



OPEN Effects of long-term fertilizer practices on rhizosphere soil ammonia oxidizer community structure under the double-cropping rice field


Haiming Tang , Li Wen, Lihong Shi, Kaikai Cheng, Geng Sun, Mei Sun & Weiyan Li

Ammonia oxidation plays a vital role in regulating soil nitrogen (N) cycle in agricultural soil, which is significantly influenced by different fertilizer regimes. However, there is still need to further investigate the effects of different fertilizer managements on rhizosphere soil ammonia-oxidizing archaea (AOA) and bacteria (AOB) community in the double-cropping rice field. Therefore, the effects of different long-term (37 years) fertilizer managements on rhizosphere soil potential nitrification activity (PNA), AOA and AOB community structure, and its relationship under the double-cropping rice system in southern of China were studied in the present paper. The field experiment included without fertilizer input as a control (CK), inorganic fertilizer (MF), rice straw and inorganic fertilizer (RF), 30% organic manure and 70% inorganic fertilizer (OM). This result indicated that rhizosphere soil organic carbon (SOC), total N and ammonium N ($\text{NH}_4^+\text{-N}$) contents in paddy field with RF and OM treatments were increased. Rhizosphere soil PNA, potential nitrification rate (PNR) and abundance of AOB in paddy field with MF treatment were increased, and abundance of AOA in paddy field with RF and OM treatments were increased, respectively. The result also showed that rhizosphere soil diversity index of AOA and AOB with RF and OM treatments were enhanced, compared with CK treatment. Rhizosphere soil AOB and AOA community composition was dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria* with all fertilizer treatments. There had significantly positively correlation between the abundance of AOA and SOC, total N, and $\text{NH}_4^+\text{-N}$ contents. However, there had significantly negatively correlation between soil pH and abundance of AOA, soil PNA, PNR. As a result, long-term application of rice straw and organic manure was benefit for increasing community structure of rhizosphere soil ammonia oxidizer under the double-cropping rice system in southern of China.

Keywords Rice, Rhizosphere soil, Fertilizer regime, Ammonia-oxidizing archaea, Ammonia-oxidizing bacteria

It is widely believed that microbial ammonia oxidation plays an important role in regulating nitrogen (N) cycle of agricultural soil, which were regulated nitrous oxide (N_2O) and N_2 gas emission in the global¹. Therefore, the biogeochemical cycling of carbon (C), N, phosphorus (P), and sulfur (S) is primarily influenced by soil microbes^{2,3}. The rhizosphere, which is the volume of soil adjacent to and affected by plant root⁴, plays a vital role in regulating plant growth and soil fertility⁵. Meanwhile, it was a benefit practices for increasing soil fertility and crop yield with fertilizer input, which strongly influence on soil biochemical and biological properties. Therefore, the effects of different fertilizer managements on activity and community structure of soil ammonia-oxidizing archaea (AOA) and bacteria (AOB) were focus on by more and more researcher in recent years^{5,6}.

In the previous studies, these results indicated that composition and community structure of soil AOA and AOB were mainly affecting by different field managements, such as cropping system, tillage, fertilizer regime, irrigation and so on. Fertilizer regime was playing a vital role in regulating AOA and AOB community in paddy field. Previous results proved that soil ammonia oxidizer population, potential nitrification activity (PNA) and potential nitrification rate (PNR) were changed under applied with inorganic fertilizer and organic manure conditions⁷⁻⁹. Some results showed that AOB population and nitrification activity were enhanced with inorganic

Hunan Soil and Fertilizer Institute, Changsha 410125, China.  email: tanghaiming66@163.com

fertilizer practices^{8,10}, whereas other result indicated that nitrification activity and abundances of AOA and AOB with organic manure management were higher than that of inorganic fertilizer management¹¹. Rhizosphere is a zone of intense microbial activity that could profound influences on soil nutrient cycling and provides soil nutrient for plant growth¹². Some results indicated that nitrifying population^{13,14} and PNA^{7,9} in rhizosphere soil with inorganic fertilizer, crop residue and organic manure input practices were increased. However, most researches were conducted on bulk soil or based on short-term experiment, there is still need to further investigate the change of rhizosphere soil ammonia oxidation in paddy field under long-term fertilization condition.

Rice (*Oryza sativa* L.) is the main agricultural land use in the tropical and subtropical monsoon regions¹⁵. It was a vital factor in maintaining or increasing soil quality and fertility of paddy field with different fertilizer regimes, including manure, crop residue and chemical fertilizer. In the previous studies, our result indicated that soil chemical properties (e.g., soil pH, total N, SOC contents) and rice yield were significantly changed under different long-term fertilizer conditions^{16,17}. Furthermore, rhizosphere soil total N content, organic N fraction and N mineralization in the double-cropping rice field were obviously increased with manure and crop residue input managements, which in turn influence the function and community structure of AOA and AOB. However, it is still not known how about rhizosphere soil PNA and PNR, diversity and community of AOA and AOB responds to different fertilizer managements under the double-cropping rice system in southern of China. Therefore, the objectives of this study were: (1) to explore the effects of different fertilizer treatments on rhizosphere soil PNA and PNR in the double-cropping rice field, (2) to investigate the rhizosphere soil diversity and community of AOA and AOB in paddy field; (3) to analysis the relationship between soil chemical properties and community of ammonia oxidation under the double-cropping rice system in southern of China.

