Received: 22 April 2019 | Revised: 14 September 2019 | Accepted: 16 September 2019

DOI: 10.1002/mbo3.942

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

Effects of applying inorganic fertilizer and organic manure for 35 years on the structure and diversity of ammonia-oxidizing archaea communities in a Chinese Mollisols field

Jianli Ding[1,2] | Mingchao Ma[2] | Xin Jiang[2] | Yao Liu[3] | Junzheng Zhang[4] | Linna Suo[1] | Lei Wang[1] | Dan Wei[1] | Jun Li[2]

[1]Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

[2]Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China

[3]National Center for Science and Technology Evaluation, MOST, Beijing, China

[4]School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, China

Correspondence
Dan Wei, Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences Beijing 100097, China.
Email: wd2087@163.com

Jun Li, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
Email: lijun01@caas.cn

Funding information
The Natural Science Foundation of Heilongjiang Province, Grant/Award Number: No. ZD2018003; Postdoctoral Research Funds of Beijing, Grant/Award Number: ZZ2019-48; National Natural Science Foundation of China, Grant/Award Number: 41771284

Abstract

In this study, we investigated the physicochemical properties of soil, and the diversity and structure of the soil ammonia-oxidizing archaea (AOA) community, when subjected to fertilizer treatments for over 35 years. We collected soil samples from a black soil fertilization trial in northeast China. Four treatments were tested: no fertilization (CK); manure (M); nitrogen (N), phosphorus (P), and potassium (K) chemical fertilizer (NPK); and N, P, and K plus *M* (MNPK). We employed 454 high-throughput pyrosequencing to measure the response of the soil AOA community to the long-term fertilization. The fertilization treatments had different impacts on the shifts in the soil properties and AOA community. The utilization of manure alleviated soil acidification and enhanced the soybean yield. The soil AOA abundance was increased greatly by inorganic and organic fertilizers. In addition, the community Chao1 and ACE were highest in the MNPK treatment. In terms of the AOA community composition, Thaumarchaeota and Crenarchaeota were the main AOA phyla in all samples. Compared with CK and M, the abundances of Thaumarchaeota were remarkably lower in the MNPK and NPK treatments. There were distinct shifts in the compositions of the AOA operational taxonomic units (OTUs) under different fertilization management practices. OTU51 was the dominant OTU in all treatments, except for NPK. OTU79 and OTU11 were relatively abundant OTUs in NPK. Only *Nitrososphaera* AOA were tracked from the black soil. Redundancy analysis indicated that the soil pH and soil available P were the two main factors that affected the AOA community structure. The abundances of AOA were positively correlated with the total N and available P concentrations, and negatively correlated with the soil pH.

KEYWORDS
amoA gene, black soil, long-term fertilization, pyrosequencing, qPCR

Jianli Ding and Mingchao Ma contributed equally to this work.

1 | INTRODUCTION

Black soil, also known as Mollisols, is a unique soil with excellent characteristics and high fertility, which is well suited to plant growth. Black soil is found in four main areas throughout the world, one of which is located in northeast China. This area covers approximately 1.03 million square kilometers, and it produces about 225–250 billion kilograms of commercial grain each year. It is China's largest commercial grain production base. However, long-term continuous cropping and unreasonable farming practices (such as long-term excessive and unreasonable fertilizer application) have led to the degradation of the black soil farmland (Zhou et al., 2016) and have affected the various microorganisms related to the nitrogen (N) cycle process in black soil (Yin et al., 2015). Ammonia oxidization is a key step in the soil N cycle and is the primary and limiting stage in nitrification (Kowalchuk & Stephen, 2001). In recent decades, many studies have suggested that ammonia-oxidizing bacteria (AOB) are the dominating participants in ammonia oxidation. However, this view changed after the identification of ammonia-oxidizing archaea (AOA) (Gao, Chang, et al., 2018c; Leininger et al., 2006; Li, Chapman, Nicol, & Yao, 2018). Thus, research into the roles of AOA and AOB in the ammonia oxidization process has intensified (Hou, Cao, Song, & Zhou, 2013; Wang, Wang, Feng, Zhai, & Zhu, 2011). A common method for determining the abundance and diversity of AOA and AOB in soil samples involves the extraction of DNA. Ammonia monooxygenase is the key enzyme that catalyzes the first reaction in ammonia oxidization. The *amoA* gene encodes the alpha subunit of ammonia monooxygenase (Kowalchuk & Stephen, 2001). Thus, the *amoA* gene has been employed widely as a molecular biomarker to measure the AOA and AOB composition and diversity (Junier et al., 2010).

In ocean, lake, and soil environments, the *amoA* gene copy numbers from AOA are tens or even thousands of times greater than those from AOB (Li, Weng, Huang, Su, & Yang, 2015). Studies have shown that AOA prefer areas with low ammonium concentrations, whereas AOB prefer areas with high ammonium concentrations (Gao, Liu, Li, Chen, & Liang, 2018a; Ouyang, Norton, & Stark, 2017). When AOA and AOB coexist in most agricultural soils, they differ in terms of their responses to environmental disturbances (i.e., niche specialization) and resource utilization (i.e., niche differentiation) (Gao, Chang, et al., 2018c; Ouyang, Norton, Stark, Reeve, & Habteselassie, 2016). AOA are suitable for a wider range of aerobic conditions according to some studies (Chen, Zhu, Xia, Shen, & He, 2008; Li et al., 2015). Indeed, the abundances of AOB are lower than those of AOA in many acidic soils (Zhang, Wang, Li, He, & Zhang, 2015a). Researchers have speculated that in some environments, such as acidic soils, the contribution of AOB to nitrification may be less than that of AOA (Lehtovirta-Morley et al., 2014; Wang et al., 2015). Hence, it is very important to study the AOA community structure in soil that has undergone long-term fertilizer treatment to further understand the nitrification process and its mechanism.

Previous studies have suggested that the main differences in AOA in terms of their abundance, activity, and community structures can be explained by the soil physicochemical properties and agricultural management strategies (Ciccolini, Bonari, Ercoli, & Pellegrino, 2016; Sun, Guo, Wang, & Chu, 2015; Wang et al., 2015). Long-term fertilization in different soils affects the composition and abundance of AOA, such as in paddy soil (Gao, Cao, et al., 2018b; Gu et al., 2017). He et al. (2018) and Zhang, Wang, et al. (2015a), Zhang, Chen, Dai, Sun, and Wen (2015b) showed that the long-term application of biochar could alter the composition and affect the abundance of ammonia oxidizers, especially in acid soils. The long-term application of N fertilizer also dramatically increases the abundance of AOA in the soil according to the analysis of the *amoA* gene (Beeckman, Motte, & Beeckman, 2018; Ouyang, Evans, Friesen, & Tiemann, 2018). Under continuous maize cultivation for 23 years, Xue et al. (2016) showed that the fertilization regime can cause changes in the structure and composition of the archaea community in an experimental black soil, where chemical fertilizer had the greatest effect on the AOA composition, whereas the application of chemical fertilizer plus manure neutralized the changes in the community induced by the chemical fertilizer. Compared with an acidic red soil, black soil was acidified due to the long-term application of chemical fertilizer (Zhou et al., 2015), and it had higher organic matter (OM) and ammonia nitrogen contents. These differences in the substrates for AOA are very important. In this study, we investigated the changes in the structure and diversity of AOA under long-term treatment (more than 35 years), where four fertilizer treatments were tested: no fertilization (CK), manure (M), chemical fertilizer (NPK), and NPK plus *M* (MNPK). The soil AOA structure, diversity, and abundance were determined using marked sequencing and quantitative PCR (qPCR) of the archaeal *amoA* genes. Correlations among black soil properties and the AOA structure and diversity were analyzed.

