

# Archaea Are the Major Responsive Ammonia Oxidizers in a Paddy Soil Following Fertilization and Irrigation

Limin Wang

Fujian Academy of Agricultural Sciences

Dongfeng Huang (✉ [hdf\\_2169395@126.com](mailto:hdf_2169395@126.com))

Fujian Academy of Agricultural Sciences

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## Research Article

**Keywords:** AOA, SOC, PAO

**Posted Date:** December 23rd, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-130507/v1>

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**Version of Record:** A version of this preprint was published at Scientific Reports on July 15th, 2021. See the published version at <https://doi.org/10.1038/s41598-021-93898-y>.

# Abstract

Because ammonia-oxidizing archaea (AOA) are ubiquitous and highly abundant in almost all terrestrial soils, they play an important role in soil nitrification. However, the changes in the structure and function of AOA communities and their specific environmental drivers in paddy soils under different fertilization and irrigation regimes remains unclear. In this study, we investigated archaeal abundance, activity and community composition in acid paddy soils by a 10-year field experiment. Results indicated that the highest potential ammonia oxidation (PAO) ( $0.011 \mu\text{g NO}_2^- \text{-N g}^{-1} \text{ d.w.day}^{-1}$ ) was found in T<sub>2</sub> (optimal irrigation and fertilization) - treated soils, whereas the lowest PAO ( $0.004 \mu\text{g NO}_2^- \text{-N g}^{-1} \text{ d.w.day}^{-1}$ ) in T<sub>0</sub> (traditional irrigation)- treated soils. Compared with the T<sub>0</sub> - treated soil, the T<sub>2</sub> treatment significantly ( $P < 0.05$ ) increased AOA abundances. Furthermore, the abundance of AOA was significantly ( $P < 0.01$ ) positively correlated with pH, soil organic carbon (SOC), and PAO. Meanwhile, pH and SOC content were significantly ( $P < 0.05$ ) higher in the T<sub>2</sub> - treated soil than those in the T<sub>1</sub> (traditional irrigation and fertilization)- treated soil. In addition, these two edaphic factors further influenced the AOA community composition. The archaeal phylum *Crenarchaeota* and genus *Candidatus Nitrosotalea* were mainly found in the T<sub>2</sub>-treated soils. Phylogenetic analysis revealed that most of the identified OTUs of AOA were mainly affiliated with *Crenarchaeota*. Together, our findings confirmed that T<sub>2</sub> might ameliorate soil chemical properties, regulate the AOA community structure, increase the AOA abundance, enhance PAO and consequently maintain optimum rice yields in a subtropical paddy field.

# Introduction

The oxidation of ammonia to nitrite is the first step in the soil nitrification process, which was driven by ammonia-oxidizing bacteria (AOB) and archaea (AOA) <sup>1,2</sup>. For example, some researches showed that AOA might make more contributions than AOB in microbial ammonia oxidation through the enzyme ammonia monooxygenase (*amoA*) <sup>3,4</sup>. Conversely, other reports found that AOB played a more critical role than AOA <sup>5,6</sup>. This was because that these two ammonia oxidizers had different ecological niche partitioning <sup>7</sup>. Thus AOA and AOB play distinct roles in ammonia oxidation process under different soil and crop management systems. In this study, electrophoretic analysis of these two ammonia-oxidizers found that AOA, but not AOB, *amoA* genes were detected in paddy soils. Similarly, Gao et al. (2018) reported that archaea were the predominant and responsive ammonia oxidizing prokaryotes in the same type of rice paddy soil <sup>3</sup>. Hence, a follow-up measurement of AOA only were done in the studied paddy soils.

The subtropical paddy soil was classified as typical Hapli-Stagnic Anthrosols characterized by low nutrient capital, low pH value, high P fixation that severely constrained rice production <sup>8</sup>. Rice production must, however, increase by 1% annually due to an increase in the population <sup>9</sup>. High rice yields mainly depend on higher inputs of nitrogen (N) and phosphorus (P) fertilizers, which inevitably increases the risk of potential eutrophication in the surrounding water bodies. Therefore, the optimizal water and fertilizer management was commonly used to increase soil nutrient bioavailability from rice plant, to achieve high yield, and to improve soil environment, and consequently, altered AOA populations <sup>10,11</sup>. For example, soil organic carbon (SOC), nitrate N ( $\text{NO}_3^- \text{-N}$ ) and ammonium N ( $\text{NH}_4^+ \text{-N}$ ) had a big impact on soil ammonia-oxidizing archaeal

composition<sup>3,12</sup>. Meanwhile, the ammonia oxidizers-driven potential ammonia oxidation (PAO) exhibited a strong response to water supply and fertilizer input<sup>13</sup>. Most of the present researches focused on the archaeal community change in response to alterations in edaphic properties induced by either irrigation management or fertilizer application alone, but few reports have evaluated their responses to different water and fertilizer management, especially in subtropical paddy soils. How the irrigation- and fertilization-induced heterogeneity in soil physicochemical properties further affects archaeal community remains unclear in subtropical paddy soils. Hence, studies on the characteristics of AOA in the paddy soil under water and fertilizer coupling are urgently needed to bridge this knowledge gap. Because AOA are ubiquitously distributed and extremely diverse in soils, high-throughput sequencing (HTS) is used to reveal soil ammonia-oxidizing archaeal diversity at a high-resolution. The approach can detect soil microbial taxa at very low levels. Additionally, the functional *amoA* gene is detected and quantified by an accurate quantitative real-time PCR<sup>14</sup>. These technologies provide opportunities to assess the abundance, composition, and activity of AOA communities in response to environmental changes associated with fertilization and irrigation.

Thus a 10-year field experiment was conducted to estimate the effects of water and fertilizer application on AOA communities and their environmental drivers. Here we would test the following hypotheses: (i) the AOA community would be modified by soil factors associated with fertilization and irrigation, and (ii) alterations in AOA communities would further influence PAO activity.

## Results

### Edaphic characteristics, potential ammonia oxidation, and soil productivity.

Rice grain yields in the T<sub>2</sub> treatment increased by 0.92 times, stover yields increased by 1.26 times compared to those in the T<sub>0</sub> treatment, respectively (Fig. 1A, B). However, there were no significant differences in the stover and grain yields between T<sub>2</sub> and T<sub>1</sub> treatments (Fig. 1A, B). Meanwhile, soil pH values varied between 5.97 and 6.24 under different fertilization and irrigation regimes, and thus the soils were acidic (Table 2). Meanwhile, soil pH and SOC content were significantly ( $P < 0.05$ ) higher in the T<sub>2</sub> treatment than those in the other two treatments. Compared with the T<sub>0</sub> treatment, the T<sub>1</sub> and T<sub>2</sub> treatments significantly ( $P < 0.05$ ) increased in soil NH<sub>4</sub><sup>+</sup>-N content. Nevertheless, no apparent differences occurred in the TN concentration in paddy soils under the different fertilization and irrigation regimes. There was a significant ( $P < 0.05$ ) increase in soil NO<sub>3</sub><sup>-</sup>-N content in the T<sub>1</sub>-treated soils compared to that in the T<sub>0</sub>-treated soils. In addition, the PAO in the T<sub>0</sub> treatment significantly ( $P < 0.05$ ) decreased compared with that in the T<sub>1</sub> and T<sub>2</sub> treatments, but the PAO in the later two treatments was not significantly different (Table 2).

### Soil ammonia-oxidising archaea community.

*Archaeal abundance and alpha diversity.* The AOA abundance was estimated by quantifying their *amoA* gene copy numbers. In this study, archaeal *amoA* gene copy numbers ( $5.74 \times 10^7$  *amoA* gene copies g<sup>-1</sup> dry soil) were higher in the T<sub>2</sub>-treated soils than those in the other two treatments (Fig. 2). In addition, the rarefaction curves reached saturation, indicating that the generated sequences were enough to reflect the diversity of archaeal *amoA* genes (Fig. 3). The diversity indices of the AOA-related OTUs did not differ significantly ( $P$ )

0.05) under fertilization and irrigation regimes (Table 4). The major reasons for no treatment effects on ammonia-oxidizing archaeal diversity mainly depended on their response to natural environments and not artificial factors, such as fertilization and irrigation.