Materials and methods

Sites and cropping system

The field experiment was beginning from 1986. It was located in Ningxiang City (28°07' N, 112°18' E) of Hunan Province, China. Under a continental monsoon climate, the annual mean precipitation and potential evapotranspiration is 1553 mm and 1354 mm, respectively. The monthly mean temperature is 17.2 °C. Soil chemical characteristics at 0–20 cm layer beginning of this field experiment, cropping system were described as by Tang et al.¹⁶.

Experimental design

The field experiment included four treatments: without fertilizer input as a control (CK), inorganic fertilizer (MF), rice straw and inorganic fertilizer (RF), 30% organic manure and 70% inorganic fertilizer (OM). The experiment design ensured all fertilizer treatments received the same level of nitrogen (N), phosphorus pentoxide (P₂O₅), potassium oxide (K₂O) (the amount of N, P₂O₅, K₂O in chemical fertilizer plus that from rice residue or manure) during the early rice and late rice whole growing season, respectively. The kinds of N, P₂O₅, K₂O chemical fertilizer included urea, ordinary superphosphate and potassium chloride, respectively. Other more detail information about the fertilizer managements and field practices were described as by Tang et al.¹⁶.

Soil sample collected

Soil samples were collected at tillering stage of late rice in 25 August 2022. Rhizosphere soil was operationally defined as soil adhering to rice root after gentle shaking. In order to obtain enough rhizosphere soil for analysis at laboratory, twenty rice plants were randomly selected from each plot, and these rhizosphere soils were pooled to form one composite sample. Thus, three composite soil samples of each fertilizer treatment were collected at sampling time, and the total number of 12 composite soil samples were collected at tillering stage of late rice. The fresh soil samples were placed immediately in ice box and transported to the laboratory. Plant root and small stone were removed, one part of soil samples were through a 2-mm sieve then stored at room temperature until chemical analysis, and another part of soil samples were stored at –20 °C until molecular analysis.

Soil laboratory analysis

Soil chemical properties analysis

Soil pH was measured with a compound electrode (PE-10, Sartorius, Germany) using a soil to water ratio of 1:2.5. Soil organic carbon (SOC) content was determined by dichromate oxidation, while total N content was measured using a vario MACRO cube element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Soil ammonium N (NH₄⁺-N) and nitrate N (NO₃⁻-N) contents were determined by extracting the soil with 0.01 M CaCl₂ solution (1:10, w/v) for 30 min and then determining the NH₄⁺ and NO₃⁻ concentrations using a flow injection autoanalyzer (FLA star 5000 Analyzer, Foss, Denmark).

Soil potential nitrification activity (PNA) and potential nitrification rate (PNR)

Soil PNA (μg NO₃⁻-N g⁻¹ h⁻¹) was determined with the shaken slurry method described as by Hart et al.¹⁸, which evaluate the maximum nitrate production rate of a soil sample. Soil PNR was investigated with griess reagent colorimetry method described as by Kurola et al.¹⁹.

DNA extraction and PCR amplification

Soil total DNA was extracted using a Fast DNA SPIN Kit for soil (MP Biomedicals, Illkirch, France) according to the manufacturer's instruction. DNA was finally eluted with 100 μL of the DNA elution solution included in the kit. Soil DNA extraction was characterized by using electrophoresis on 0.7% (wt/vol) agarose gels.

Polymerase chain reaction (PCR) reaction mixture included 12.5 μL 2 × EasyTaq PCR SuperMix (TransGen Biotech, Beijing, China), 0.5 μM of each primer, 1 μL of 10-fold diluted DNA template, and diluted to a final volume of 25 μL. PCR reaction was performed using the AOB-specific primers CTO654r and CTO189f²⁰, AOA

amoA genes were amplified using primers CrenamoA616r and CrenamoA23f²¹. The size and quality of soil PCR products were verified with 1.5% (wt/vol) agarose.

Quantitative real-time PCR

In order to investigate of soil sample ammonia-oxidizing archaea community, bacteria community was analysis using the primers CrenamoA616r and CrenamoA23f, CTO654r and CTO189f, respectively²². Quantitative real-time PCR for AOB and AOA was conduct in triplicate using an ABI 7500 Real-Time PCR System (Applied Biosystems) under the following thermocycling conditions: 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 34 s at 60 °C. The amplification specificity of AOA was confirmed by generating a melting curve. Standard curves ranging from 1×10^2 to 1×10^7 copies were prepared by 10-fold serial dilution of known copy numbers of plasmid DNA possessing the genes of interest. In order to calculate abundances of AOA and AOB genes, it was assumed that AOA carry one copy of the *amoA* gene per cell²³, and AOB contain one copy of 16 S rRNA per cell²⁴. The sequences of AOA and AOB were then compared with those available in the National Center for Biotechnology Information (NCBI) GenBank database using the BLAST algorithm. Sequence analysis and operational taxonomic units (OTUs) identification were according to the method described as by Fagen et al.²⁵. Matches were filtered at 80% length fraction and OTUs were classified at 97% identity level. Richness diversity, Shannon diversity and Chao 1 diversity index were used to calculate the diversity of ammonia-oxidizing archaea and bacteria community using the Mothur software²⁶.

