Original article

Relationships between ammonia-oxidizing communities, soil methane uptake and nitrous oxide fluxes in a subtropical plantation soil with nitrogen enrichment

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ABSTRACT

Ammonia-oxidizers play an essential role in nitrogen (N) transformation and nitrous oxide (N₂O) emission in forest soils. It remains unclear if ammonia-oxidizers affect interaction between methane (CH₄) uptake and N₂O emission. Our specific goal was to test the impacts of changes in ammonia-oxidizing communities elicited by N enrichment on soil CH₄ uptake and N₂O emission. Based on a field experiment, two-forms (NH₄Cl and NaNO₃) and two levels (40 and 120 kg N ha⁻¹ yr⁻¹) of N were applied in the subtropical plantation forest of southern China. Soil CH₄ and N₂O fluxes, the abundance and structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) communities were measured using static chamber-gas chromatography, quantitative PCR (qPCR), and terminal-restriction fragment length polymorphism (T-RFLP). Nitrogen addition tended to inhibit soil CH₄ uptake, but significantly promoted soil N₂O emission; moreover, these impacts were more significant with NH₄⁺ – N than with NO₃⁻ – N addition. NH₄Cl addition significantly changed ammonia-oxidizer abundance with an increase in AOA and a decrease in AOB. Nitrogen additions significantly decreased the relative abundance of 329 bp and 421 bp of archaeal *amoA* gene. Negative relationships occurred between soil CH₄ uptake and AOA abundance and between soil CH₄ uptake and AOA/AOB ratio; however, a positive relationship was found between soil N₂O emission and AOA abundance. These results indicate that a shift in abundance and composition of ammonia-oxidizing communities is closely linked to changes in soil CH₄ uptake and N₂O emission under N enrichment. Furthermore, AOA communities play a contrasting role from AOB communities for regulating the fluctuation between soil CH₄ and N₂O fluxes.

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1. Introduction

Methane (CH₄) and nitrous oxide (N₂O) are two potent greenhouse gases, and their global warming potential (GWP) is 25 and 298 times as high as carbon dioxide (CO₂), respectively [1]. The

contribution of accumulated CH₄ and N₂O in the atmosphere to overall global warming is more than 25% of total CO₂ equivalent [1]. Global change strongly affects the capacity of undisturbed soils (i.e., forests, grasslands, shrubs) to act as atmospheric CH₄ sink and N₂O source [2]. Reactive nitrogen (N) content in the atmosphere and N deposition rate globally caused by human activities has increased by 11 fold and 2.5 fold since the 1860s, respectively [3]. Overall, the increased N deposition input to terrestrial ecosystems improves the net primary productivity, but inhibits CH₄ uptake and promotes N₂O emission in soils [4,5]. Considering the effects of N deposition on soil CH₄ uptake and N₂O emission, the carbon sequestration potential elicited by N deposition would be offset from 53% to 76% [2].

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Soil N_2O mainly originates from soil nitrification and denitrification processes [6]. NH_3 oxidation to NO_2^- , the first and rate-limiting step of nitrification, is catalyzed by two groups of prokaryotes, including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) [7–9]. CH_4 in the soil can be oxidized by both methane monooxygenase (MMO) and ammonia monooxygenase (AMO) that are synthesized by methanotrophs and ammonia-oxidizers, respectively [10]. Due to the homology of MMO and AMO enzymes [11], the same habitats [12,13], and a variety of analog substrates [14], both methanotrophs and AOB are simultaneously able to oxidize CH_4 and NH_3 in soils [11]. Pure culture studies provide the direct evidence for nitrification by methanotrophs [15] and for CH_4 oxidation by nitrifiers [16,17]. Moreover, a negative relationship between CH_4 oxidation and NH_3 oxidation is found, which could be partly due to competition for O_2 in soils [18]. Nevertheless, the above mechanisms are rarely examined and verified in the field. It is essential to explore the links between ammonia-oxidizers and soil CH_4 consumption and N_2O production, so as to clarify the mechanism responsible for the trade-off between CH_4 uptake and N_2O emission under N enrichment.