2 | MATERIALS AND METHODS

2.1 | Soil sampling

The soil samples were collected from the trial site, located in Harbin, Heilongjiang Province, China (N 45°40′, E 126°35′), which is affiliated with Heilongjiang Academy of Sciences. This region has a mean annual temperature of 3.5°C and annual precipitation of 575 mm, with a typical monsoon climate. The site had a total area of 7,000 m^2 and 96 plots (32 treatments with triplicates of each treatment). Plots were randomly arranged, and cement plates were inserted between the plots. The tillage practices comprised shallow plowing combined with subsoiling and rotary tillage combined with subsoiling. The study site had been subjected to wheat–maize–soybean crop rotation since 1980, and the samples were collected in the 35th year of use (soybean was planted in this year). The experimental site was flat, and the soil properties were homogeneous. The basic soil physicochemical properties (1980) were as follows: OM 26.7 g/kg, total *N* (TN) 1.47 g/kg, total phosphorus (TP) 1.07 g/kg, available N 151.1 mg/kg, available phosphorus (AP) 51.0 mg/kg, available potassium (AK) 200 mg/kg, and pH 7.22 (Ding et al., 2016). Further details of this long-term field experiment were reported by Wei et al. (2008). Four fertilization experimental treatments were tested

comprising no fertilization (CK); manure (M); *N* (urea used as N), P, and K chemical fertilizer (NPK); and N, P, and K plus *M* (MNPK). The doses of chemical fertilizers were 150 kg N/ha, 75 kg P_2O_5/ha, and 75 kg K_2O/ha for the wheat and maize plots, and 75 kg N/ha, 150 kg P_2O_5/ha, and 75 kg K_2O/ha for the soybean plots. The dose of horse manure as an organic amendment was approximately 18,600 kg manure/ha. The nutrient contents of the horse manure determined as averages in a calendar year were 0.56%, 0.63%, and 0.89% for N, P_2O_5, and K_2O, respectively. When the soybean was harvested in September 2014, we selected three plots from each of the four fertilization treatments (total of 12 plots) to collect soil samples.

We collected soil from a depth of 5–25 cm at 10 points (plow layer) and mixed them together to obtain a soil sample for the plot. Samples were stored in an icebox and returned to the laboratory for examination. Each sample was separated into two parts, where one was stored at −80℃ for biological analysis and the other was employed for physical and chemical analyses. The soil nitrate nitrogen (NN) and ammonium nitrogen (AN) contents were measured using the fresh soil samples. Other soil properties were analyzed after drying the samples at room temperature and passing through a 2.0-mm mesh.

2.2 | Soil properties and soybean yield

The soil pH, NN, AN, AP, AK, OM, and TN were determined using the methods described by Ding et al. (2017). Briefly, the soil pH was measured with a pH meter using a 1:1 sample:water extract. Inorganic *N* (NN and AN) was determined by flow injection analysis. Soil TN was measured with the micro-Kjeldahl method. Soil AP was analyzed using the Mo-Sb colorimetric method. The soil AK was determined by flame photometry. Soil OM was measured using the $K_2Cr_2O_7$-capacitance method. The soybean yield was estimated after harvesting soybeans from 10 m^2 of the central area of the plot. After threshing and drying, the dry weight was determined for the soybeans harvested from the sampling area in order to calculate the soybean yield per plot.

2.3 | DNA extraction and qPCR analysis

We extracted the soil total community DNA (TC DNA) with a Power Soil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, except the additional incubation step at 65°C was increased by 10 min (Fierer et al., 2012). In order to obtain the whole community DNA for the subsequent analyses, each soil sample was extracted to obtain six replicates and these replicates were then mixed. The DNA quality and concentration (A260/A280) were estimated using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, Rockwood, TN, USA). Each TC DNA sample was stored at −80°C until further analyses.

The primers comprising *Arch-amoA*F (5′-STAATGGTCTGGCTTAGACG-3′) and *Arch-amoA*R (5′-GCGGCCATCCATCTGTATGT-3′) were used for amplifying the archaeal *amoA* gene (Gan et al., 2016). The plasmids were prepared as described by Ding et al. (2016). Quantitative analysis of the archaeal *amoA* gene was conducted with an ABI 7,500 Real-Time PCR Detection System (Applied Biosystems, Waltham, MA, USA). The reaction mixture (20 μL) comprised 2 × FastFire qPCR PreMix (FastFire qPCR PreMix, Tiangen Biotech, China), 1 × ROX Reference Dye, 1 ml of 1/10 diluted DNA, and 10 nM of each primer. The optimum amplification conditions were as follows: initial denaturation at 95℃ for 60 s, 40 cycles of denaturation at 95℃ for 5 s and annealing at 60℃ for 32 s, and melting curve analysis. A plasmid containing the archaeal *amoA* gene was 10-fold serially diluted to generate the standard curve.

2.4 | 454 high-throughput pyrosequencing and bioinformatics analyses

We performed 454 high-throughput pyrosequencing as described by Zhang, Chen, et al. (2015b). The primers were the same as those used for qPCR. Briefly, the AOA *amoA* genes with barcoded primers were amplified in triplicate with an ABI 9,700 thermocycler (ABI, Foster City, CA, USA). The PCR amplification cycle comprised the following: 120 s at 95℃, followed by 35 cycles of denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 30 s, with a final elongation step for 300 s at 72℃. The mixture of triplicate PCR products was verified by 1% agarose gel electrophoresis and then purified using an AxyPrep DNA Gel Extraction Kit (Axygen, Union City, CA, USA). Finally, the purified PCR products were used to generate the amplicon libraries. The DNA library was sequenced with a Roche GS-FLX Titanium Sequencer (Roche Diagnostics Corporation, Branford, CT, USA). The pyrosequencing data were deposited in the NCBI Short Reads Archive Database (SRA accession: PRJNA512072).

The pyrosequencing data were analyzed with the QIIME pipeline version 1.8.0 (Caporaso et al., 2010). We excluded low-quality reads in an initial quality filtering step. First, the reads with ambiguous bases > 0 and average sequence quality < 25 were removed. The lowest sample number after rarefaction was 11,517. The average length of the valid sequences used for alignment was 418 bp. Chimeric sequences were then identified using UCHIME (Edgar, 2010). Operational taxonomic units (OTUs) were defined by clustering at 97% similarity. The rarefaction and diversity indices were calculated after clustering the OTUs. The OTUs were taxonomically classified based on the Fungene reference database (Release 7.3 http://fungene.cme.msu.edu/) (Wang, Garrity, Tiedje, & Cole, 2007), and the confidence level was set at 80%. The α-diversity of AOA was calculated using mothur (Schloss, Gevers, & Westcott, 2011) (1.31.2, http://www.mothur.org/) and with the following four parameters: Shannon and Simpson diversity indices, and Chao1 and the abundance-based coverage estimator (ACE) as richness indices. Phylogenetic trees were constructed using MEGA 6.0 with the neighbor-joining method.