Statistical analysis

These results of every investigate items were presented as average value and standard error. The significance of differences between different fertilizer treatments was conducted using analysis of variance (ANOVA). The relationship between soil chemical properties, soil potential nitrification activity, soil potential nitrification rate and abundances of AOA and AOB were analyzed using the Pearson correlation test. All statistical analyses were calculated by using SAS 9.3 software package²⁷ and a probability value of 0.05 was considered to indicate statistical significance.

Results

Soil chemical properties

This result indicated that rhizosphere soil chemical characteristics in paddy field were obvious changed under different long-term fertilizer conditions (Table 1). The result showed that soil pH value in paddy field with OM and RF treatments was significantly ($p < 0.05$) higher than that of CK treatment. SOC, soil NO_3^- -N and NH_4^+ -N contents with MF, RF and OM treatments were significantly ($p < 0.05$) higher than that of CK treatment. The result showed that soil total N content in paddy field with OM and RF treatments was significantly ($p < 0.05$) higher than that of MF and CK treatments (Table 1).

Soil potential nitrification rate and potential nitrification activity

The effects of different fertilizer treatments on rhizosphere soil potential nitrification activity (PNA) and potential nitrification rate (PNR) in paddy field were showed as in Fig. 1. This result showed that rhizosphere soil PNA with all fertilizer treatments ranged from 1.96 to 3.11 $\mu\text{g NO}_3^-$ -N $\text{g}^{-1} \text{h}^{-1}$ (Fig. 1a). Soil PNA with MF treatment was significantly ($p < 0.05$) higher than that of RF, OM and CK treatments. Soil PNA with RF and OM treatments was significantly ($p < 0.05$) higher than that of CK treatment.

This result showed that rhizosphere soil PNR with all fertilizer treatments ranged from 0.34 to 0.51 $\mu\text{g g}^{-1} \text{h}^{-1}$ (Fig. 1b). Soil PNR with MF treatment was significantly ($p < 0.05$) higher than that of RF, OM and CK treatments. Soil PNR with RF and OM treatments was significantly ($p < 0.05$) higher than that of CK treatment, but there were no significantly difference ($p > 0.05$) in soil PNR between RF and OM treatments.

Abundances of AOA and AOB genes

The effects of different fertilizer treatments on abundances of rhizosphere soil AOA and AOB genes in paddy field were showed as in Fig. 2. This result showed that abundance of AOA with all fertilizer treatments ranged from 81.79 to 143.75 copies $\times 10^7$ cells g^{-1} soil (Fig. 2a). Abundance of AOA with RF and OM treatments was significantly ($p < 0.05$) higher than that of MF and CK treatments. Compared with CK treatment, abundance of AOA with MF, RF and OM treatments increased by 1.25, 1.62 and 1.76 times, respectively.

Treatments	pH	SOC (g kg ⁻¹)	Total N (g kg ⁻¹)	NO ₃ ⁻ -N (g kg ⁻¹)	NH ₄ ⁺ -N (g kg ⁻¹)
MF	6.32 ± 0.17ab	21.46 ± 0.68c	2.07 ± 0.06c	0.17 ± 0.01c	0.16 ± 0.01c
RF	6.70 ± 0.17a	24.82 ± 0.72b	2.45 ± 0.07b	0.21 ± 0.01b	0.20 ± 0.01b
OM	6.81 ± 0.18a	29.62 ± 0.71a	3.08 ± 0.08a	0.27 ± 0.01a	0.23 ± 0.01a
CK	6.21 ± 0.16b	19.68 ± 0.56d	1.91 ± 0.06c	0.13 ± 0.01d	0.12 ± 0.01d

Table 1. Effects of different long-term fertilizer treatments on soil chemical characteristics in the double-cropping rice field. Values were presented as mean ± standard error. Different lowercase letters in the same column were indicated significantly at difference in 0.05 levels. The same as below. MF, inorganic fertilizer; RF, rice straw and inorganic fertilizer; OM, 30% organic manure and 70% inorganic fertilizer; CK, without fertilizer input as a control.