### ***Archaeal community composition.***

A total of 154,733 archaeal sequence reads, ranging from 12,837 to 21,639 per soil sample after QIIME quality filtering, were taxonomically classified into five phyla and six individual genera (Fig. 6). The Venn diagram indicated that the numbers of ammonia-oxidizing archaeal OTUs at a 97 % sequence identity were 37, 33, and 41 in the  $T_0$ -,  $T_1$ - and  $T_2$ - treated soils, respectively (Fig. 4). Only 36.67% of the total archaeal OTUs were shared in three soils treated by fertilization and irrigation (Fig. 4). Additionally, the proportion of shared OTUs in AOA between  $T_1$ - and  $T_2$ - treatments was 64.70% in their total sequences (Fig. 4). Furthermore, a query coverage was > 99.99%, suggesting that this study captured the dominant OTUs of AOA in each soil sample (Table 4). The dominant ammonia-oxidizing archaeal phylum in paddy soils was *Crenarchaeota* (the sequence number at the phylum level varied from 59.36% to 75.62% in soils), while the rare archaeal phylum was characterized by low *Thaumarchaeota* (Fig. 6A). Meanwhile, fertilization and irrigation altered ammonia-oxidizing archaeal community structure using PLS - DA approach (Fig. 5). A total 34.21% of the variations in the archaeal community composition could be mainly explained by soil pH, SOC, and PAO (Fig. 8). Moreover,  $T_2$  resulted in the prevalence of the archaeal phylum *Crenarchaeota*, which accounted for 75.62% of the total ammonia-oxidizing *archaea* (Fig. 6A). However,  $T_1$  decreased the relative abundance of the phylum *Thaumarchaeota* by 78.38% and 73.33%, respectively, compared to that in the  $T_0$  and  $T_2$  treatments (Fig. 6A). Meanwhile, the relative abundance of the genus *Candidatus Nitrosotalea* in the  $T_2$  treatment increased by 4.86 and 19.50 times, respectively, as compared with that in the  $T_0$  and  $T_1$  treatments. In contrast, the  $T_1$  and  $T_2$  treatments decreased the abundance of the genus *Nitrososphaera* by 77.62% and 70.15% compared with that under the  $T_0$  treatment, respectively (Fig. 6B).

Phylogenetic tree for the *amoA* gene of AOA was constructed based on the values of OTUs of the top 10 most abundant species, which indicated that most of ammonia-oxidizing archaeal OTUs were affiliated with *Crenarchaeota* cluster and unclassified\_k\_\_norank\_d\_\_*Archaea* cluster, accounting for 64.12% and 21.76% of total reads, respectively (Fig. 7). In addition, the OTUs 21, 38, 40, 50, and 51 belonged to cluster *Crenarchaeota*, and the OTUs 35, 39, 49, and 52 for cluster unclassified\_k\_\_norank\_d\_\_*Archaea*, respectively (Fig. 7).

### **Relationships between ammonia-oxidizing archaeal communities and edaphic characteristics.**

Correlation analysis showed that the AOA abundance had positive correlation with soil pH ( $r = 0.9744$ ,  $P < 0.01$ ), SOC ( $r = 0.9863$ ,  $P < 0.01$ ), and PAO ( $r = 0.8523$ ,  $P < 0.01$ ), but had negative relationship with TN ( $r = -0.9823$ ,  $P < 0.01$ ) and  $\text{NO}_3^-$ -N ( $r = -0.6765$ ,  $P < 0.05$ ) contents (Table 3). Furthermore, soil pH ( $r^2 = 0.6703$ ,  $P = 0.03$ ), SOC content ( $r^2 = 0.6881$ ,  $P = 0.012$ ), and PAO ( $r^2 = 0.5689$ ,  $P = 0.048$ ) were significantly ( $P < 0.05$ ) positively correlated with ammonia-oxidizing archaeal community structure using a db - RDA (Fig. 8). The first ordination db - RDA axis (axis 1, horizontal), which was strongly related to pH and SOC, explained 22.84% of the total variability in ammonia-oxidizing archaeal community structure. The second ordination db - RDA axis (axis 2, vertical) was mainly related to PAO, explained 16.70% of the variability in the archaeal community

structure (Fig. 8). Meanwhile, the relative abundance of the genus unclassified\_k\_\_norank\_d\_\_Archaea was significantly ( $P < 0.05$ ) positively related to TN content, but was significantly ( $P < 0.05$ ) negatively correlated with SOC content (Fig. 9).

## Discussion

The AOA abundance was reflected by the AOA *amoA* gene copy number. In this study, the AOA *amoA* gene copy number significantly ( $P < 0.05$ ) increased in the  $T_2$  - treated soil, but no apparent difference occurred in the  $T_1$  - treated soil, in comparison with that in the  $T_0$  - treated soil (Fig. 2). This finding demonstrated that the growth of AOA communities was significantly stimulated by the optimized fertilizer and water management practice. However, this result did not support the hypotheses that the oligotrophic nature of AOA makes them important for nutrient-limited soil ecosystems<sup>14,15</sup>. Additionally, these findings were inconsistent with those reported by Fang et al. (2019), who found that there was no obvious difference in the AOA *amoA* gene copy number between the NPK and CK treatments<sup>10</sup>. These inconsistent results mainly depended on soil types, rice cultivars, and agricultural practices<sup>11,16</sup>. Meanwhile, the AOA *amoA* gene copy number ranged from  $0.28 \times 10^7$  to  $5.74 \times 10^7$  per gram dry soil (Fig. 2), which was about 10-fold higher than the value obtained by Fang et al. (2019)<sup>10</sup>. The difference in the *amoA* gene copy numbers might arise from different sampling times<sup>17</sup>. In summary, it is difficult to accurately predict the AOA abundance by straightforward ways under different environmental conditions.

This study also indicated that different fertilization and irrigation treatments formed distinct ammonia-oxidizing archaeal community compositions based on PLS - DA (Fig. 5). This agreed with a previous report showing the proper combination of fertilization rate and irrigation frequency could regulate soil ammonia-oxidizing archaeal community composition<sup>11</sup>. In addition, the most predominant OTUs of AOA had affiliation to *Crenarchaeota* (Fig. 7), which differed from the previous findings indicating that the dominant OTUs of AOA fell into *Nitrososphaera* in many soil systems<sup>10,17</sup>. The difference in AOA community structure in different types of soils reveals a separation and selectivity of AOA induced by their own growth characteristics and habitat conditions. Moreover, these findings indicated that AOA members were dominated by distinct ammonia-oxidizing archaeal species under different environmental conditions<sup>17,18</sup>. In comparison with the other two treatments, the  $T_2$  treatment increased the abundances of both ammonia-oxidizing archaeal phylum *Crenarchaeota* and genus *Candidatus Nitrosotalea* in soils. The genus *Candidatus Nitrosotalea* are obligate acidophiles, and thus are abundant in acidic soils<sup>19</sup>. In general, these findings indicated a possible niche differentiation for AOA populations in which different soil microsites supported different AOA species.

The changes in ammonia-oxidizing archaeal abundance and community composition, in turn could result in altered rates and/or controls of corresponding functions. This study suggested that the AOA-specific PAO in the  $T_1$ - and  $T_2$ -treated soils was significantly ( $P < 0.05$ ) greater than that in the  $T_0$ -treated soils (Table 2). The result is similar to that obtained by previous studies indicating that enhanced PAO in soils was observed in the NPK treatments compared to that without fertilizer application<sup>10</sup>. These findings demonstrated that fertilization and irrigation enhanced soil PAO by increasing AOA abundances, particularly in the  $T_2$  - treated soils (Table 3). The analyses supported that the hypothesis that an increase in AOA abundances could

enhance PAO in soils<sup>20</sup>. Furthermore, the stimulation of PAO might increase N availability for rice plants and resulted in higher paddy soil productivity<sup>10,17</sup>, as confirmed in this study (Fig. 1A). However, long-term fertilization and irrigation had a small impact on ammonia-oxidizing archaeal diversity in paddy soils (Table 4). The reasons for nonsignificant effects of fertilization and irrigation treatments on the ammonia-oxidizing archaeal diversity might depend on their response to natural conditions (e.g., soil temperature, spatial pattern, and floristic composition)<sup>15,21,22</sup>. Taken together, this study indicated that long-term fertilization and irrigation could significantly influence the abundance, activity and community structure of AOA rather than their diversity in a paddy soil.