In China, the forest plantations cover an area of 6.26×10^7 ha, accounting for 31.8% of China's forest area and ranking first in the world [19]. Approximately 63% of forest plantations are distributed in the subtropical region of southern China [20]. This region has a high atmospheric N deposition rate ranging from 30 to $73 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ [21]. The humid subtropical forests are considered to be N-rich compared with the boreal and temperate forests, and respond differently to N enrichment [22]. Exogenous N inputs to the subtropical forests can significantly decrease soil CH_4 uptake [21,23] and increase N loss via NO_3^- – N leaching [24], as well as via gaseous N emission (N_2O , NO, and N_2) [25,26]. Experimental N deposition increases [27,28], decreases [29,30] or does not change [31–34] soil AOA abundance. The effects of N additions on soil AOB abundance are controversial, including positive [30,34,35], negative [36], and neutral effects [27,28]. Moreover, few studies on the contrasting effects of soil NH_4^+ – N and NO_3^- – N enrichment on ammonia-oxidizer communities are available [37]. Few studies have investigated the relationships between ammonia-oxidizers and N_2O emission in grassland [29,38] and agricultural soils [39]. Little information is available on links between ammonia-oxidizers and soil CH_4 uptake and N_2O emission in the subtropical forest plantation under N enrichment.

In this study, our objectives were (1) to examine the effects of NH_4^+ and NO_3^- fertilization on soil CH_4 and N_2O fluxes, as well as on the abundance and structure of ammonia-oxidizer communities in the subtropical plantation soils; and (2) to evaluate the relationships between the abundance of ammonia-oxidizer communities and soil CH_4 and N_2O fluxes. Since AOA adapts well to a lower pH environment and requires a lower NH_3 concentration compared with AOB [40], we hypothesized that (1) AOA dominated the soil ammonia oxidation process in the acid subtropical plantation forest soil, and NH_4^+ – N fertilization could have a greater effect on soil ammonia-oxidizer communities than NO_3^- – N fertilizations; and (2) N additions would inhibit CH_4 uptake and promote N_2O emissions through changing the abundance and structure of ammonia-oxidizer communities. To test these two hypotheses, we *in situ* investigated soil CH_4 and N_2O fluxes, and ammonia-oxidizer communities using field observation and a molecular biology method based on a N addition experiment.

2. Materials and methods

2.1. Site description

This experiment was conducted in a subtropical slash pine

(*Pinus elliottii*) plantation at the Qianyanzhou Ecological Station ($26^\circ 44' 39'' \text{N}$, $115^\circ 03' 33'' \text{E}$), located in Taihe city, Jiangxi province, China. The monsoon climate dominates the region with a mean annual temperature of 17.9°C and a mean annual precipitation of 1505 mm. Little rainfall and high temperatures in late summer often result in seasonal droughts [41]. Slash pine was planted in 1985 with a mean diameter at breast height (DBH) of 16.0 cm and total living biomass of 104.13 t ha^{-1} [42]. Soils are typically red soils, classified as Cambosols (IUSS classification), which were derived from sandstone and sandy conglomerate [43]. Topsoil (0–20 cm) organic C is 20.44 g kg^{-1} , total N is 1.10 g kg^{-1} , total phosphorus is 1.12 g kg^{-1} , pH is 4.26, and soil bulk density is 1.54 g cm^{-3} .

2.2. Experimental design

The N addition experiment is a randomized block design with three replicates. To simulate the effects of deposited NH_4^+ and NO_3^- on soil microbial communities and biogeochemical cycles, two fertilizers (NH_4Cl and NaNO_3) were added at two rates: 40 and $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. There were five treatments, including a control, low- NH_4Cl , low- NaNO_3 , high- NH_4Cl , and high- NaNO_3 , per block respectively. A total of 15 plots ($20 \text{ m} \times 20 \text{ m}$) were established, each surrounded by a 10-m-wide buffer strip. Each month, 509.6 g and 1528.8 g NH_4Cl , as well as 809.5 g and 2428.5 g NaNO_3 were weighted and dissolved in 40 L of water for low and high N treatments, respectively. N fertilizer solutions were sprayed below the canopy during the first week of every month, and the control plots only received equivalent amounts of water, which is equivalent to an increase in annual precipitation of 1.2 mm [23]. The experiment was carried out over 1.5 years beginning in May 2012 and ending in October 2013.