2.5 | Statistical analysis

Significant differences in the abundances of AOA were detected by ANOVA. The relationships between the soil properties and the relative abundances and diversity indices for AOA were tested based on

TABLE 1 General physicochemical properties of soils (mean ± standard deviation*) for each treatment

Variable	CK	M	MNPK	NPK
AP(mg/kg)	2.89 ± (0.90)^{a}	13.86 ± (0.98)^{a}	103.1 ± (20.48)^{b}	94.59 ± (7.01)^{b}
AK(mg/kg)	157.17 ± (29.27)^{a}	190.2 ± (1.48)^{a}	171.78 ± (9.30)^{a}	166.88 ± (15.02)^{a}
NN(mg/kg)	2.36 ± (1.02)^{a}	4.44a ± (0.62)^{b}	6.84 ± (0.63)^{c}	4.72 ± (1.12)^{bc}
AN(mg/kg)	34.85 ± (0.57)^{a}	37.47 ± (6.41)^{a}	41.27 ± (4.82)^{a}	35.80 ± (7.46)^{a}
OM(g/kg)	24.46 ± (0.25)^{a}	27.67 ± (0.12)^{c}	25.65 ± (0.31)^{b}	24.92 ± (0.32)^{a}
TN(g/kg)	1.18 ± (0.02)^{a}	1.23 ± (0.06)^{ab}	1.36 ± (0.11)^{ab}	1.42 ± (0.08)^{b}
pH(1:1 H_2O)	6.48 ± (0.06)^{c}	6.59 ± (0.05)^{c}	5.88 ± (0.16)^{b}	5.53 ± (0.03)^{a}

Abbreviations: AP, available phosphorus; AK, available potassium; NN, nitrate nitrogen; AN, ammonium nitrogen; OM, organic matter; TN, total nitrogen; CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure.
*Values followed by different letters are significantly different ($p < .05$) according to Tukey's multiple comparison test.

Pearson's correlation coefficients (Ramirez, Craine, & Fierer, 2012). Data analyses were conducted with SPSS version 19.0 (IBM Inc., USA). Interactions between the AOA community and environmental factors were detected using CANOCO 5.0 (Microcomputer Power, Ithaca, NY) for redundancy analysis.

3 | RESULTS

3.1 | Soil properties and soybean yield

Compared with the CK treatment, the AP, TN, and NN contents all increased uniformly in the fertilization treatments (Table 1). The OM concentrations in M and MNPK were higher than CK. The pH was lowest in the NPK treatment. The soil AP contents differed significantly in the four treatments, for example, 103.1 and 94.59 mg/kg in MNPK and NPK, respectively. Furthermore, fertilization improved soybean production, where the soybean production with MNPK (2,421.73 kg/ha) was greater than that under NPK (2,283.67 kg/ha) (Figure 1). Soybeans can fix N biologically, but inorganic N fertilization decreased biological N fixation by soybeans (Ding et al., 2016). Thus, the yield was highest with M, followed by MNPK and then NPK.

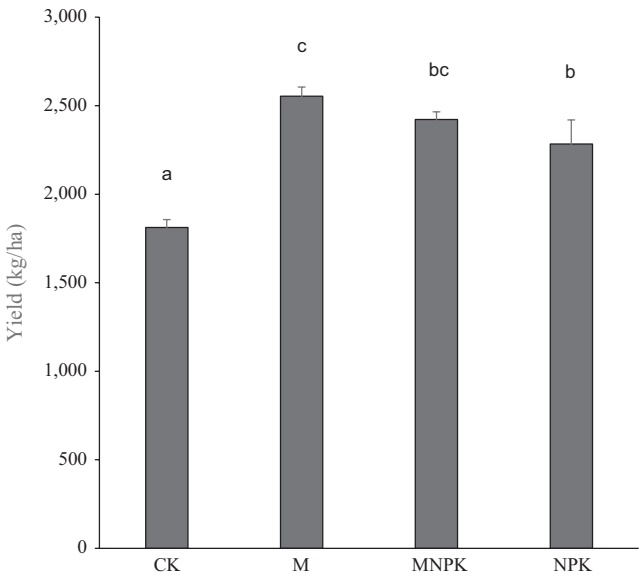

FIGURE 1 Soybean yield with each treatment. CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure

3.2 | Copy numbers of *amoA* gene

The long-term fertilization regimes affected the sizes of the AOA communities based on the AOA *amoA* gene copy numbers. The *amoA* gene copy numbers in the treatments ranged from 2.36×10^5 to 3.43×10^6 gene copies per ng DNA (Figure 2). The *amoA* gene copy numbers did not differ significantly in CK and *M* ($p < .05$), but they were lower in MNPK than NPK ($p < .05$). Moreover, Pearson's correlation coefficients

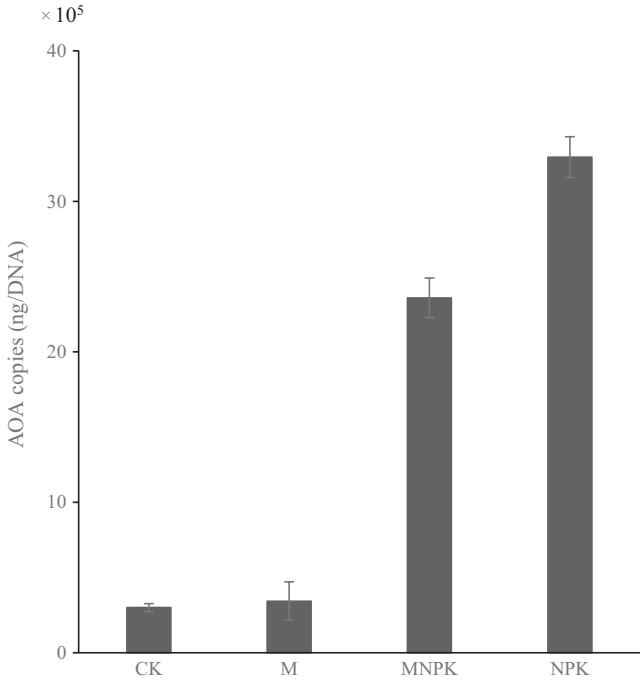

FIGURE 2 AOA *amoA* gene copy numbers detected by qPCR in soil samples from different fertilization treatments. CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure

between the soil properties and AOA *amoA* gene copy numbers (Table 3) showed that the AOA *amoA* gene copy numbers were positively correlated with the soil TN ($r = 0.691$, $p < .05$) and AP concentrations ($r = 0.799$, $p < .01$), but negatively correlated with the soil pH ($r = -0.792$, $p < .01$).

3.3 | AOA community diversity

The community characteristics were determined by 454 high-throughput pyrosequencing based on the AOA *amoA* gene. The coverage, diversity, and richness indices obtained for the AOA *amoA* gene are shown in Table 2. The coverage exceeded 99% in all of the treatments, and 153 OTUs were detected. The order of abundance in the treatments for the detected OTUs was as follows: MNPK > NPK > *M* > CK, which was consistent with the diversity of the sequences based on the community diversity indices and richness indices. Moreover, the community Chao1 and ACE indices were higher in MNPK (133.33 and 136.0, respectively) than those in the NPK treatment (115.0 and 115.33, respectively). Furthermore, the community Shannon index value was higher in MNPK (2.73) compared with the NPK treatment (2.55). There were clear significant differences ($p < .05$) between the fertilizer regimes (Table 2). In addition, the relationships between the α-diversity and the soil properties are shown in Table 3. The Simpson index values were negatively correlated with the soil NN ($r = -0.846$, $p < .01$) and AP ($r = -0.667$, $p < .05$). The Shannon, ACE, and Chao1 indices were positively correlated with NN.