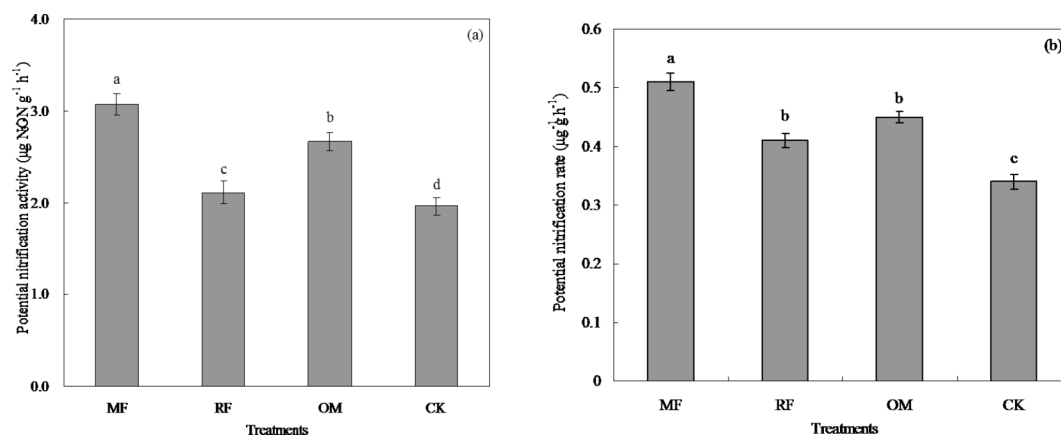


Fig. 1. Potential nitrification activity (a) and potential nitrification rate (b) in rhizosphere soil under different fertilizer treatments. MF: inorganic fertilizer; RF: rice straw and inorganic fertilizer; OM: 30% organic manure and 70% inorganic fertilizer; CK: without fertilizer input as a control. Vertical bars represent the standard error. Different lowercase letters were indicated significantly at difference among fertilizer treatments in 0.05 levels. The same as below.

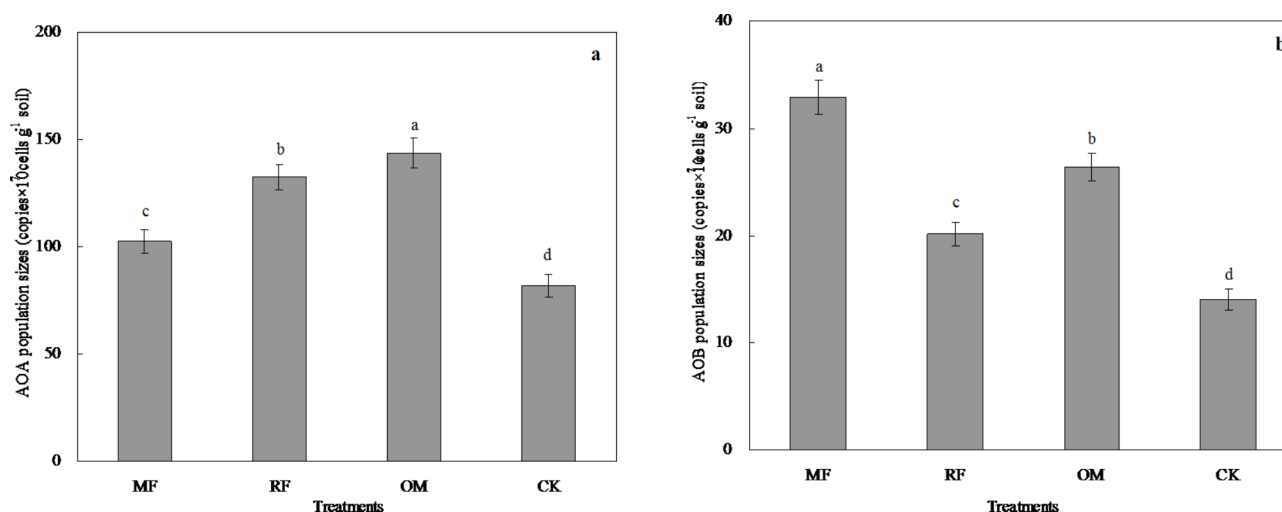


Fig. 2. Effects of different long-term fertilizer treatments on abundances of AOA (a) and AOB (b) in rhizosphere soil under the double-cropping rice system.

This result showed that abundance of AOB with all fertilizer treatments ranged from 14.05 to 32.87 copies $\times 10^7$ cells g^{-1} soil (Fig. 2b). Abundance of AOB with MF treatment was significantly ($p < 0.05$) higher than that of RF, OM and CK treatments. Compared with CK treatment, abundance of AOB with MF, RF and OM treatments increased by 2.34, 1.44 and 1.88 times, respectively.

Diversity index of AOA and AOB

The effects of different fertilizer treatments on rhizosphere soil diversity index of AOA and AOB were showed as in Table 2. Soil Richness diversity index of AOA with RF and OM treatments was significantly ($p < 0.05$) higher than that of CK treatment, soil Shannon and Chao 1 diversity index of AOA with OM treatment was significantly ($p < 0.05$) higher than that of CK treatment, respectively.

This result indicated that rhizosphere soil Richness and Shannon diversity index of AOB with OM treatment was significantly ($p < 0.05$) higher than that of MF and CK treatments. Soil Chao 1 diversity index of AOB with RF and OM treatments was significantly ($p < 0.05$) higher than that of MF and CK treatments, but there had no significantly ($p > 0.05$) difference in soil Chao 1 diversity index of AOB between OM and RF treatments (Table 2).