The variation in the AOA community structure and abundance might be due to their responses to altered edaphic properties associated with different fertilization and irrigation managements. A correlation analysis revealed that the AOA abundance was negatively related to TN and NO<sub>3</sub><sup>-</sup>-N contents in the acidic paddy soil (Table 3). Conversely, positive correlations existed between the AOA abundance and TN, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N contents<sup>10</sup>. We found that various studies produced inconsistent findings, perhaps because of different sampling times<sup>17</sup>. Meanwhile, the AOA abundance was positively correlated with SOC content in the acidic paddy soil (Table 3). Likewise, Zhang et al. (2013) reported that the AOA abundance had a significant positive relationship with SOC content, which supported the idea of heterotrophic growth of the archaeal community<sup>23</sup>. Additionally, the AOA abundance was positively related to pH (Table 3). However, the opposite results were previously obtained by Gao et al. (2018) and Fang et al. (2019), who found that there was a negative relationship between soil pH with the AOA abundance<sup>3,10</sup>. Additionally, pH also showed no significant correlations with AOA abundance in alkaline soils<sup>24</sup>. These inconsistent results indicated that there were correlative variations in the AOA abundance and pH value in response to soil acidity-alkalinity gradients. In addition, soil pH ( $r^2 = 0.6703$ ,  $P = 0.03$ ) and SOC ( $r^2 = 0.6881$ ,  $P = 0.012$ ) were the most predominant edaphic factors for the AOA community structure under different fertilization and irrigation regimes using db - RDA (Fig. 8). Nonetheless, Fang et al. (2019) found that soil pH was nonsignificantly correlated with the AOA community composition<sup>10</sup>. The distinct responses of the AOA populations to soil pH were perhaps these AOA communities grew well in their own narrow pH ranges<sup>25</sup>. Besides directly pH-selecting for acidophilic or neutrophilic ammonia oxidizers, pH could also influence soil nutrient availability, which indirectly mediated the AOA community composition. For instance, SOC, especially dissolved organic carbon (DOC) was another important edaphic factor driving the AOA community structure in acid soils<sup>25</sup>. In general, these studies further demonstrated that the abundance and community composition of AOA were shifted by altered soil properties associated with different fertilization and irrigation treatments, which might be the important factors responsible for variations in PAO.

## Conclusions

Our results showed that AOA (ammonia-oxidizing archaea) played the vital role in ammonia oxidation in acid paddy soils. The T<sub>2</sub> (optimal irrigation and fertilization) treatment increased the AOA *amoA* gene copy number. Additionally, *Crenarchaeota* was the dominant phylum of AOA communities. Furthermore, an increase in the abundances of the phylum *Crenarchaeota* and genus *Candidatus Nitrosotalea* was observed in the T<sub>2</sub> treatment compared with those in the other two treatments. Meanwhile, SOC (soil organic C), pH, and PAO

(potential ammonia oxidation) were the highest in the  $T_2$  - treated soils. The  $T_2$  - treated soils had clear differences in the AOA community composition compared with that in the  $T_0$  (traditional irrigation) - and  $T_1$  (traditional irrigation and fertilization) - treated soils. Moreover, the AOA abundance and community composition were mainly driven by soil pH and SOC. Meanwhile, the AOA abundance showed a significant positive relationship with PAO. Furthermore, the  $T_2$  treatment increased rice yield compared to the  $T_0$  and  $T_1$  treatments. Taken together, these results suggest that the  $T_2$  treatment should be utilized to mediate the archaeal community structure, promote ammonia oxidation rate, improve soil nutrient availability and thus maintain rice yield in the subtropical paddy field.

## Methods

*Experiment design and sample collection.* A fertilization and irrigation experiment was established in 2008 and double cropping rice (*Oryza sativa* L.) was planted annually at Baisha Experimental Station (119°04'10"E, 26°13'31"N), Minhou County, Fuzhou City, Fujian Province, China. The early and late cultivars of rice (*O. sativa* L.) are conventional rice varieties 78-30 and 428, respectively, from south China. This region has a subtropical monsoonal climate with an average annual air temperature of 19.5 °C and mean annual precipitation of 1350 mm. The soil is a typic Hapli-Stagnic Anthrosol (USDA soil system). At the beginning of the experiment, the soil had a pH 6.19, 14.16 g kg<sup>-1</sup> soil organic matter (SOM), 0.66 g kg<sup>-1</sup> total N (TN), 0.30 g kg<sup>-1</sup> total P (TP), 3.8 mg kg<sup>-1</sup> NO<sub>3</sub><sup>-</sup>-N, 12 mg kg<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N, 3.358 and 0.83 mg kg<sup>-1</sup> of available P (AP) and K (AK), respectively, in the 0 – 20 cm soil layer. A randomized complete block design with three treatments was conducted in 9 plots (4.0 m long × 5.0 m wide). Each treatment had three duplicates. The treatments included control (no fertilization with traditional irrigation,  $T_0$ ), traditional fertilization with traditional irrigation ( $T_1$ , based on local practices), and optimum fertilization with water-saving irrigation ( $T_2$ , based on both fertilizer recommendation from local agriculture committee and water saving by shallow intermittent irrigation). The water and fertilizer practices used in this experiment are described in Table 1. The chemical compound fertilizer (15% N, 15% P<sub>2</sub>O<sub>5</sub>, 15% K<sub>2</sub>O) was produced by China Petroleum and Chemical Co., Ltd. N, P, and K fertilizers were applied in the form of urea (46.4 % N), superphosphate (12 % P<sub>2</sub>O<sub>5</sub>), and potassium chloride (60 % K<sub>2</sub>O) and used according to the amount of these fertilizers as shown in Table 1. The 100% of the total amount of P, 60% of N, and 40% of K fertilizers were used as basal fertilizers before transplanting of rice seedlings, and the 40% N and 60% K fertilizers as topdressing fertilizers after tillering, respectively (Table 1). Traditional irrigation was needed to be maintained at a depth of 1.0 – 6.0 cm during the rice-growing season, and water-saving irrigation at a depth of -3.0 – 3.0 cm in the field. To avoid water and fertilizer exchange between adjacent experimental units, a cement concrete border, with dimensions (length × width × height) 40 cm × 30 cm × 20 cm, was constructed. The early rice was transplanted with a 20.0 cm × 23.0 cm row spacing on 21 April and harvested on 25 July 2018. The late rice was transplanted with the same row spacing on 30 July and harvested on 1 December 2018.

The rice straw and grain were sampled. In addition, five samples of 0–20 cm soil layer were collected and mixed from each plot after late rice harvest. The fresh soil samples were transported immediately on ice to the laboratory. Plant residues and stones were manually removed from soil samples. The soil samples were then

mixed and sieved to < 2.0 mm. One subsample was stored at - 80 °C for soil microbial analysis, while the other subsample was air-dried for chemical analysis.

*Plant, soil physiochemical properties, and Potential ammonia oxidation (PAO).* The rice straw and grain yields were measured at harvest from each plot, separately (rice grain weights were adjusted to 13.5% moisture content). In addition, the rice grain and straw materials were dried at 60 °C for 72 h, and weighed. Meanwhile, soil moisture was calculated as the difference between oven - dry (24 h at 105 °C) and fresh weight. Soil pH was analyzed by a pH meter (EL20 K, Mettler - Toledo, Greifensee, Switzerland) in a 1:2.5 (m:v) soil - water suspension. The SOC content was measured by means of the oxidation-reduction titration. The TN content was analyzed using a Kjeldahl digestion. Both  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in fresh soils were extracted with 2 M KCl (1:10 (m:v) soil/extract), and the extract was analyzed by using UV spectrophotometry<sup>26</sup>. The PAO activity was measured according to Kurolo et al. (2005)<sup>27</sup> with minor modifications. Briefly, 5 g of fresh soil was incubated in 20 mL phosphate-buffered saline (PBS) and 1 mM of  $(\text{NH}_4)_2\text{SO}_4$  at room temperature in the dark for 24 h. Then 10 mg  $\text{L}^{-1}$  of  $\text{KClO}_3$  addition inhibited nitrite oxidation. At the end of incubation, soil  $\text{NO}_2^-$ -N was extracted with 5 mL of 2 M KCl. The optical density (at 540 nm) of the supernatant was determined after each centrifugation in order to calculate the  $\text{NO}_2^-$ -N content using sulfonamide and naphthylethylene diamide as reagents. The PAO activity was estimated by the slope of the  $\text{NO}_2^-$ -N accumulation.

*Soil ammonia-oxidizing archaeal analysis.* DNA was extracted from 0.25 g of fresh soils per sample by the E.Z.N.A Soil DNA Kit (Omega Bio-tek, Norcross, Georgia, USA) on the basis of the manufacturer's instructions<sup>28</sup>. The DNA purity and concentration were detected using a Nanodrop 2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA).