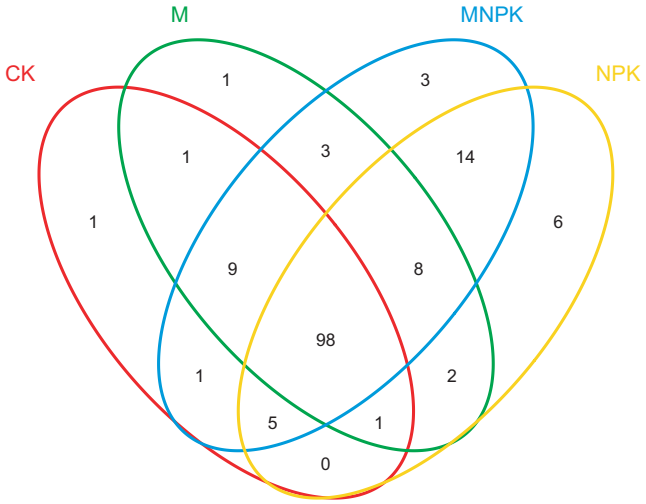

FIGURE 3 Venn diagram of AOA *amoA* genes based on the OTUs in different fertilization treatments. CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure

3.4 | AOA composition and phylogenetic analysis

According to the shared and unique OTUs, the AOA communities in the four fertilizer regimes were compared using the Venn diagrams. In total, 153 AOA-related OTUs were detected in CK, M, MNPK, and NPK (Figure 3). The number of shared OTUs was 98

TABLE 2 Diversity indices, coverage, and OTUs (mean ± standard deviation*) in soils with each treatment

Variable	CK	M	MNPK	NPK
ACE	98.67 ± (4.62)^{a}	111 ± (6.93)^{ab}	136.0 ± (3.61)^{c}	115.33 ± (6.93)^{b}
Chao1	98.33 ± (7.57)^{a}	112.33 ± (9.87)^{a}	133.33 ± (4.16)^{b}	115.0 ± (6.0)^{ab}
Shannon	2.28 ± (0.11)^{a}	2.52 ± (0.01)^{b}	2.73 ± (0.01)^{c}	2.55 ± (0.02)^{b}
Simpson	0.25 ± (0.03)^{b}	0.2 ± (0.0)^{a}	0.16 ± (0.0)^{a}	0.18 ± (0.0)^{a}
Coverage	0.9990	0.9986	0.9987	0.9986
OTU	92 ± (3)^{a}	99 ± (3)^{ab}	115 ± (3)^{c}	106 ± (4)^{b}

Abbreviations: CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure.
*Values followed by different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

TABLE 3 Pearson's correlation coefficients between diversity indices, AOA sequence copy numbers, and soil properties

	AP	AK	NN	AN	pH	OM	TN
ACE	0.580*	−0.02	0.765**	0.319	−0.282	0.117	0.321
Chao1	0.51	0.039	0.730**	0.247	−0.247	0.158	0.31
Shannon	0.650*	0.072	0.868**	0.411	−0.393	0.304	0.496
Simpson	−0.667*	−0.071	−0.846**	−0.384	0.471	−0.301	−0.548
AOA gene copy numbers	0.799**	−0.328	0.499	0.211	−0.792**	−0.345	0.691*

Abbreviations: AP, available phosphorus; AK, available potassium; NN, nitrate nitrogen; AN, ammonium nitrogen; OM, organic matter; TN, total nitrogen.
*Correlation is significant at $p < .05$.
**Correlation is significant at $p < .01$.

(64.0% of the total). The distributions of the main OTUs in each treatment are shown in Figure 4a. The OTUs were selected based on their relative abundances (1%) in the four fertilizer regimes. The total number of selected OTUs was greater than 90% of the total OTUs, and thus, they reflected the overall distribution. Clear shifts in the AOA community compositions under different fertilization treatments were detected by analyzing the distributions of the OTUs as follows. In CK and M, OTU51 and OTU121 were the dominant OTUs. In MNPK, OTU121, OTU51, and OTU7 were relatively abundant OTUs. In NPK, the main OTUs were OTU121, OTU7, OTU79, and OTU11. Thaumarchaeota and Crenarchaeota were the dominant AOA phyla (Figure 4b). The main AOA *amoA* OTUs in the different fertilization treatments were assembled in a phylogenetic tree (Figure 5), and only *Nitrososphaera* AOA were tracked from the black soil. The main AOA *amoA* OTUs were assigned to two clusters, where OTU79 and OTU45 were distributed in cluster 2, and the other 23 OTUs in cluster 1. The two clusters were closely related. The abundances of Thaumarchaeota were far lower in MNPK and NPK compared with those in CK and M. Furthermore, the relationships between the main AOA *amoA* OTUs and the soil properties are shown in Table 4. Eleven OTUs were negatively correlated with the soil pH, and 10 OTUs were positively correlated with the soil pH. In addition, 10 OTUs were negatively correlated with the soil AP and 12 OTUs were positively correlated with the soil AP.

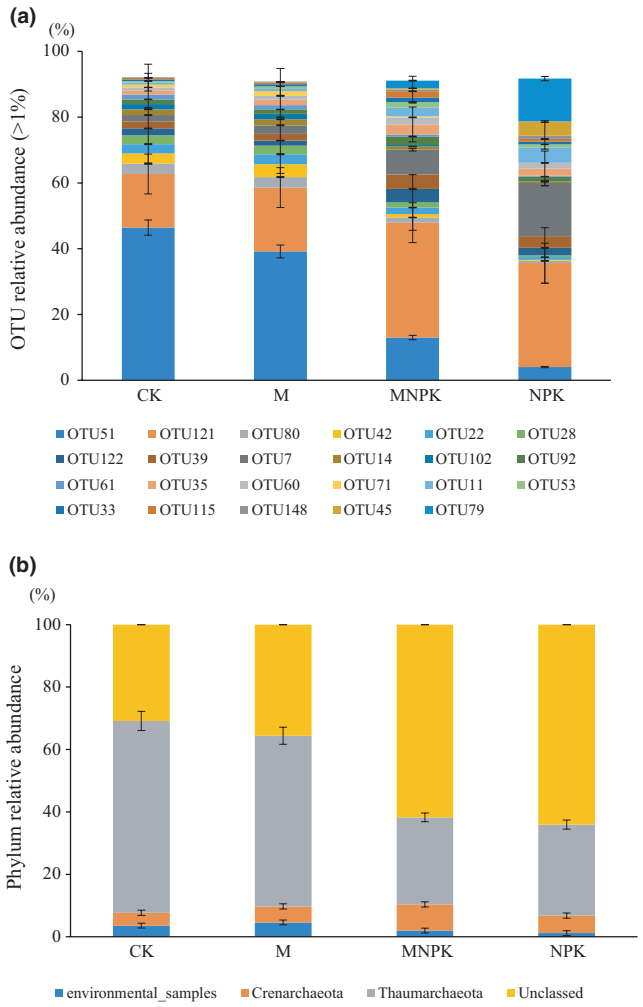

FIGURE 4 Relative abundances based on the AOA community compositions in soils under different fertilization treatments at the OTU level (a) and main phylum level (b). CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure

3.5 | Correlations between selected soil properties and AOA taxa

According to redundancy analysis (Figure 6), all of the selected soil properties explained 97.0% of the variation in the AOA community composition among the samples. Axis 1 and axis 2 obtained by redundancy analysis explained 96.43% of the total variance, where axis 1 explained 86.0% and axis 2 explained 10.43%. The main contributor to the shifts in the AOA community was the soil pH (F = 38.6, p = .002), which explained 79.4% of the variation. The remaining soil properties affected the AOA community composition in the following order: AP > AN > TN > AK > OM. Furthermore, the plots for CK, M, MNPK, and NPK grouped well and they were separated from the NPK plot.

