impacted by soil acidification. Combined with our previous results that AOA abundance was significantly decreased by soil acidification and AOB abundance was higher in the acidified treatments compared with Control (Sun et al., 2015a), our results suggest that the contribution of AOB to ammonia oxidation in acid environments may be underestimated. Compared with the single factor of pH in shaping AOA community, soil carbon and available phosphorus were also significantly correlated with AOB abundance and diversity, suggesting that soil organic carbon and phosphorus are also important for the AOB community.

Acknowledgments

We thank Yuntao Li, Congcong Shen, Huaibo Sun, Yu Shi, Xingjia Xiang, and Dawei Ma for assistance in soil sampling, Zhibin Guo and Keke Hua for managemant of the filed. The first author, Ruibo Sun, appreciates his soul mate Jessy for shining his life. This study was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15010101), the National Natural Science Foundation of China (31870480), and the National Key Research and Development Program of China (2017YFD0800604).

Electronic supplementary material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s42832-019-0016-8 and is accessible for authorized users.

Reference

Abascal, F., Zardoya, R., Telford, M.J., 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic Acids Research 38, W7-13.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Carey, C.J., Dove, N.C., Beman, J.M., Hart, S.C., Aronson, E.L., 2016. Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. Soil Biology & Biochemistry 99, 158–166.

Cytryn, E., Levkovitch, I., Negreanu, Y., Dowd, S., Frenk, S., Silber, A., 2012. Impact of short-term acidification on nitrification and nitrifying bacterial community dynamics in soilless cultivation media. Applied and Environmental Microbiology 78, 6576–6582.

De Boer, W., Gunnewiek, P.J.A.K., Veenhuis, M., Bock, E., Laanbroek, H.J., 1991. Nitrification at low pH by aggregated chemolithotrophic bacteria. Applied and Environmental Microbiology 57, 3600–3604.

De'Ath, G., 2002. Multivariate regression trees: a new technique for modeling species-environment relationships. Ecology 83, 1105–1117.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics (Oxford, England) 26, 2460–2461.

Erguder, T.H., Boon, N., Wittebolle, L., Marzorati, M., Verstraete, W., 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiology Reviews 33, 855–869.

Geets, J., Boon, N., Verstraete, W., 2006. Strategies of aerobic ammonia-oxidizing bacteria for coping with nutrient and oxygen fluctuations. FEMS Microbiology Ecology 58, 1–13.

Gubry-Rangin, C., Nicol, G.W., Prosser, J.I., 2010. Archaea rather than bacteria control nitrification in two agricultural acidic soils. FEMS Microbiology Ecology 74, 566–574.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B.C., James, P., Schloter, M., Griffiths, R.I., Prosser, J.I., Nicol, G.W., 2011. Niche specialization of terrestrial archaeal ammonia oxidizers. Proceedings of the National Academy of Sciences 108, 21206.

Gubry-Rangin, C., Novotnik, B., Mandič-Mulec, I., Nicol, G.W., Prosser, J.I., 2017. Temperature responses of soil ammonia-oxidising archaea depend on pH. Soil Biology and Biochemistry 106, 61–68

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M., DeLong, E.F., 2006. Pathways of Carbon Assimilation and Oxidation Suggested by Environmental Genomic Analyses of Marine Crenarchaeota. Plos Biology 4, e95.

Hatzenpichler, R., 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Applied and Environmental

Microbiology 78, 7501–7510.

Hayatsu, M., Tago, K., Uchiyama, I., Toyoda, A., Wang, Y., Shimomura, Y., Okubo, T., Kurisu, F., Hirono, Y., Nonaka, K., Akiyama, H., Itoh, T., Takami, H., 2017. An acid-tolerant ammonia-oxidizing γ-proteobacterium from soil. ISME Journal 11, 1130–1141.

Hu, H.W., Zhang, L.M., Dai, Y., Di, H.J., He, J.Z., 2013. pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing. Journal of Soils and Sediments 13, 1439–1449.

Huang, R., Wu, Y., Zhang, J., Zhong, W., Jia, Z., Cai, Z., 2011. Nitrification activity and putative ammonia-oxidizing archaea in acidic red soils. Journal of Soils and Sediments 12, 420–428.

Jia, Z., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environmental Microbiology 11, 1658–1671.

Kelly, J.J., Policht, K., Grancharova, T., Hundal, L.S., 2011. Distinct responses in ammonia-oxidizing archaea and bacteria after addition of biosolids to an agricultural soil. Applied and Environmental Microbiology 77, 6551–6558.