The archaeal *amoA* gene was amplified by the primers Arch-*amoA*F (5' STAATGGTCTGGCTTAGACG 3') and Arch-*amoA*R (5'GCGGCCATCCATCTGTATGT 3')<sup>29</sup>. Base on the preliminary experiment, the reaction systems and cycling conditions such as DNA amounts, annealing temperatures, and circular times were further optimized. The preliminary PCR amplification was performed for 27 cycles. All PCR products after 2.0% (w/v) agarose gel electrophoresis on a 2.0% (w/v) agarose gel is used to verify ammonia-oxidizing bacterial and archaeal size and quality. The band numbers and relative intensities of PCR products were analyzed using Quantity One analysis software (Bio-Rad). However, only the ammonia-oxidizing archaeal community was found and further analyzed under the following conditions. Each 20- $\mu\text{L}$  qPCR reaction mixture contained 10  $\mu\text{L}$  2X Taq Plus Master Mix (VazymeBiotech, Nanjing, China), 0.8  $\mu\text{L}$  forward and reverse primers (5  $\mu\text{M}$ ), 1  $\mu\text{L}$  DNA template and 7.4  $\mu\text{L}$  ddH<sub>2</sub>O. The qPCR of ammonia-oxidizing archaeal *amoA* gene was conducted on an ABI 7300 thermocycler (Applied Biosystems, California, USA) at 95 °C for 5 min, 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min. The purified PCR products were ligated into the plasmid pMD19-T Vector (Takara, Dalian, China) carrying AOA *amoA* insert. Individual clones were grown in Luria-Bertani (LB) medium at 37 °C for 18 h. Plasmid DNA from a 5-mL culture was extracted with a TIANprep Mini Plasmid Kit (Tiangen, Beijing, China) and quantified by a Nanodrop 2000 UV-Vis spectrophotometer<sup>17</sup>. Each sample and each standard were quantified in triplicate. The qPCR was performed with three times, and the amplification efficiency of the qPCR was 89.09% ( $R^2 = 0.9998$ ) for AOA. The cell numbers of AOA were calculated from the quantified numbers of the *amoA* gene on the basis of the fact that each cell in AOA contains one copy of the *amoA* genes<sup>30</sup>.

The primers Arch-*amoA*F and Arch-*amoA*R<sup>29</sup> were used to amplify *amoA* gene fragments by a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). The PCR reactions for AOA were performed with the following conditions: denaturation at 95 °C for 3 min, 27 cycles of 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR reaction mixture (20 µL) was comprised of 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of 5 µM each primer, 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. The PCR products were run on 2% agarose gels and purified with an AxyPrep DNA Gel Extraction Kit (Axygen, USA), and quantified using QuantiFluor™-ST (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. The purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2 × 300) using an Illumina MiSeq platform (Illumina, San Diego, USA) on the basis of the standard protocols by the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China)<sup>3</sup>.

Raw fastq files were demultiplexed, filtered using Trimmomatic software (Version 3.29) and merged by FLASH (Version 1.2.7) with the criteria: (i) The sequences were trimmed at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Sequences with mismatches to either the primer (> 2) or with ambiguous bases (> 1) were discarded. (iii) Sequences with their overlap > 10 bp or length < 200 bp were deleted. The remaining sequences were selected for chimeras by UCHIME<sup>31</sup>. The high-quality sequences were assigned to operational taxonomic units (OTUs) according to 97% sequence identity by the UPARSE pipeline. The taxonomy of each *amoA* gene sequence was identified by the Ribosomal Database Project (RDP) Classifier tool (<http://rdp.cme.msu.edu/>) against the *fgr/amoA* database (GeneBank Release 7.3 <http://fungene.cme.msu.edu>) with a confidence threshold of 70%. Furthermore, the rarefaction curve and other OTUs-based parameters, including coverage estimators (ACE), Chao1, Shannon-Wiener index (H'), and Simpson's index (D) were analyzed by the mothur software package<sup>32</sup>. Chao1 and ACE were used to evaluate the ammonia-oxidizing archaeal community richness on the basis of the degree of sequence dissimilarity. H' and D were used to evaluate the diversity within each individual sample<sup>33</sup>. In addition, a Venn diagram showing the number of shared and unique archaeal OTUs was used to describe the similarities and differences among the archaeal communities associated with three treatments. A heatmap analysis was performed to compare the relative abundance of the top 10 archaeal genera. Moreover, a heatmap of relationship between the relative genus abundances of ammonia-oxidizing archaea and soil properties (e.g., pH, SOC, and TN) was conducted by Canoco for Windows 4.5 package. In addition, environmental factors were selected by the functions of envfit (permu = 999) and vif.cca, and the environmental factors such as SOC, TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and PAO with *P* < 0.05 or vif < 10 were retained. The distance - based redundancy analysis (db-RDA) and partial least squares discriminant analysis (PLS - DA) were processed by R software (version 3.2.1). The phylogenetic analysis on the basis of the sequences acquired from this study and reference sequences from the NCBI GenBank was made using the software MEGA version 5.0<sup>34</sup> to construct a phylogenetic tree by the neighbor-joining method. All bioinformatics analyses for soil ammonia-oxidizing archaeal communities were performed on online "I-Sanger" (<http://www.i-sanger.com/>) developed by Majorbio Bio-Pharm Technology Co. Ltd. All original nucleotide sequence reads were deposited at the NCBI Sequence Read Archive (SRA) with the accession number of SRP293735.

One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to estimate the statistical significance of the differences of edaphic characteristics, rice yields, archaeal *amoA* gene abundance and

alpha-diversity under different water and fertilizer regimes by SAS Version 8.02 (SAS Institute Inc, Carey, North Carolina, USA). All data were expressed as mean  $\pm$  SD ( $n=3$ ).

## Declarations

### Acknowledgements

This work was supported by the Special Fund of Fundamental Scientific Research at Nonprofit Research Institutions in Fujian (2018R1022-4), Innovation Team in Fujian Academy of Agricultural Sciences (STIT2017-2-10), Fuzhou Science and Technology Support Program (2018-G-65), and the Youth Talent Program of Fujian Academy of Agricultural Sciences (YC2019006). Moreover, we gratefully acknowledge the support of Fujian Key Laboratory of Agro - products Quality & Safety.

### Author Contributions

L. W. carried out the field experiment and analyzed the data. D. H. designed the experiment. All co-authors contributed to write the manuscript.

### Additional Information

**Competing financial interests:** The authors declare no competing financial interests.

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## Tables

**Table 1. Amounts of fertilizer in the field.**

Fertilizer (kg ha <sup>-1</sup> )		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
Base fertilizer	Nitrogen (N)	0	75	144
	Phosphorus (P)	0	75	120
	Potassium (K)	0	75	96
Top-dressing fertilizer	N	0	198	96
	P	0	60	0
	K	0	60	144

Notes: T<sub>0</sub> = Traditional irrigation; T<sub>1</sub> = Traditional irrigation and fertilization practice; T<sub>2</sub> = Water-saving irrigation and optimizing fertilization.

**Table 2. Soil chemical properties and potential ammonia oxidation as influenced by fertilization and irrigation in 2018.**

Treatment	pH	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	PAO (µg NO <sub>2</sub> <sup>-</sup> -N g <sup>-1</sup> d.w.day <sup>-1</sup> )
T <sub>0</sub>	5.97 ±0.08b	15.164 ±0.099b	2.178 ±0.247a	13.196 ±0.767b	35.138 ±6.117b	0.004 ±0.002b
T <sub>1</sub>	6.01 ±0.08b	15.334 ±0.128b	2.000 ±0.117a	18.738 ±2.941a	59.635 ±5.525a	0.010 ±0.002a
T <sub>2</sub>	6.24 ±0.11a	15.982 ±0.178a	1.839 ±0.155a	6.365 ±0.956c	51.547 ±7.429a	0.011 ±0.004a

Notes: T<sub>0</sub>= Traditional irrigation; T<sub>1</sub>= Traditional irrigation and fertilization practice; T<sub>2</sub>= Water-saving irrigation and optimizing fertilization. SOC: soil organic carbon; N: nitrogen; TN: total N; NO<sub>3</sub><sup>-</sup> - N: nitrate N; NH<sub>4</sub><sup>+</sup> - N: ammonium N; PAO: potential ammonia oxidation. Values (means ± SD) with different lower-case letters in a column are significantly different at  $P \leq 0.05$  according to the Duncan test.