4 | DISCUSSION

4.1 | Alleviation of soil acidification

The 35-year application of inorganic fertilizer increased the acidification of the soil, but organic manure effectively alleviated the soil acidification, probably because it contained organic acids, carbonates, and bicarbonates (Ding et al., 2017). Furthermore, there were large amounts of carboxyl and phenolic hydroxyl groups in the organic acids, which may relieve the soil acidity and enhance the soil pH value (Ding et al., 2016).

4.2 | Changes in the abundance of the AOA *amoA* gene

Long-term fertilization stimulated the growth of AOA in the black soil. Compared with no fertilization, the AOA *amoA* gene number was increased in the fertilizer treatments because of the stimulating effects of the inorganic fertilizer and the inorganic plus organic fertilizers, but particularly the former, possibly because there was a negative correlation between the abundance of AOA and the soil pH. The long-term use of inorganic fertilizer led to a decrease in the soil pH, and thus, the abundance of AOA was highest with the inorganic fertilizer. The AOA *amoA* gene copy number was not affected by fertilization in some previous studies. Ouyang et al. (2016) reported that AOA was affected less by N fertilizer in a calcareous Millville silt loam, and Tao, Wakelin, Liang, and Chu (2017) found no significant effects of a mineral fertilizer and mineral fertilizer plus cattle manure on the AOA *amoA* copy numbers in a cultivated gray desert soil. However, archaeal *amoA* copies in root layer soil with high N were significantly

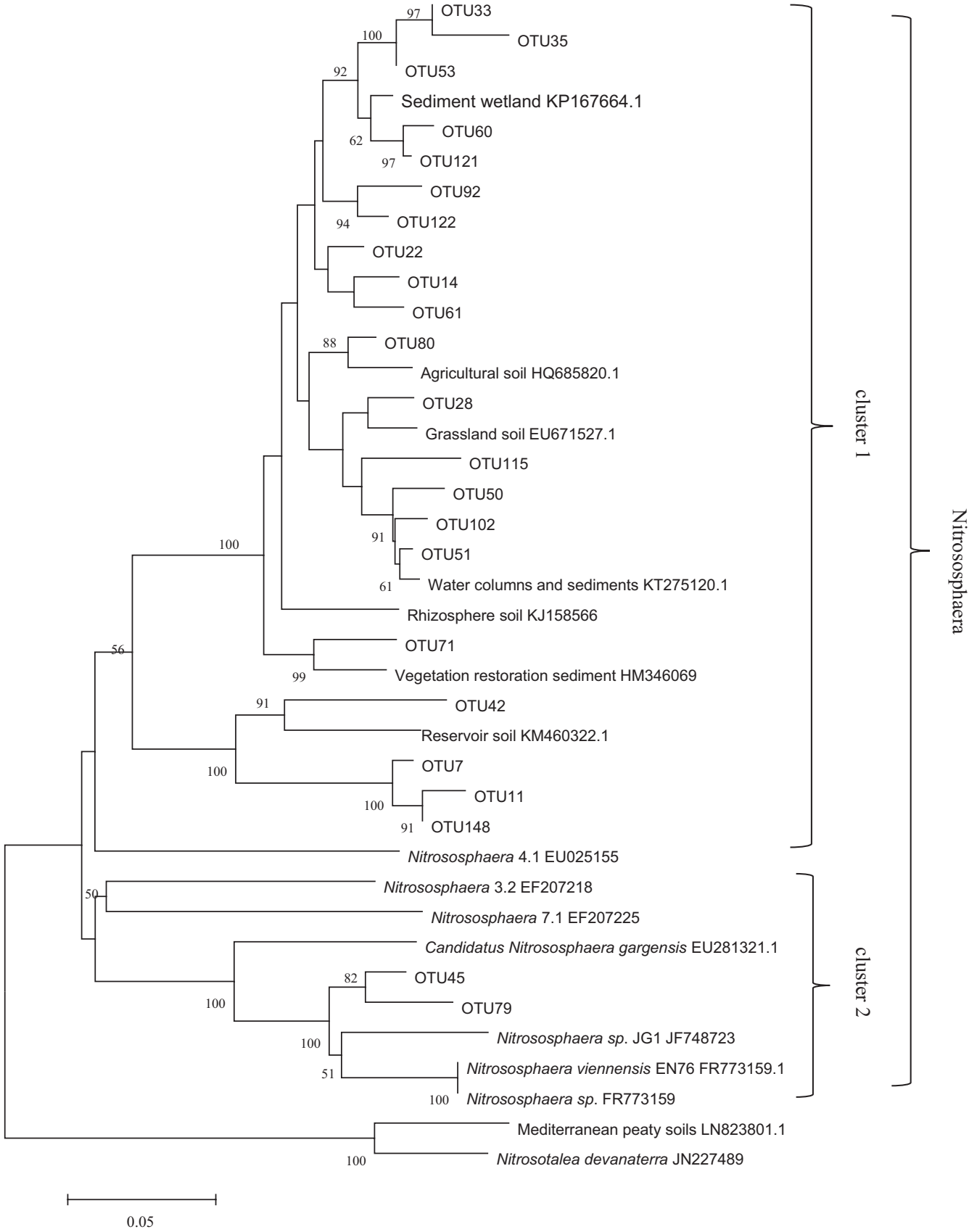

FIGURE 5 Neighbor-joining phylogenetic tree obtained based on AOA *amoA* gene fragment sequences from black soil. OTUs were selected based on their relative abundances > 1%

TABLE 4 Pearson's correlation coefficients between soil properties and main AOA *amoA* OTUs

	pH	OM	TN	NN	AN	AP	AK
OTU7	−0.944**	−0.341	0.842**	0.393	0.029	0.797**	−0.117
OTU11	−0.975**	−0.355	0.892**	0.449	0.057	0.844**	−0.128
OTU14	0.973**	0.38	−0.871**	−0.473	−0.038	−0.841**	0.22
OTU18	0.920**	0.214	−0.829**	−0.584*	−0.105	−0.900**	0.068
OTU22	0.953**	0.446	−0.827**	−0.429	−0.118	−0.841**	0.155
OTU28	0.964**	0.405	−0.850**	−0.462	−0.034	−0.845**	0.166
OTU35	−0.816**	−0.068	0.755**	0.770**	0.264	0.951**	−0.044
OTU39	−0.897**	−0.259	0.792**	0.733**	0.282	0.961**	−0.173
OTU42	0.965**	0.5	−0.776**	−0.541	−0.149	−0.915**	0.314
OTU45	−0.864**	−0.366	0.790**	0.183	−0.081	0.640*	−0.136
OTU51	0.947**	0.209	−0.875**	−0.630*	−0.189	−0.946**	0.061
OTU53	−0.754**	−0.011	0.697*	0.790**	0.291	0.894**	0.011
OTU60	−0.861**	−0.223	0.848**	0.597*	0.188	0.919**	−0.111
OTU61	0.960**	0.284	−0.886**	−0.588*	−0.107	−0.935**	0.052
OTU68	0.424	0.624*	−0.3	0.468	0.454	−0.076	0.337
OTU71	0.918**	0.676*	−0.693*	−0.38	−0.012	−0.833**	0.354
OTU79	−0.923**	−0.368	0.846**	0.288	−0.02	0.747**	−0.132
OTU80	0.985**	0.352	−0.884**	−0.535	−0.095	−0.910**	0.167
OTU92	−0.553	−0.2	0.407	0.657*	0.386	0.793**	−0.097
OTU102	0.972**	0.355	−0.885**	−0.565	−0.171	−0.920**	0.139
OTU115	−0.767**	−0.186	0.686*	0.787**	0.342	0.911**	−0.053
OTU121	−0.938**	−0.234	0.848**	0.680*	0.205	0.973**	−0.083
OTU122	−0.695*	−0.344	0.537	0.644*	0.398	0.836**	−0.221

Abbreviations: AP, available phosphorus; AK, available potassium; NN, nitrate nitrogen; AN, ammonium nitrogen; OM, organic matter; TN, total nitrogen.