Keluskar, R., Nerurkar, A., Desai, A., 2013. Mutualism between autotrophic ammonia-oxidizing bacteria (AOB) and heterotrophs present in an ammonia-oxidizing colony. Archives of Microbiology 195, 737–747.

Kerou, M., Offre, P., Valledor, L., Abby, S.S., Melcher, M., Nagler, M., Weckwerth, W., Schleper, C., 2016. Proteomics and comparative genomics of *Nitrososphaera viennensis* reveal the core genome and adaptations of archaeal ammonia oxidizers. Proceedings of the National Academy of Sciences of the United States of America 113, E7937–E7946

Kim, J.G., Park, S.J., Sinninghe Damsté, J.S., Schouten, S., Rijpstra, W.I.C., Jung, M.Y., Kim, S.J., Gwak, J.H., Hong, H., Si, O.J., Lee, S., Madsen, E.L., Rhee, S.K., 2016. Hydrogen peroxide detoxification is a key mechanism for growth of ammonia-oxidizing archaea. Proceedings of the National Academy of Sciences of the United States of America 113, 7888–7893.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33, 1870–1874.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809.

Liu, H., Li, J., Zhao, Y., Xie, K., Tang, X., Wang, S., Li, Z., Liao, Y., Xu, J., Di, H., Li, Y., 2018. Ammonia oxidizers and nitrite-oxidizing bacteria respond differently to long-term manure application in four paddy soils of south of China. Science of the Total Environment 633, 641–648.

Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461, 976–979.

Meng, H., Katayama, Y., Gu, J.D., 2017. More wide occurrence and dominance of ammonia-oxidizing archaea than bacteria at three Angkor sandstone temples of Bayon, Phnom Krom and Wat Athvea in Cambodia. International Biodeterioration & Biodegradation 117, 78–88.

Milligan, G.W., 1985. Cluster-Analysis for Researchers- Romesburg, Hc. Journal of Classification 2, 133–137.

Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10, 2966–2978.

Norman, J.S., Barrett, J.E., 2014. Substrate and nutrient limitation of ammonia-oxidizing bacteria and archaea in temperate forest soil. Soil Biology & Biochemistry 69, 141–146.

Nunes-Alves, C., 2016. Microbial ecology: Do it yourself nitrification. Nature Reviews Microbiology 14, 61–61.

Ouyang, Y., Norton, J.M., Stark, J.M., Reeve, J.R., Habteselassie, M. Y., 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biology & Biochemistry 96, 4–15.

Pan, H., Xie, K.X., Zhang, Q.C., Jia, Z.J., Xu, J.M., Di, H.J., Li, Y., 2018. Archaea and bacteria respectively dominate nitrification in lightly and heavily grazed soil in a grassland system. Biology and Fertility of Soils 54, 41–54.

Pester, M., Rattei, T., Flechl, S., Gröngröft, A., Richter, A., Overmann, J., Reinhold-Hurek, B., Loy, A., Wagner, M., 2012. *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of *amoA* genes from soils of four different geographic regions. Environmental Microbiology 14, 525–539.

Prosser, J.I., Nicol, G.W., 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environmental Microbiology 10, 2931–2941.

Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 20, 523–531.

Racz, L., Datta, T., Gole, R., 2010. Effect of organic carbon on ammonia oxidizing bacteria in a mixed culture. Bioresource Technology 101, 6454–6460.

Rice, M.C., Norton, J.M., Valois, F., Bollmann, A., Bottomley, P.J., Klotz, M.G., Laanbroek, H.J., Suwa, Y., Stein, L.Y., Sayavedra-Soto, L., Woyke, T., Shapiro, N., Goodwin, L.A., Huntemann, M., Clum, A., Pillay, M., Kyrpides, N., Varghese, N., Mikhailova, N., Markowitz, V., Palaniappan, K., Ivanova, N., Stamatis, D., Reddy, T.B.K., Ngan, C.Y., Daum, C., 2016. Complete genome of *Nitrosospira briensis* C-128, an ammonia-oxidizing bacterium from agricultural soil. Standards in Genomic Sciences 11, 46.

Rudisill, M.A., Turco, R.F., Hoagland, L.A., 2016. Fertility practices and rhizosphere effects alter ammonia oxidizer community structure and potential nitrification activity in pepper production soils. Applied Soil Ecology 99, 70–77.

Schleper, C., 2010. Ammonia oxidation: different niches for bacteria and archaea? ISME Journal 4, 1092–1094.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing

and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.