**Table 3. Correlation coefficients between soil properties and the abundance of soil ammonia-oxidising archaea (AOA).**

Item	pH	SOC	TN	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	PAO
AOA abundance	0.9744**	0.9863**	-0.9823**	0.5294	-0.6765*	0.8523**

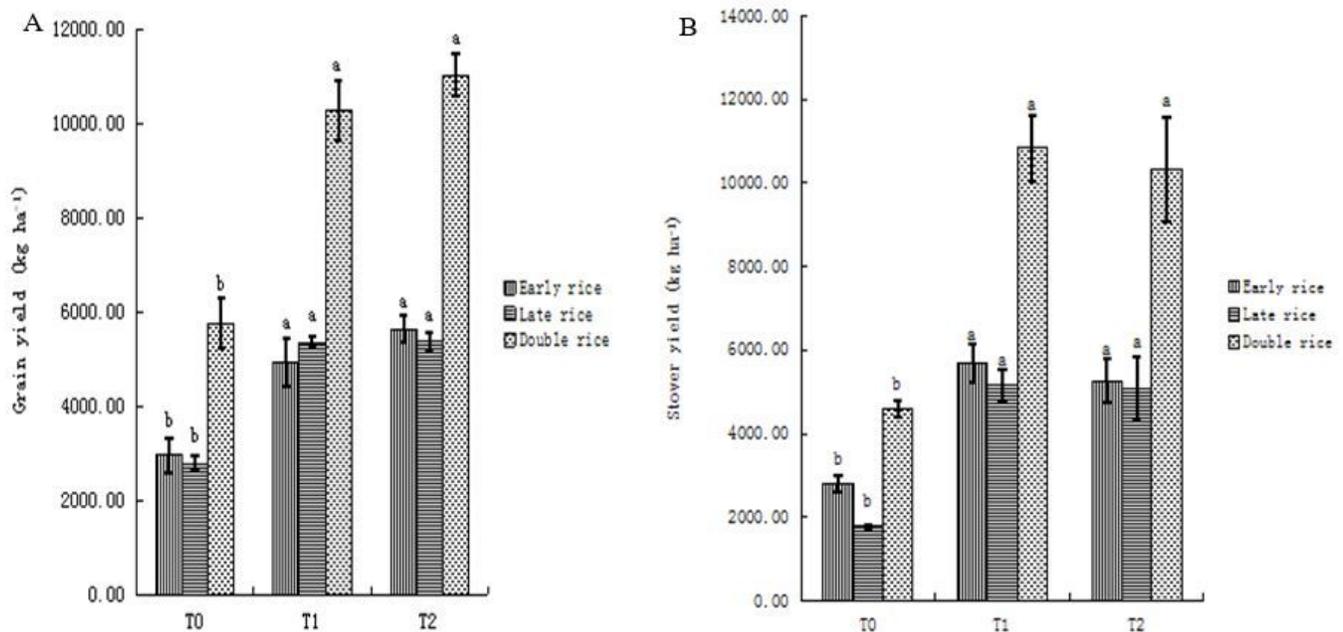
Notes: SOC: soil organic carbon; N: nitrogen; TN: total N; NO<sub>3</sub><sup>-</sup> - N: nitrate N; NH<sub>4</sub><sup>+</sup> - N: ammonium N; PAO: potential ammonia oxidation. \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 4. Diversity of soil ammonia-oxidising archaea (AOA) as affected by fertilization and irrigation in 2018.**

Treatment	OTUs ( $\times 10^4$ )	Coverage (%)	Richness		Diversity	
			Ace	Chao	Shannon	Simpson
T <sub>0</sub>	1.6573 $\pm$ 0.2812a	99.9975 $\pm$ 0.4330a	23 $\pm$ 3a	23 $\pm$ 3a	2.052 $\pm$ 0.181a	0.195 $\pm$ 0.045a
T <sub>1</sub>	1.7049 $\pm$ 0.4413a	99.9985 $\pm$ 0.2656a	18 $\pm$ 5a	18 $\pm$ 4a	1.885 $\pm$ 0.399a	0.218 $\pm$ 0.103a
T <sub>2</sub>	1.7956 $\pm$ 0.3252a	99.9902 $\pm$ 0.4557a	26 $\pm$ 3a	25 $\pm$ 2a	1.692 $\pm$ 0.169a	0.287 $\pm$ 0.067a

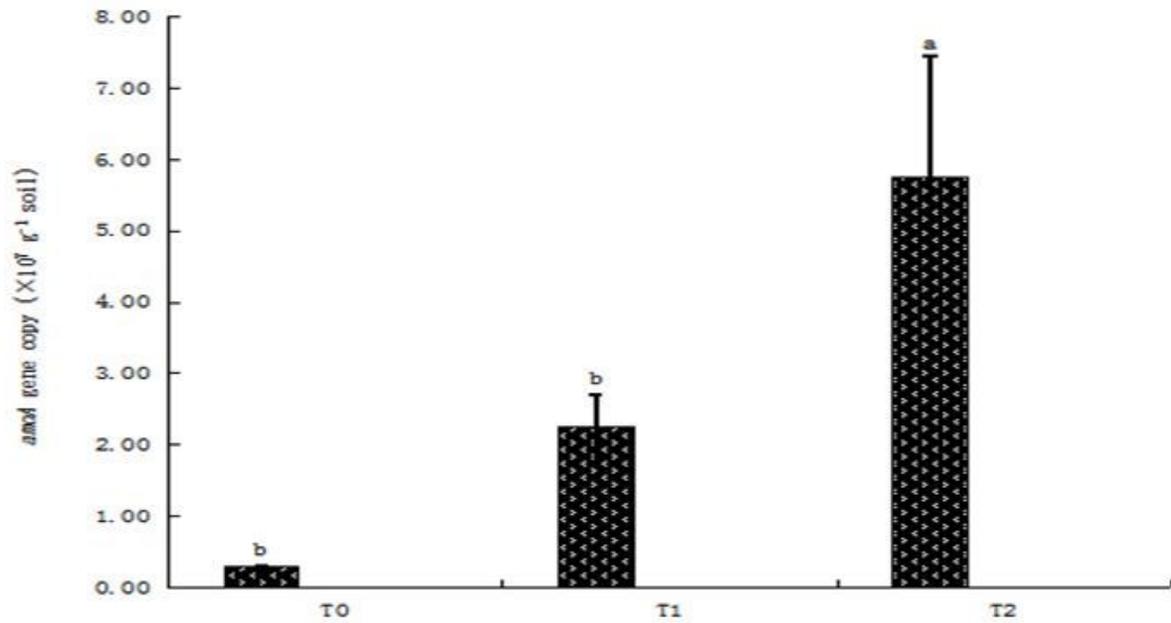
Notes: T<sub>0</sub> = Traditional irrigation; T<sub>1</sub> = Traditional irrigation and fertilization practice; T<sub>2</sub> = Water-saving irrigation and optimizing fertilization. Operational taxonomic units (OTUs). Values (means  $\pm$  SD) with different lower-case letters in a column are significantly different at  $P \leq 0.05$  according to the Duncan test.