*Correlation is significant at $p < .05$.

**Correlation is significant at $p < .01$.

higher than those in soils with no fertilization and low *N* (Song & Lin, 2014. The application of N fertilizer stimulated the growth of AOA in a yellow clay paddy soil (Yao et al., 2016). Ai et al. (2013) found that the abundance of AOA increased with the OM inputs in a long-term field trial in a calcareous fluvoaquic soil.

A previous study also showed that nitrification activity is positively correlated with the abundance of AOA in some acidic soils (Li et al., 2018). In our study, the abundance of AOA was negatively related to the soil pH, thereby demonstrating that the distribution of AOA was influenced by the soil pH (Liu et al., 2018). The inorganic fertilizer decreased the soil pH and greatly increased the abundance of AOA. The long-term application of inorganic fertilizer, especially N fertilizer, can increase the abundance of AOA (Carey, Dove, Beman, Hart, & Aronson, 2016; Ouyang et al., 2018). However, the abundance of AOA was inhibited by organic fertilizer when we applied organic fertilizer plus inorganic fertilizer to the black soil. Moreover, the abundance of AOA was increased by the TN and AP contents in the long-term fertilizer treatments in black soil, where they had positive relationships. However, Chen et al. (2017) showed that the abundance of AOA in alpine meadows was negatively correlated with the soil TN. This difference may have been related to other factors.

4.3 | Effects on AOA α-diversity and community composition

Gao, Chang, et al. (2018c) showed that the AOA community diversity was reduced by the application of chemical fertilizer to a paddy soil. However, we found that fertilization for 35 years increased the AOA α-diversity index in black soil. The soil AOA α-diversity was greatly enhanced by the application of inorganic and organic fertilizations, but especially the organic plus inorganic fertilizer, and this result was consistent with that obtained by Han et al. (2018). The AOA α-diversity indices were significantly positively correlated with the soil NN content of the black soil.

Li, Han, He, Zhang, and Zhang (2019) found that the AOA community structure was influenced little by the application of chemical fertilizer. By contrast, we showed that the long-term application of fertilizers to a black soil altered the AOA community structure. Clear shifts in the AOA community composition under the different fertilization strategies were detected by analyzing the

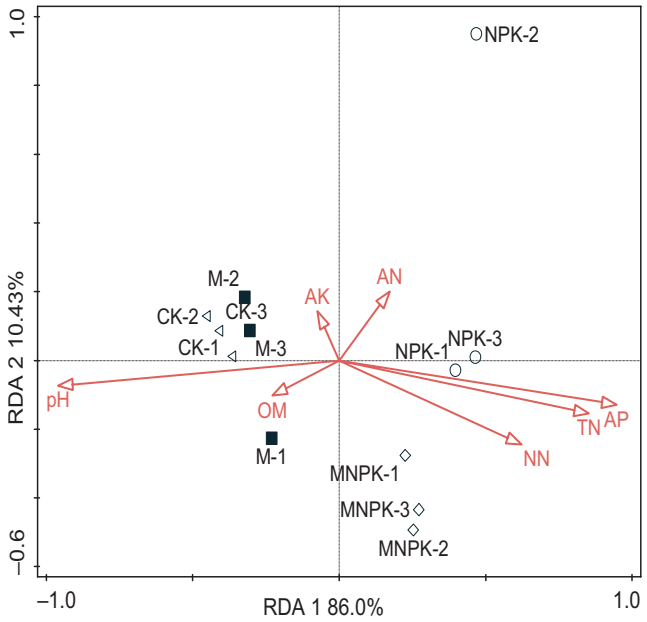

FIGURE 6 Redundancy analysis of the AOA community structure and relationships with soil factors. AP, available phosphorus; AK, available potassium; NN, nitrate nitrogen; AN, ammonium nitrogen; OM, organic matter; TN, total nitrogen; CK, no fertilizer; M, manure only; NPK, inorganic fertilizer; MNPK, inorganic fertilizer with manure

distributions of the AOA OTUs. OTU51 was the dominant OTU in CK, M, and MNPK, but not in NPK. OTU79 and OTU11 were relatively abundant OTUs in NPK. In addition, redundancy analysis clearly demonstrated that the CK, M, and MNPK treatments were distinctly separate from the NPK treatment, thereby suggesting that the change in the AOA community associated with inorganic fertilizer could have been partially attributable to the greater soil mineral N content and the relatively low pH (Ouyang et al., 2017; Tao et al., 2017).

We constructed a phylogenetic tree according to the AOA *amoA* gene fragment sequences (Figure 5), which showed that the main groups comprised *Nitrososphaera* AOA, and they have been detected frequently in many soil environments in previous studies (Ke, Angel, Lu, & Conrad, 2013; Prosser & Nicol, 2012; Shi et al., 2018). A previous study showed that the *Nitrososphaera* cluster was the richest soil AOA lineage in acidic soil and alkaline soil (Oishi et al., 2012). Several studies (Koch et al., 2015; Palatinszky et al., 2015) have shown that the *Nitrososphaera* cluster in farmland soils has a direct linear relationship with nitrification activity, and members of this cluster have a strong genetic capacity to utilize various ammonia sources. In the present study, the diverse fertilization strategies had different effects on the relative abundances of AOA at the phylum level. Thaumarchaeota and Crenarchaeota were the main AOA phyla (Figure 4b). The abundances of Thaumarchaeota were very low in MNPK and NPK compared with those in CK and M. The appearance of Thaumarchaeota as the dominant taxon in the AOA community may have led to a shift in the ammonia-oxidizing capacity (Deignan, Pawlik, & Erwin, 2018). The phylum Crenarchaeota was highly abundant in the black soil. Previous studies reported that members of Crenarchaeota dominate soils with higher pH values in forests (Nicol, Campbell, Chapman, & Prosser, 2007), thereby agreeing with the results obtained in the present study.

5 | CONCLUSION

We studied a black soil in northeast China and investigated the responses of the soil AOA community structure and diversity to long-term fertilizer practices. The changes were primarily due to shifts in the soil pH. Compared with NPK, MNPK alleviated the acidification of the soil. Based on the diversity indices (ACE, Chao1, and Shannon), the soil AOA α-diversity was improved more with MNPK than NPK. According to redundancy analysis and the AOA composition, inorganic plus organic fertilizer may be beneficial for maintaining the stability of the original AOA community composition in the soil. Our results highlight the effects of organic manure as an amendment for use with inorganic fertilizer to facilitate the long-term stable development of Chinese Mollisols.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No. 41771284), the Natural Science Foundation of Heilongjiang Province (No. ZD2018003), and the Postdoctoral Research Funds of Beijing (ZZ2019-48). We wish to thank Baoku Zhou of the Heilongjiang Academy of Agricultural Sciences for assistance with fieldwork.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

AUTHORS CONTRIBUTION

J. D. and M. M. designed and executed the experiments. X. J., Y. L., and J. Z. provided technical and theoretical support. L. S. and L. W. analyzed all of the data and produced the figures. J. D. and M. M. wrote and revised the manuscript. D. W. and J. L. were responsible for the overall supervision of the study. All authors read and approved the final manuscript.