Bacteria and archaea	Diversity parameters	Treatments			
		MF	RF	OM	CK
AOA	Richness indices	12.52 ± 0.37b	12.96 ± 0.38a	13.25 ± 0.38a	12.27 ± 0.35b
	Shannon indices	2.59 ± 0.07ab	2.63 ± 0.07ab	2.79 ± 0.08a	2.48 ± 0.06b
	Chao 1 indices	5.26 ± 0.16b	5.62 ± 0.16b	6.29 ± 0.18a	4.47 ± 0.13c
AOB	Richness indices	9.37 ± 0.27b	9.82 ± 0.28b	10.43 ± 0.31a	8.98 ± 0.26c
	Shannon indices	2.68 ± 0.07b	2.95 ± 0.08ab	3.12 ± 0.09a	2.74 ± 0.07b
	Chao 1 indices	3.37 ± 0.10b	3.72 ± 0.11a	3.89 ± 0.11a	2.94 ± 0.08c

Table 2. Soil diversity index of AOA and AOB with different long-term fertilizer treatments. AOA, soil ammonia-oxidizing archaea; AOB, soil ammonia-oxidizing bacterial. Different lowercase letters in the same line indicated significantly difference at $p < 0.05$. The same as below.

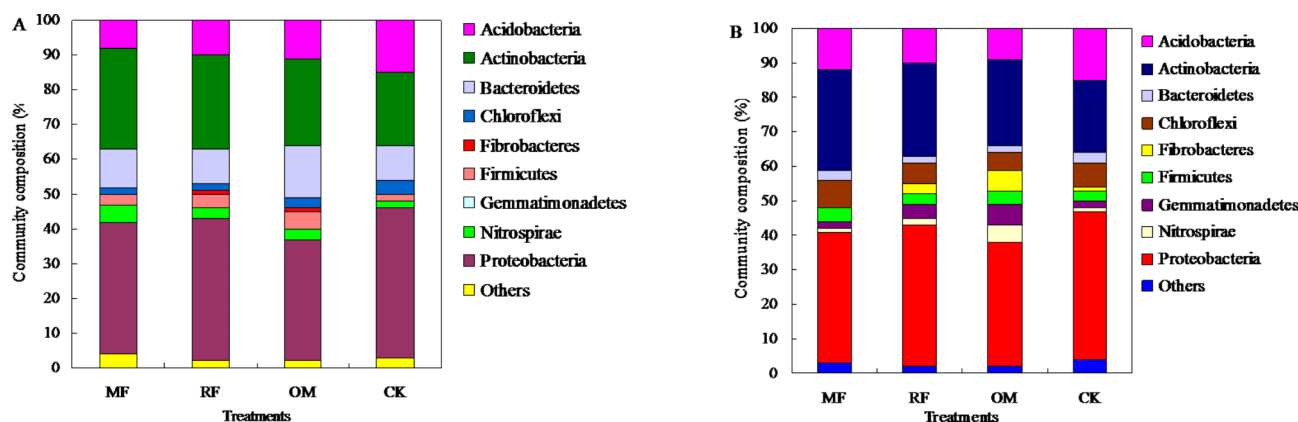


Fig. 3. Community compositions of AOA (a) and AOB (b) in rhizosphere soil under different long-term fertilizer treatments.

Items	pH	SOC	TN	NH ₄ -N	NO ₃ -N	AOA	AOB
AOB	- 0.425	0.301	0.327	0.287	- 0.195	0.285	—
AOA	- 0.702**	0.706**	0.715**	0.734**	0.406	—	0.284
PNA	- 0.611*	0.352	0.438	0.243	- 0.275	0.347	0.872**
PNR	- 0.605*	0.326	0.375	0.136	- 0.231	0.284	0.836**

Table 3. Correlation between soil chemical properties, potential nitrification activity (PNA), potential nitrification rate (PNR) and abundances of AOA and AOB. TN: soil total nitrogen content; SOC: soil organic carbon. AOB: soil ammonia-oxidizing bacterial; AOA: soil ammonia-oxidizing archaea; PNA: soil potential nitrification activity; PNR: potential nitrification rate. *, ** were significantly at the 0.05 and 0.01 level, respectively.

Community structure of AOA and AOB

This result indicated that rhizosphere soil ammonia-oxidizing archaea community composition was *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Nitrospirae*. The *Proteobacteria* and *Actinobacteria* occupy absolute advantage in rhizosphere soil with all fertilizer treatments (Fig. 3A).

The main community composition rhizosphere soil ammonia-oxidizing bacterial community composition was *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Firmicutes* with all fertilizer treatments. The *Proteobacteria*, *Actinobacteria* and *Acidobacteria* occupy absolute advantage in rhizosphere soil with MF, RF and OM treatments. This result showed that community composition of soil *Actinobacteria* with MF, RF, OM treatments were higher than that of CK treatment (Fig. 3B).