## Figures



**Figure 1**

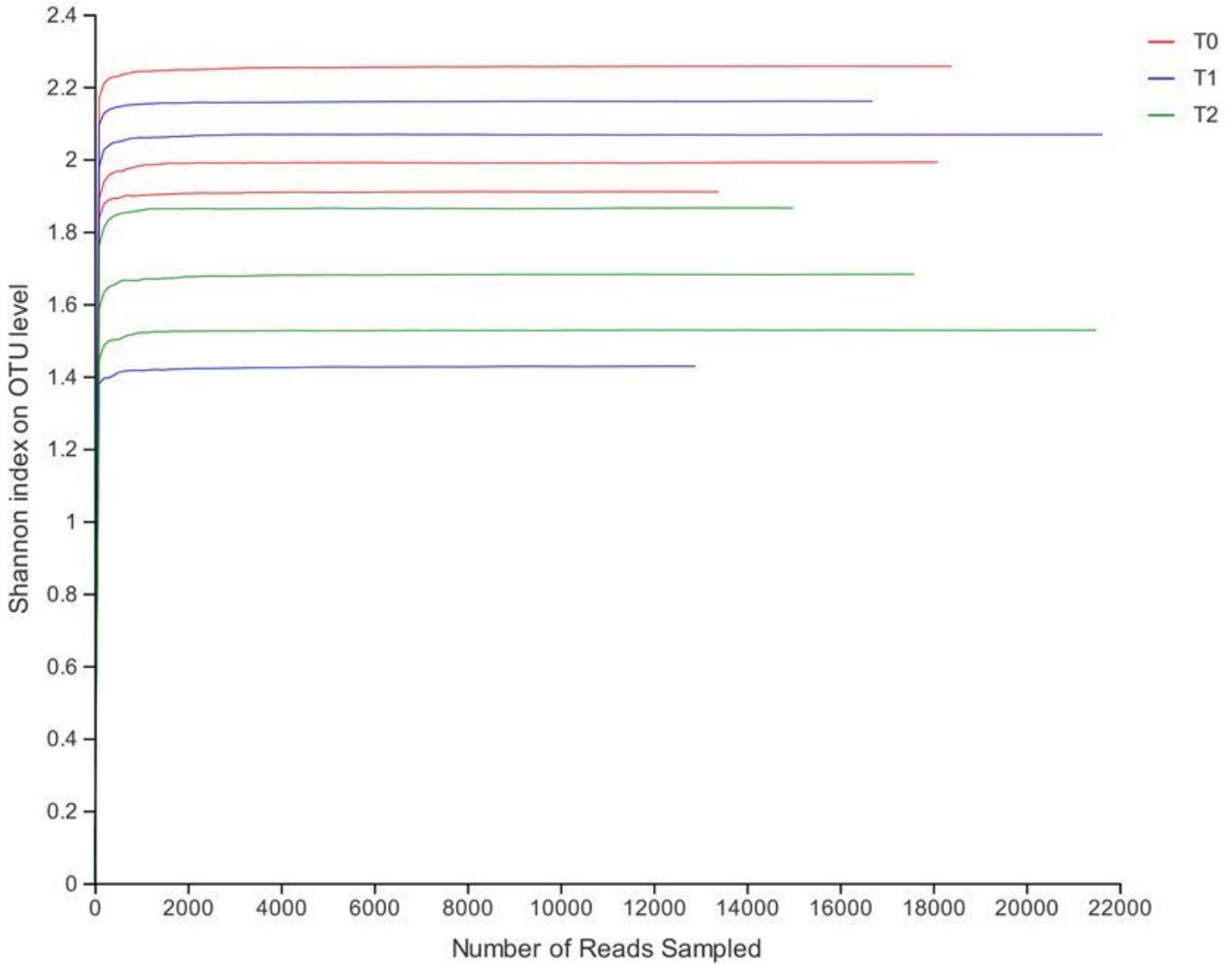
Grain (A) and stover (B) yield of rice as influenced by fertilization and irrigation in 2018. Notes: T<sub>0</sub> = Traditional irrigation; T<sub>1</sub> = Traditional irrigation and fertilization practice; T<sub>2</sub> = Water-saving irrigation and optimizing fertilization. The different lower-case letters indicate significant differences between different treatments at  $P \leq 0.05$  according to Duncan multiple range test. Error bars show SE (n=3).



**Figure 2**

Abundances of soil ammonia-oxidizing archaeal amoA genes in response to different fertilization and irrigation. The different letters indicate significant differences (Duncan's test,  $P < 0.05$ ). Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization.

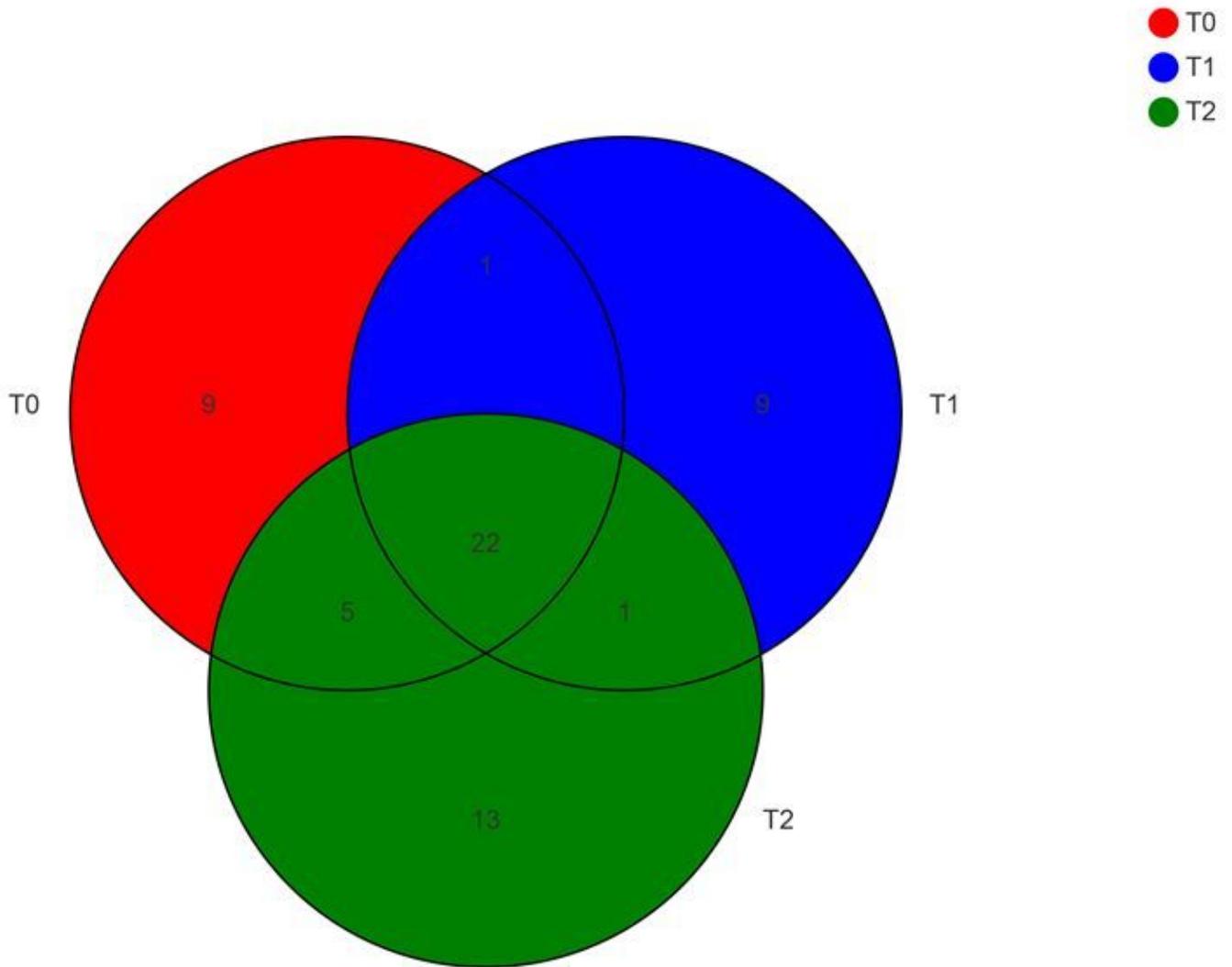
### Shannon curves



**Figure 3**

Shannon-Wiener curves of soil ammonia-oxidizing archaea (AOA) for the normalized number of reads at the 97% level of similarity as influenced by fertilization and irrigation in 2018. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization.