ETHICAL APPROVAL

None required.

DATA AVAILABILITY STATEMENT

The raw pyrosequencing data were deposited at GenBank under the project accession number: PRJNA512072. On request, additional raw data can be obtained from the corresponding author.

ORCID

Jianli Ding https://orcid.org/0000-0002-5024-931X

Mingchao Ma https://orcid.org/0000-0003-2609-321X

Dan Wei https://orcid.org/0000-0002-5261-7825

REFERENCES

Ai, C., Liang, G., Sun, J., Wang, X., He, P., & Zhou, W. (2013). Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biology & Biochemistry*, *57*, 30–42. https://doi.org/10.1016/j.soilbio.2012.08.003

Beeckman, F., Motte, H., & Beeckman, T. (2018). Nitrification in agricultural soils: Impact, actors and mitigation. *Current Opinion Biotechnology.*, *50*, 166–173. https://doi.org/10.1016/j.copbio.2018.01.014

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

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.

Che, R., Wang, F., Wang, W., Zhang, J., Zhao, X., Rui, Y., … Cui, X. (2017). Increase in ammonia-oxidizing microbe abundance during degradation of alpine meadows may lead to greater soil nitrogen loss. *Biogeochemistry*, *136*(3), 341–352. https://doi.org/10.1007/s10533-017-0399-5

Chen, X., Zhu, Y., Xia, Y., Shen, J., & He, J. (2008). Ammonia-oxidizing archaea: Important players in paddy rhizosphere soil. *Environmental Microbiology*, *10*, 1978–1987. https://doi.org/10.1111/j.1462-2920.2008.01613.x

Ciccolini, V., Bonari, E., Ercoli, L., & Pellegrino, E. (2016). Phylogenetic and multivariate analyses to determine the effect of agricultural land-use intensification and soil physico-chemical properties on N-cycling microbial communities in drained Mediterranean peaty soils. *Biology and Fertility of Soils*, *52*(6), 811–824. https://doi.org/10.1007/s00374-016-1121-9

Deignan, L. K., Pawlik, J. R., & Erwin, P. M. (2018). Agelas wasting syndrome alters prokaryotic symbiont communities of the Caribbean brown tube sponge, Agelas tubulata. *Microbial Ecology*, *76*, 459–466. https://doi.org/10.1007/s00248-017-1135-3

Ding, J., Jiang, X., Guan, D., Zhao, B., Ma, M., Zhou, B., … Li, J. (2017). Influence of inorganic fertilizer and organic manure application on fungal communities in a long-term field experiment of Chinese Mollisols. *Applied Soil Ecology*, *111*, 114–122. https://doi.org/10.1016/j.apsoil.2016.12.003

Ding, J., Jiang, X., Ma, M., Zhou, B., Guan, D., Zhao, B., … Li, J. (2016). Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. *Applied Soil Ecology*, *105*, 187–195. https://doi.org/10.1016/j.apsoil.2016.04.010

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., … Caporaso, J. G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences*, *109*, 21390–21395. https://doi.org/10.1073/pnas.1215210110

Gan, X.-H., Zhang, F.-Q., Gu, J.-D., Guo, Y.-D., Li, Z.-Q., Zhang, W.-Q., … Wang, Y.-F. (2016). Differential distribution patterns of ammonia-oxidizing archaea and bacteria in acidic soils of Nanling National Nature Reserve forests in subtropical China. *Antonie Van Leeuwenhoek*, *109*, 237–251. https://doi.org/10.1007/s10482-015-0627-8

Gao, D. W., Liu, F. Q., Li, L., Chen, C. H., & Liang, H. (2018a). Diversity and community structure of ammonia oxidizers in a marsh wetland of the northeast China. *Applied Microbiology and Biotechnology*, *102*, 8561. https://doi.org/10.1007/s00253-018-9225-9

Gao, S., Cao, W., Zou, C., Gao, J., Huang, J., Bai, J., … Dou, F. (2018b). Ammonia-oxidizing archaea are more sensitive than ammonia-oxidizing bacteria to long-term application of green manure in red paddy soil. *Applied Soil Ecology*, *124*, 185–193. https://doi.org/10.1016/j.apsoil.2017.09.041

Gao, S.-J., Chang, D.-N., Zou, C.-Q., Cao, W.-D., Gao, J.-S., Huang, J., … Thorup-Kristensen, K. (2018c). KristianThorup-Kristensenh. Archaea are the predominant and responsive ammonia oxidizing prokaryotes in a red paddy soil receiving green manures. *European Journal of Soil Biology*, *88*, 27–35. https://doi.org/10.1016/j.ejsobi.2018.05.008

Gu, Y., Wang, Y., Lu, S., Xiang, Q., Yu, X., Zhao, K. E., … Zhang, X. (2017). Long-term fertilization structures bacterial and Archaeal communities along soil depth gradient in a paddy soil. *Frontiers in Microbiology*, *8*, 1516. https://doi.org/10.3389/fmicb.2017.01516

Han, S., Li, X., Luo, X. S., Wen, S. L., Chen, W. L., & Huang, Q. Y. (2018). Nitrite-oxidizing bacteria community composition and diversity are influenced by fertilizer regimes, but are independent of the soil aggregate in acidic subtropical red soil. *Frontiers in Microbiology*, *9*, 885. https://doi.org/10.3389/fmicb.2018.00885

He, L. L., Bia, Y. C., Zhao, J., Pittelkow, C. M., Zhao, X., Wang, S. Q., & Xing, G. X. (2018). Population and community structure shifts of ammonia oxidizers after four-year successive biochar application to agricultural acidic and alkaline soils. *Science of the Total Environment*, *619–620*, 1105–1115. https://doi.org/10.1016/j.scitotenv.2017.11.029

Hou, J., Cao, X., Song, C., & Zhou, Y. (2013). Predominance of ammonia-oxidizing archaea and nirK-gene-bearing denitrifiers among ammonia-oxidizing and denitrifying populations in sediments of a large urban eutrophic lake (Lake Donghu). *Canadian Journal of Microbiology*, *59*, 456–464.

Junier, P., Molina, V., Dorador, C., Hadas, O., Kim, O.-S., Junier, T., … Imhoff, J. F. (2010). Phylogenetic and functional marker genes to study ammonia-oxidizing microorganisms (AOM) in the environment. *Applied Microbiology & Biotechnology*, *85*(3), 425–440. https://doi.org/10.1007/s00253-009-2228-9

Ke, X., Angel, R., Lu, Y., & Conrad, R. (2013). Niche differentiation of ammonia oxidizers and nitrite oxidizers in rice paddy soil. *Environmental Microbiology*, *15*, 2275–2292. https://doi.org/10.1111/1462-2920.12098

Koch, H., Lucker, S., Albertsen, M., Kitzinger, K., Herbold, C., Spieck, E., … Daims, H. (2015). Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(36), 11371–11376. https://doi.org/10.1073/pnas.1506533112

Kowalchuk, G. A., & Stephen, J. R. (2001). Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annual Review of Microbiology*, *55*, 485–592. https://doi.org/10.1146/annurev.micro.55.1.485

Lehtovirta-Morley, L. E., Ge, C., Ross, J., Yao, H., Nicol, G. W., & Prosser, J. I. (2014). Characterisation of terrestrial acidophilic archaeal ammonia oxidisers and their inhibition and stimulation by organic compounds. *FEMS Microbiology Ecology*, *89*, 542–552. https://doi.org/10.1111/1574-6941.12353