Segal, L.M., Miller, D.N., McGhee, R.P., Loecke, T.D., Cook, K.L., Shapiro, C.A., Drijber, R.A., 2017. Bacterial and archaeal ammonia oxidizers respond differently to long-term tillage and fertilizer management at a continuous maize site. Soil & Tillage Research 168, 110–117.

Stahl, D.A., de la Torre, J.R., 2012. Physiology and diversity of ammonia-oxidizing archaea. Annual Review of Microbiology 66, 83–101.

Sun, R., Dsouza, M., Gilbert, J.A., Guo, X., Wang, D., Guo, Z., Ni, Y., Chu, H., 2016a. Fungal community composition in soils subjected to long-term chemical fertilization is most influenced by the type of organic matter. Environmental Microbiology 18, 5137–5150.

Sun, R., Guo, X., Wang, D., Chu, H., 2015a. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. Applied Soil Ecology 95, 171–178.

Sun, R., Zhang, X.X., Guo, X., Wang, D., Chu, H., 2015b. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology & Biochemistry 88, 9–18.

Tang, Y., Zhang, X., Li, D., Wang, H., Chen, F., Fu, X., Fang, X., Sun, X., Yu, G., 2016. Impacts of nitrogen and phosphorus additions on the abundance and community structure of ammonia oxidizers and denitrifying bacteria in Chinese fir plantations. Soil Biology & Biochemistry 103, 284–293.

Tourna, M., Freitag, T.E., Nicol, G.W., Prosser, J.I., 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environmental Microbiology 10, 1357–1364.

Verhagen, F.J.M., Laanbroek, H.J., Woldendorp, J.W., 1995. Competition for Ammonium between Plant-Roots and Nitrifying and Heterotrophic Bacteria and the Effects of Protozoan Grazing. Plant and Soil 170, 241–250.

Verhamme, D.T., Prosser, J.I., Nicol, G.W., 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME Journal 5, 1067–1071.

Wang, X., Wang, C., Bao, L., Xie, S., 2015. Impact of carbon source amendment on ammonia-oxidizing microorganisms in reservoir riparian soil. Annals of Microbiology 65, 1411–1418.

Wang, Y., Zhu, G., Song, L., Wang, S., Yin, C., 2014. Manure fertilization alters the population of ammonia-oxidizing bacteria rather than ammonia-oxidizing archaea in a paddy soil. Journal of Basic Microbiology 54, 190–197.

Wessén, E., Nyberg, K., Jansson, J.K., Hallin, S., 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. Applied Soil Ecology 45, 193–200.

Xiang, X., He, D., He, J.S., Myrold, D.D., Chu, H., 2017. Ammonia-oxidizing bacteria rather than archaea respond to short-term urea amendment in an alpine grassland. Soil Biology & Biochemistry 107, 218–225.

Xu, X., Liu, X., Li, Y., Ran, Y., Liu, Y., Zhang, Q., Li, Z., He, Y., Xu, J., Di, H., 2017. High temperatures inhibited the growth of soil bacteria and archaea but not that of fungi and altered nitrous oxide production mechanisms from different nitrogen sources in an acidic soil. Soil Biology & Biochemistry 107, 168–179.

Yao, H., Gao, Y., Nicol, G.W., Campbell, C.D., Prosser, J.I., Zhang, L., Han, W., Singh, B.K., 2011. Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. Applied and Environmental Microbiology 77, 4618–4625.

Ying, J., Li, X., Wang, N., Lan, Z., He, J., Bai, Y., 2017. Contrasting effects of nitrogen forms and soil pH on ammonia oxidizing microorganisms and their responses to long-term nitrogen fertilization in a typical steppe ecosystem. Soil Biology & Biochemistry 107, 10–18.

Zhalnina, K., de Quadros, P.D., Camargo, F.A., Triplett, E.W., 2012. Drivers of archaeal ammonia-oxidizing communities in soil. Frontiers in Microbiology 3, 210.

Zhang, L.M., Hu, H.W., Shen, J.P., He, J.Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. ISME Journal 6, 1032–1045.

Zhang, L.M., Offre, P.R., He, J.Z., Verhamme, D.T., Nicol, G.W., Prosser, J.I., 2010. Autotrophic ammonia oxidation by soil thaumarchaea. Proceedings of the National Academy of Sciences of the United States of America 107, 17240–17245.

Zheng, Y., Hou, L., Newell, S., Liu, M., Zhou, J., Zhao, H., You, L., Cheng, X., 2014. Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze estuary. Applied and Environmental Microbiology 80, 408–419.