Correlations of soil properties with community structures of AOA and AOB

There had significantly positively ($p < 0.01$) correlation between abundance of AOA and SOC, total N, NH₄-N contents (Table 3). Meanwhile, there had significantly negatively ($p < 0.05$) correlation between abundance of AOB and soil potential nitrification activity, potential nitrification rate. However, there had significantly negatively ($p < 0.05$) correlation between soil pH and abundance of AOA, soil potential nitrification activity,

potential nitrification rate. There had not correlation between abundance of AOA and soil PNA, PNR. There had significantly positively ($p < 0.01$) correlation between abundance of AOB and soil PNA, PNR.

Discussions

In the previous studies, these results indicated that rhizosphere soil microbial activity was affected during N transformation processes in paddy field^{7,14}. In this study, our result showed that rhizosphere soil potential nitrification activity (PNA) and potential nitrification rate (PNR) in paddy field were obvious changed in different fertilizer treatments (Fig. 1), implying a profound fertilization effect on rhizosphere soil ammonia oxidation. Meanwhile, soil PNA and PNR in paddy field with MF treatment were significantly increased, compared with RF, OM and CK treatments. These results were consistent with previous results of paddy field in China²⁸. The reason maybe attributed to that higher resource of rhizodeposition after mineralization may provide more substrate for ammonia oxidizers and stimulate microbial growth^{28,29}. On the other hand, soil nitrification and ammonia oxidizers were frequently influenced by the rice varieties and climate factors, which were regarded as important environmental factors responsible for microbial nitrification³⁰. Furthermore, there had significantly positively correlation between abundance of AOB and soil PNA, PNR. Meanwhile, there had significantly negatively correlation between soil pH and soil PNA, PNR. These results suggested that soil pH and abundance of AOB were important drivers in regulating soil PNA and PNR under the double-cropping rice system in southern of China.

Our result showed that abundance of AOA was higher than that of AOB in rhizosphere soil, which were agree with previous reported by Chen et al.²⁸. However, the community structure and abundance of AOB were significantly correlated with soil PNA and PNR, while those of AOA were not (Table 2; Fig. 2), suggesting that soil nitrification were primarily driven by AOB in the double-cropping rice paddy field. The predominance of bacterial nitrification were consistent with previous result³¹, but inconsistent with report for acidic soil in paddy field³², suggesting that soil texture, soil characteristics and soil pH were obvious difference from the present experimental field. Further results suggested that archaea can be well adapted to extreme conditions, such as soil type, soil pH, low ammonia availability, and climate conditions^{19,33}, while the opposite response were detected for bacteria¹¹. These soil texture and soil characteristics were main explain why AOB but not AOA play a vital role in regulating soil nitrification in this field experiment (pH from 6.21 to 6.81) (Table 1) under long-term fertilizer condition.

In the present study, our result indicated that abundance of AOA in rhizosphere soil were higher than that of AOB in paddy field, which were consistent with the previous result¹¹. The reason maybe attributed to that soil pH of paddy field was greatly impacted by fertilizer managements (Table 1), possibly owing to strong carbonate buffering². It had proved that soil pH had a considerable effect on activity of AOB and other microbial processes that they mediate⁶. In this study, the result indicated that abundance of AOB with MF treatment were significantly increased compared with RF and OM treatments, which were agree with previous study²¹, who found that N-induced stimulation of AOB under long-term inorganic fertilizer input condition. The reason was attributed to that dependence of monooxygenase on NH_3 , which would be ionized exponentially to NH_4^+ with decreasing pH in paddy field under long-term inorganic fertilizer input condition, but soil acid buffering capacity and soil pH were increased under long-term rice straw and organic manure input conditions¹¹. Meanwhile, this result indicated that abundance of AOA with RF and OM treatments were higher than that of MF and CK treatments, which suggested there had higher soil organic carbon, total N contents and soil pH with organic manure and rice straw input practices, may stimulate growth of the AOA community. Compared with RF and OM treatments, abundance of AOA with MF treatment were decreased, which suggested there had lower soil organic carbon, total N contents and soil pH with long-term inorganic fertilizer input practice, may restricted growth of the AOA community²⁸. Positively correlation between abundance of AOA and SOC, total N contents were also observed in the present study (Table 3), which suggested that AOA and AOB have alternative growth strategies for mixotrophic or heterotrophic growth¹⁰.

Previous studies results indicated that soil functional community involved in ammonia oxidizer respond different to fertilizer practices change^{7,9}. In the present study, our result showed that diversity of ammonia oxidizer community (Richness index, Shannon index and Chao 1 diversity index) was significantly changed among the different fertilizer regimes. Our result indicated that long-term application of rice straw and organic manure was result in increased diversity of AOA and AOB community (Table 2). The reason maybe attributed to that soil texture and nutrient content were increased in the double-cropping rice paddy field under long-term application of rice straw and organic manure conditions^{16,17}, which decreased in the competitive niche exclusion and selection mechanism between soil ammonia-oxidizing bacterial and archaea populations. Therefore, these consequences could lead to changes in ammonia-oxidizing bacterial and archaea diversity, and enhance in the Richness, Shannon and Chao 1 indices¹⁹. Meanwhile, this result indicated that diversity of ammonia oxidizer community with MF treatment were higher than that of CK treatment. The reason maybe attributed to that application of inorganic fertilizer practice, which caused promoted soil chemical properties and rhizosphere soil environment than the without fertilizer input practice did, could promoted in multiplying soil ammonia-oxidizing bacterial and archaea^{4,11}. Therefore, this study supports the view that application of rice residue and manure treatments strongly influence on soil texture and altered soil chemical properties, causing changes in diversity and abundances of soil ammonia-oxidizing bacterial and archaea.