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., … Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, *442*, 806–809. https://doi.org/10.1038/nature04983

Li, H., Weng, B. S., Huang, F. Y., Su, J. Q., & Yang, X. R. (2015). pH regulates ammonia-oxidizing bacteria and archaea in paddy soils in southern China. *Applied Microbiology & Biotechnology*, *99*(14), 6113–6123. https://doi.org/10.1007/s00253-015-6488-2

Li, P. P., Han, Y. L., He, J. Z., Zhang, S. Q., & Zhang, L. M. (2019). Soil aggregate size and long-term fertilization effects on the function and community of ammonia oxidizers. *Geoderma*, *338*, 107–177. https://doi.org/10.1016/j.geoderma.2018.11.033

Li, Y., Chapman, S. J., Nicol, G. Y., & Yao, H. Y. (2018). Nitrification and nitrifiers in acidic soils. *Soil Biology & Biochemistry*, *116*, 290–301. https://doi.org/10.1016/j.soilbio.2017.10.023

Liu, H., Li, J., Zhao, Y., Xie, K., Tang, X., Wang, S., … 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. https://doi.org/10.1016/j.scitotenv.2018.03.108

Nicol, G. W., Campbell, C. D., Chapman, S. J., & Prosser, J. I. (2007). Afforestation of moorland leads to changes in crenarchaeal community structure. *FEMS Microbiology Ecology*, *60*, 51–59. https://doi.org/10.1111/j.1574-6941.2006.00258.x

Oishi, R., Tada, C., Asano, R., Yamamoto, N., Suyama, Y., & Nakai, Y. (2012). Growth of ammonia-oxidizing archaea and bacteria in cattle manure compost under various temperatures and ammonia concentrations. *Microbial Ecology*, *63*, 787–793. https://doi.org/10.1007/s00248-011-9971-z

Ouyang, Y., Evans, S., Friesen, M. L., & Tiemann, L. K. (2018). Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: A meta-analysis of field studies. *Soil Biology & Biochemistry*, *127*, 71–78.

Ouyang, Y., Norton, J. M., & Stark, J. M. (2017). Ammonium availability and temperature control contributions of ammonia oxidizing bacteria and archaea to nitrification in an agricultural soil. *Soil Biology & Biochemistry*, *113*, 161–172.

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. https://doi.org/10.1016/j.soilbio.2016.01.012

Palatinszky, M., Herbold, C., Jehmlich, N., Pogoda, M., Han, P., von Bergen, M., … Wagner, M. (2015). Cyanate as an energy source for nitrifiers. *Nature*, *524*, 105–108. https://doi.org/10.1038/nature14856

Prosser, J. I., & Nicol, G. W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. *Trends in Microbiology*, *108*, 4170–4175. https://doi.org/10.1016/j.tim.2012.08.001

Ramirez, K. S., Craine, J. M., & Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, *18*, 1918–1927.

Schloss, P. D., Gevers, D., & Westcott, S. L. (2011). Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. *PLoS ONE*, *6*(12), e27310. https://doi.org/10.1371/journal.pone.0027310

Shi, X. Z., Hu, H. W., Wang, J. Q., He, J. Z., Zheng, C. Y., Wan, X. H., & Hang, Z. P. (2018). Niche separation of comammox Nitrospira and canonical ammonia oxidizers in an acidic subtropical forest soil under long-term nitrogen deposition. *Soil Biology & Biochemistry*, *126*, 114–122. https://doi.org/10.1016/j.soilbio.2018.09.004

Song, Y. N., & Lin, Z. M. (2014). Abundance and community composition of ammonia-oxidizers in paddy soil at different nitrogen fertilizer rates. *Journal of Integrative Agriculture*, *13*(4), 870–880. https://doi.org/10.1016/S2095-3119(13)60426-8

Sun, R. B., Guo, X. S., Wang, D. Z., & Chu, H. Y. (2015). 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. https://doi.org/10.1016/j.apsoil.2015.06.010

Tao, R., Wakelin, S., Liang, Y. C., & Chu, G. X. (2017). Response of ammonia-oxidizing archaea and bacteria in calcareous soil to mineral and organic fertilizer application and their relative contribution to nitrification. *Soil Biology & Biochemistry*, *114*, 20–30. https://doi.org/10.1016/j.soilbio.2017.06.027

Wang, B. Z., Zhao, J., Guo, Z. Y., Ma, J., Xu, H., & Jia, Z. J. (2015). Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. *The ISME Journal*, *9*, 1062–1075. https://doi.org/10.1038/ismej.2014.194

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, S., Wang, Y., Feng, X., Zhai, L., & Zhu, G. (2011). Quantitative analyses of ammonia-oxidizing Archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. *Applied Microbiology & Biotechnology*, *90*, 779–787. https://doi.org/10.1007/s00253-011-3090-0

Wei, D., Yang, Q., Zhang, J.-Z., Wang, S., Chen, X.-L., Zhang, X.-L., & Li, W.-Q. (2008). Bacterial community structure and diversity in a black soil as affected by long-term fertilization. *Pedosphere*, *18*(5), 582–592. https://doi.org/10.1016/S1002-0160(08)60052-1.

Xue, C., Zhang, X., Zhu, C., Zhao, J., Zhu, P., & Peng, C., … Shen, Q. R. (2016). Quantitative and compositional responses of ammonia-oxidizing archaea and bacteria to long-term field fertilization. *Scientific Reports*, *6*(1), 28981. https://doi.org/10.1038/srep28981

Yao, H. Y., Huang, S., Qiu, Q. F., Li, Y. Y., Wu, L. H., Mi, W. H., & Dai, F. (2016). Effects of different fertilizers on the abundance and community structure of ammonia oxidizers in a yellow clay soil. *Applied Microbiology and Biotechnology*, *100*, 6815–6826.

Yin, C., Fan, F., Song, A., Cui, P., Li, T., & Liang, Y. (2015). Denitrification potential under different fertilization regimes is closely coupled with changes in the denitrifying community in a black soil. *Applied Microbiology & Biotechnology*, *99*, 5719–5729. https://doi.org/10.1007/s00253-015-6461-0

Zhang, M. M., Wang, B. R., Li, D. C., He, J. Z., & Zhang, L. M. (2015a). Effects of long-term N fertilizers application and liming on nitrification and ammonia oxidizers in acidic soils. *Acta Ecologica Sinica*, *35*(19), 1–11. (in Chinese).

Zhang, Y., Chen, L., Dai, T., Sun, R., & Wen, D. (2015b). Ammonia manipulates the ammonia oxidizing archaea and bacteria in the coastal sediment-water microcosms. *Applied Microbiology & Biotechnology*, *99*(15), 6481–6491. https://doi.org/10.1007/s00253-015-6524-2

Zhou, J., Guan, D., Zhou, B., Zhao, B., Ma, M., Qin, J., … Li, J. (2015). Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. *Soil Biology & Biochemistry*, *90*, 42–51. https://doi.org/10.1016/j.soilbio.2015.07.005

Zhou, J., Jiang, X., Zhou, B., Zhao, B., Ma, M., Guan, D., … Qin, J. (2016). Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biology & Biochemistry*, *95*, 135–143. https://doi.org/10.1016/j.soilbio.2015.12.012

How to cite this article: Ding J, Ma M, Jiang X, et al. Effects of applying inorganic fertilizer and organic manure for 35 years on the structure and diversity of ammonia-oxidizing archaea communities in a Chinese Mollisols field. *MicrobiologyOpen*. 2020;9:e942. https://doi.org/10.1002/mbo3.942