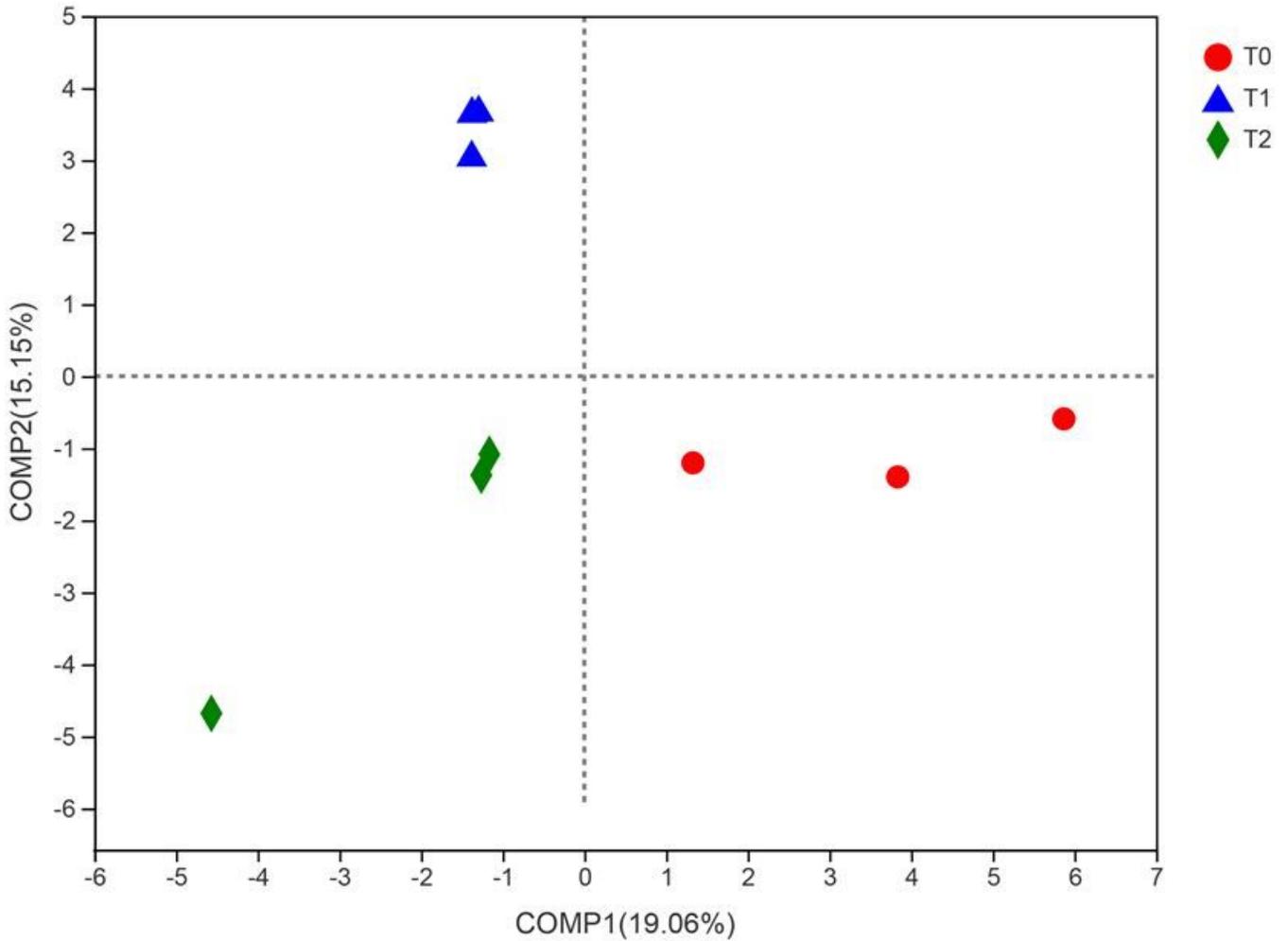
## Venn



**Figure 4**

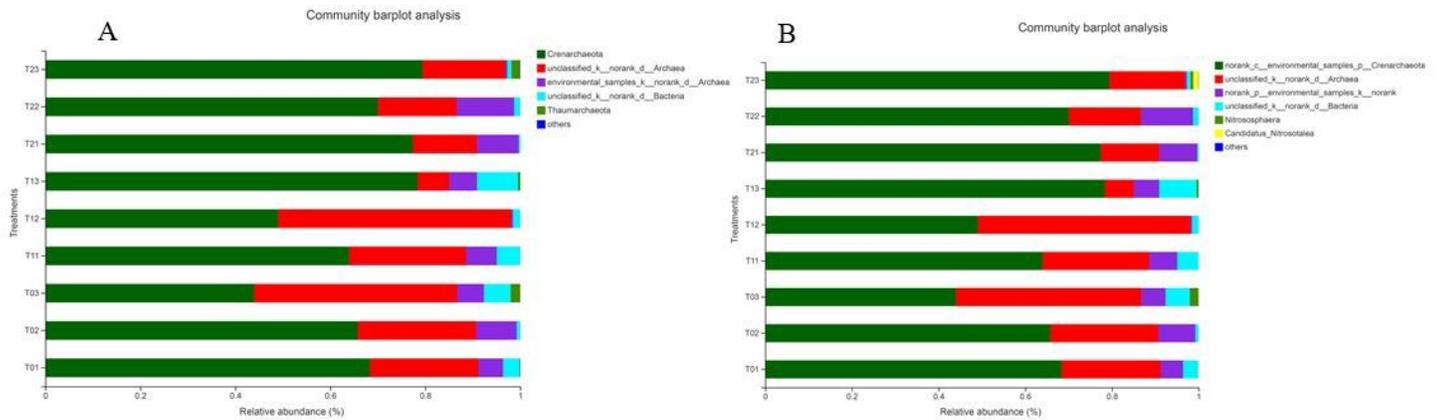
Venn diagram depicts operational taxonomic units (OTUs) of soil ammonia-oxidizing archaea (AOA) that were shared or unique for T0, T1, and T2. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization.

### PLS-DA on OTU level



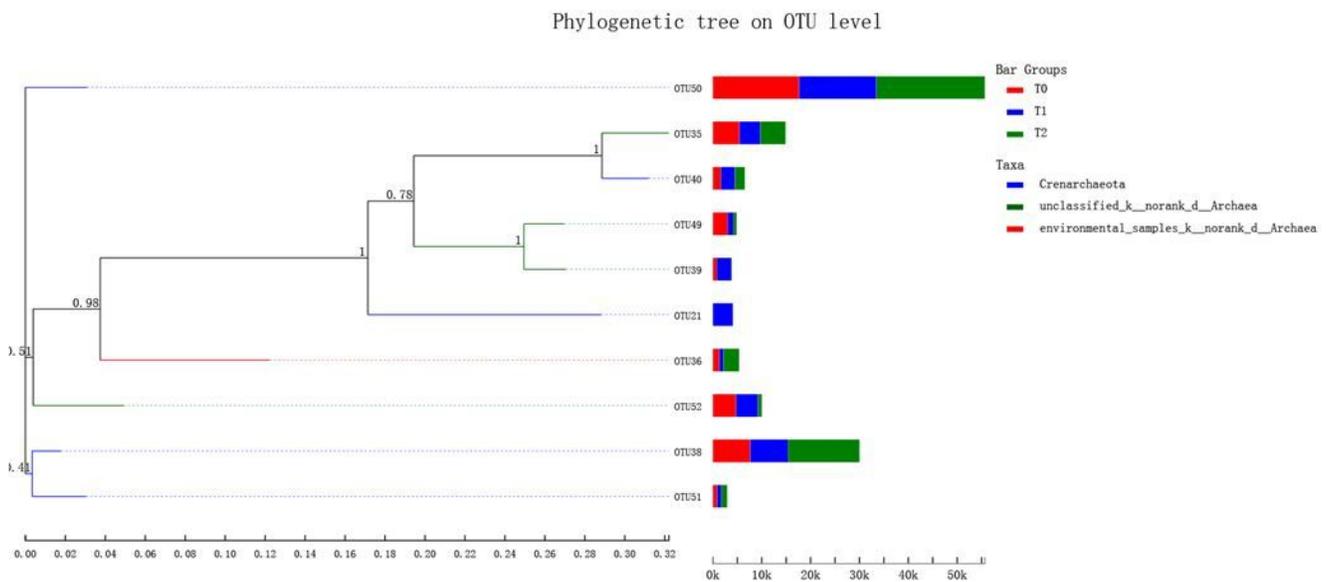
**Figure 5**

Partial least squares discriminant analysis (PLS - DA ) is an adaptation of PLS regression methods to represent differences in the community structure of ammonia-oxidizing archaea (AOA) that was associated with T0, T1, and T2. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization.