In the previous studies, these results indicated that soil AOB and AOA community composition was main influenced by different fertilizer treatments^{20,30}. In this study, the result revealed that rhizosphere soil AOB and AOA community composition were dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria* with fertilizer treatments (MF, RF and OM treatments), this findings were agree with the previous found^{7,9}. The reason maybe attributed to that *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were considered as the eutrophic bacteria in soil, which were using total N, NH_4^+ -N and NO_3^- -N contents as main N source and growth

rapidly under nutrient rich conditions. On the other hand, rhizosphere soil physical and chemical properties were important factors in influencing soil microbial abundance and community under application of fertilizer practice condition. In this study, the result revealed that ammonia oxidizer community were correlated with soil chemical properties (Table 3), implying that soil ammonia-oxidizing bacterial and archaea community in the double-cropping rice field were changed by combined effect of soil pH, SOC, total N, NH_4^+ -N, NO_3^- -N contents, soil PNA and PNR. These results were supported by the observation that AOA and AOB abundances were negatively correlated with soil pH^{33,34}, but positively correlated with SOC, total N and NH_4^+ -N contents (Table 3). However, the controlling factor in influencing on ecological function of rhizosphere soil AOB and AOA in paddy field is still need to further study.

Conclusions

Our result indicated that activity and community structure of rhizosphere soil AOB and AOA in paddy field were obvious changed under different long-term of fertilization conditions. Rhizosphere soil potential nitrification activity and potential nitrification rate in paddy field were stimulated by application of inorganic fertilizer practice. Abundances of rhizosphere soil AOA and AOB genes in paddy field with MF, RF and OM treatments were significantly increased, compared with CK treatment. Long-term (37 years) application of chemical fertilizer increase soil PNA and abundance of AOB, whereas application of rice residue and organic manure significantly enhance abundance of AOA in the double-cropping rice field. Meanwhile, this result indicated that soil Richness, Shannon and Chao 1 diversity indices of AOB and AOA were improved under manure and crop residue input conditions. Rhizosphere soil AOB and AOA community composition were dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria* with all fertilizer treatments. However, there is still need to further investigate effects of different fertilizer treatments on specific mechanistic of soil N-cycle ammonia-oxidizing bacterial and archaea based on long-term field experiment.

Data availability

Data is provided within the manuscript.

Received: 28 September 2024; Accepted: 1 January 2025

Published online: 05 January 2025

References

- Wang, Y. et al. Responses of potential ammonia oxidation and ammonia oxidizers community to arsenic stress in seven types of soil. *J. Environ. Sci.* **127**, 15–29 (2023).
- Liu, R., Yang, Y. & Zhou, J. T. X. Mining activities accelerate the decomposition of organic matter from aquatic ecosystems through soil microbes. *Land. Degrad. Dev.* **10**, 2803–2811 (2026).
- Ai, C., Liang, G., Sun, J., Wang, X. & Zhou, W. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma* **173–174**, 330–338 (2012).
- Shi, L. H. et al. Effects of long-term fertilizer practices on rhizosphere soil nitrogen mineralization in the double-cropping rice field. *J. Basic. Microb.* **63**, 781–789 (2023).
- Shen, J., Zhang, L., Zhu, Y., Zhang, J. & He, J. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environ. Microbiol.* **10**, 1601–1611 (2008).
- Verhamme, D. T., Prosser, J. I. & Nicol, G. W. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME J.* **5**, 1067–1071 (2011).
- Ai, C. et al. 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. Biol. Biochem.* **57**, 30–42 (2013).
- Chu, H., Fujii, T., Morimoto, S., Lin, X. & Yaki, K. Population size and specific nitrification potential of soil ammonia-oxidizing bacteria under long-term fertilization management. *Soil. Biol. Biochem.* **40**, 1960–1963 (2008).
- He, Z. Y. et al. Fertilization has a greater effect than rhizosphere on community structures of comammox Nitrospira in an alkaline agricultural soil. *Appl. Soil. Ecol.* **175**, 104456 (2022).
- Strauss, S. L., Reardon, C. L. & Mazzola, M. The response of ammonia-oxidizer activity and community structure to fertilizer amendment of orchard soils. *Soil. Biol. Biochem.* **68**, 410–418 (2014).
- Meng, X. et al. Fertilization regimes and the nitrification process in paddy soils: lessons for agricultural sustainability from a meta-analysis. *Appl. Soil. Ecol.* **186**, 104844 (2023).
- Berendsen, R., Pieterse, C. & Bakker, P. The rhizosphere microbiome and plant health. *Trends Plant. Sci.* **17**, 478–486 (2012).
- Hussain, Q. et al. Temporal dynamics of ammonia oxidizer (*amoA*) and denitrifier (*nirK*) communities in the rhizosphere of a rice ecosystem from Tai Lake region, China. *Appl. Soil. Ecol.* **48**, 210–218 (2011).
- Kleinedam, K. et al. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. *Chemosphere* **84**, 182–186 (2011).
- Yang, X. Y., Ren, W. D., Sun, B. H. & Zhang, S. L. Effects of contrasting soil management regimes on total and labile soil organic carbon fractions in a loess soil in China. *Geoderma* **177–178**, 49–56 (2012).
- Tang, H. M. et al. Long-term effects of NPK fertilizers and organic manures on soil organic carbon and carbon management index under a double-cropping rice system in Southern China. *Commun. Soil. Sci. Plan.* **49**, 1976–1989 (2018).
- Tang, H. M. et al. Effects of long-term fertiliser regime on soil organic carbon and its labile fractions under double cropping rice system of southern China. *Acta Agr. Scand. B-S P* **70**, 409–418 (2020).
- Hart, S. C., Stark, J. M., Davidson, E. A. & Firestone, M. K. Nitrogen mineralization, immobilisation and nitrification. In *Methods of Soil Analysis. Part 2. Microbial and Biogeochemical Properties* (eds. Weaver, R. W., Angle, J. S. & Bottomley, P. S.) 985–1018 (Soil Science Society of America, 1994).
- Kurola, J., Salkinoja-Salonen, M., Aarnio, T., Hultman, J. & Romantschuk, M. Activity, diversity and population size of ammonia-oxidising bacteria in oil-contaminated landfarming soil. *FEMS Microbiol. Lett.* **250**, 33–38 (2005).
- Kowalchuk, G. A. et al. Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Appl. Environ. Microb.* **63**, 1489–1497 (1997).
- Tourna, M., Freitag, T. E., Nicol, G. W. & Prosser, J. I. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ. Microbiol.* **10**, 1357–1364 (2008).