**Figure 6**

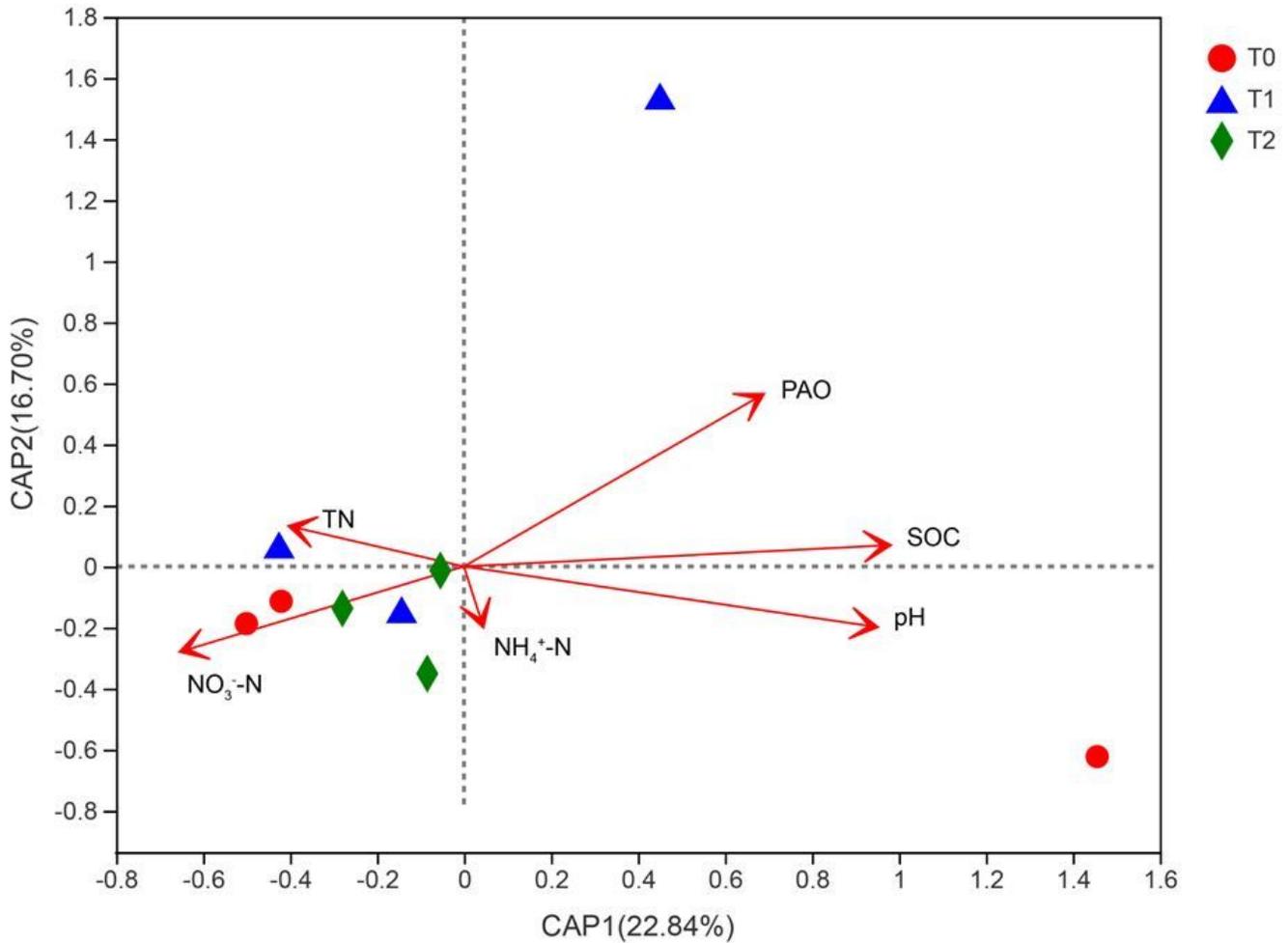
Relative abundance of soil ammonia-oxidizing archaea (AOA) phyla (A) and genera (B) under different fertilization and irrigation regimes. The relative abundance is expressed as the mean percentage of the targeted sequences to the total high-quality bacterial and fungal sequences of samples from triplicate plots of each irrigation and fertilization treatment. Notes: T0= Traditional irrigation; T1= Traditional irrigation and fertilization practice; T2= Water-saving irrigation and optimizing fertilization.



**Figure 7**

Neighbor-joining tree for representative AOA OTUs (representatives with relative abundance of the top 10 most abundant species). OTUs from this study were shown in bold, and bootstrap values of > 0.40 were shown at branch points. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization.

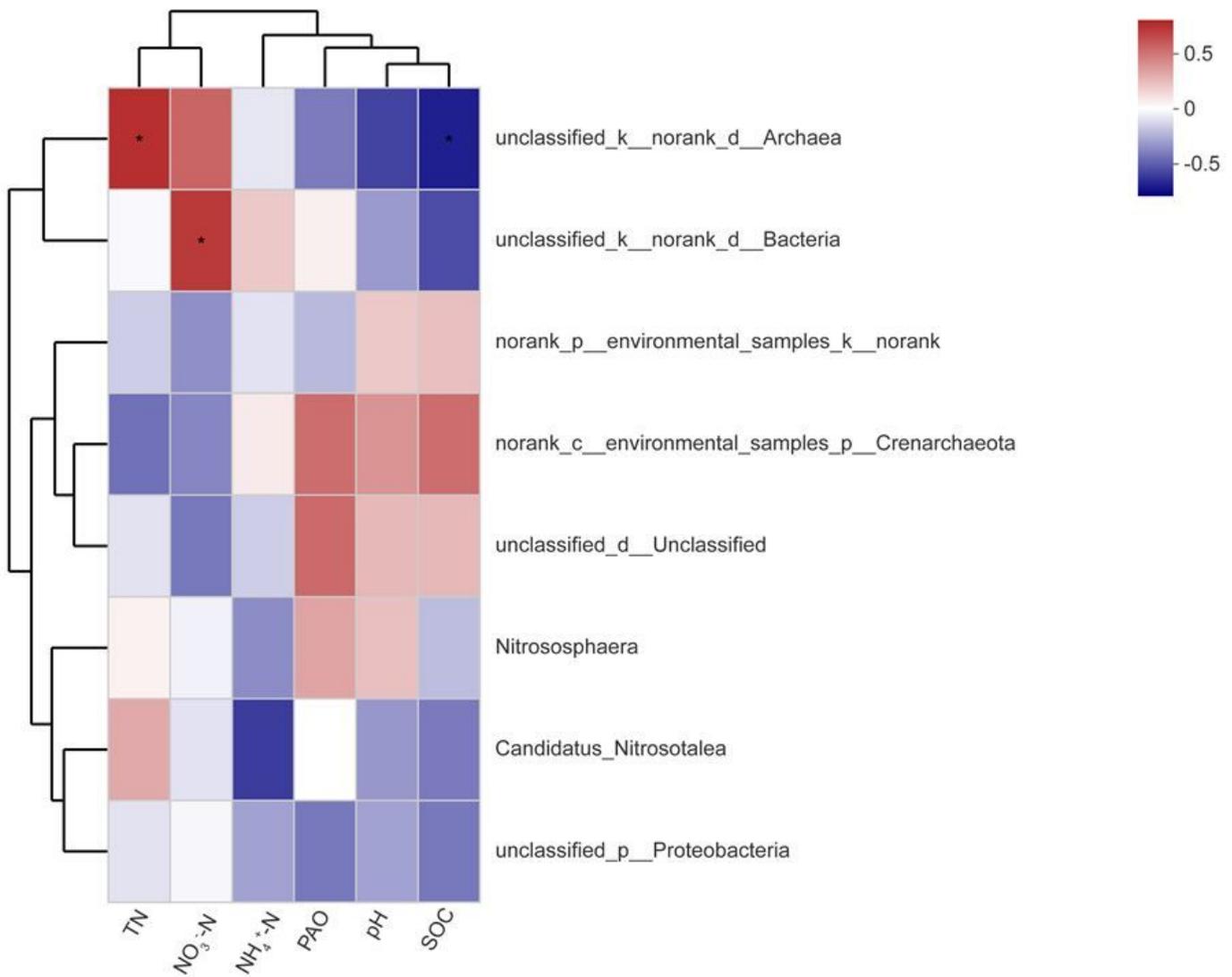
### db-RDA on OTU level



**Figure 8**

Distance-based redundancy analysis (db-RDA) of ammonia-oxidizing archaea (AOA) communities according to edaphic factors. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water - saving irrigation and optimizing fertilization. SOC: soil organic carbon; N: nitrogen; TN: total N; NO<sub>3</sub><sup>-</sup>-N: nitrate N; NH<sub>4</sub><sup>+</sup>-N: ammonium N; PAO: potential ammonia oxidation.

## Spearman Correlation Heatmap



**Figure 9**

Correlation heatmap of edaphic characteristics and relative genus abundances of ammonia-oxidizing archaea (AOA). The color intensity expresses the correlative values of edaphic characteristics with relative genus abundances. \*P < 0.05. Notes: T0 = Traditional irrigation; T1 = Traditional irrigation and fertilization practice; T2 = Water-saving irrigation and optimizing fertilization. SOC: soil organic carbon; N: nitrogen; TN: total N; NO<sub>3</sub>-N: nitrate N; NH<sub>4</sub><sup>+</sup>-N: ammonium N; PAO: potential ammonia oxidation.