22. Hermansson, A. & Lindgren, P. E. Quantification of ammonia-oxidizing bacteria in arable soil by real-time PCR. *Appl. Environ. Microb.* **67**, 972–976 (2001).
23. Aogué, H., Brink, M., Dinasquet, J. & Herndl, G. J. Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. *Nature* **456**, 788–791 (2008).
24. Coci, M. et al. Quantitative assessment of ammonia-oxidizing bacterial communities in the epiphyton of submerged macrophytes in shallow lakes. *Appl. Environ. Microb.* **76**, 1813–1821 (2010).
25. Fagen, J. R. et al. Characterization of the relative abundance of the citrus pathogen ca. *Liberibacter asiaticus* in the microbiome of its insect 525vector, *Diaphorina citri*, using high throughput 16S rRNA sequencing. *Open. Microbiol. J.* **6**, 29–33 (2012).
26. Edgar, R. C. UPARSE: highly accurate OUT sequences from microbial amplicon reads. *J. Nat. Methods.* **10**, 996 (2013).
27. SAS. *SAS Software of the SAS System for Windows* (SAS Institute Inc, 2008).
28. Chen, X. P., Zhu, Y. G., Xia, Y., Shen, J. P. & He, J. Z. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environ. Microbiol.* **10**, 1978–1987 (2008).
29. Malchair, S. et al. Do climate warming and plant species richness affect potential nitrification, basal respiration and ammonia-oxidizing bacteria in experimental grasslands? *Soil. Biol. Biochem.* **42**, 1944–1951 (2010).
30. Hei, Z. et al. Full substitution of chemical fertilizer by organic manure decreases soil N₂O emissions driven by ammonia oxidizers and gross nitrogen transformations. *Global Change Biol.* **29**, 7117–7130 (2023).
31. Jia, Z. & Conrad, R. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environ. Microbiol.* **11**, 1658–1671 (2009).
32. Yao, H. et al. Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. *Appl. Environ. Microb.* **77**, 4618–4625 (2011).
33. Nicol, G. W., Leininger, S., Schleper, C. & Prosser, J. I. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ. Microbiol.* **10**, 2966–2978 (2008).
34. Xie, J. et al. Manure combined with biochar reduces rhizosphere nitrification potential and amoA gene abundance of ammonia-oxidizing microorganisms in acid purple soil. *Appl. Soil. Ecol.* **181**, 104660 (2023).

Acknowledgements

This study was supported by the National Key Research and Development Project of China (2023YFD2301403), the Hunan Provincial Natural Science Foundation of China (2022JJ30352), the Hunan Science and Technology Talent Lift Project (2022TJ-N07), the Innovative Research Groups of the Natural Science Foundation of Hunan Province (2024CX39), and the Special Funds for the Construction of Innovative Provinces in Hunan Province (2023NK2027).

Author contributions

Haiming Tang and Li Wen wrote the main manuscript text, Kaikai Cheng and Lihong Shi prepared Figs. 1 and 2, Geng Sun prepared Fig. 3, Mei Sun and Weiyan Li prepared Table 1, and 2. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to H.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025