2.3. Measurement of soil CH_4 and N_2O fluxes

Soil CH_4 and N_2O fluxes were measured using a static opaque chamber and gas chromatography techniques [23]. In each plot, a stainless steel collar ($50 \text{ cm} \times 50 \text{ cm} \times 10 \text{ cm}$) was permanently inserted into the soil to a depth of 10 cm and remained intact. Chambers (length $\times$ width $\times$ height = $50 \text{ cm} \times 50 \text{ cm} \times 15 \text{ cm}$) were temporarily mounted onto the frames for gas sampling. The soil CH_4 and N_2O fluxes were measured twice a week and conducted between 9:00 and 11:00 am (China Standard Time, CST). Five gas samples from the chamber headspace were collected at 10 min intervals using 100 ml plastic syringes during 40 min. The concentrations of CH_4 and N_2O in the gas samples were analyzed within 24 h using gas chromatography (Agilent 7890A, USA). Soil CH_4 and N_2O fluxes were calculated based on the rate of change in concentration within the chamber, which was estimated as the slope of linear or nonlinear regression between concentration and time [44]. All the coefficients of determination (r^2) of the regression were greater than 0.90 in this study. The flux measurements began in May 2012, but only the flux data from January to October 2013 were used to perform this study.

2.4. Soil sampling, DNA extraction, and quantitative PCR

In September 2013, soil samples (0–20 cm) were collected, and in five subsamples were pooled to make one composite sample from each plot. All soil samples were passed through a 2.0 mm sieve in the field, and then transported to the lab in a biological refrigerator. Soil samples were stored at -80°C before analysis.

Soil DNA was extracted from a 0.5 g soil using the Fast DNA[®] SPIN Kit for soil (Qbiogen Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was checked on a 1% agarose gel and the DNA concentration was assessed using a

Nanodrop® ND-1000 UV–Vis Spectrophotometer (NanoDROP Technologies, Wilmington, DE, USA). Ten fold diluted DNA was used in PCR analysis.

The *amoA* genes of AOA and AOB were amplified using the primer pairs *amoA1F/amoA2R* [45] and *Arch-amoAF/Arch-amoAR* [46], respectively. Real-time PCR was performed on an iCycler iQ5 Thermo-cycler (Bio-Rad Laboratories, Hercules, CA, USA) as follows: 95 °C for 1 min, followed by 35 cycles of 10 s at 95 °C, 30 s at 53 °C for AOA and 55 °C for AOB, 1 min at 72 °C and plate read at 83 °C [34]. Amplification was performed in 25 µl reaction mixtures, including 12.5 µl SYBR® Premix Ex Taq™ (Takara Biotechnology, Dalian, China), 1 µl bovine serum albumin (25 mg ml⁻¹), 0.5 µl each primer (10 µmol l⁻¹), and 2 µl of tenfold-diluted DNA as a template [34]. Amplification of DNA fragments for the correct size was confirmed by a dissociation curve analysis and agarose gel electrophoresis. A known copy number of plasmid DNA for AOA or AOB was used to generate a standard curve. For all assays, PCR efficiency was 90–100% and the *r*² was 0.95–0.99.

2.5. Terminal restriction fragment length polymorphism (T-RFLP)

For T-RFLP analysis, the PCR amplification was performed using the same primer pairs as the qPCR assays described above, with each forward primer labeled with 6-carboxyfluorescein (FAM). The 50 µl PCR reactions contained 5 µl 10 × PCR buffer (Mg²⁺ plus), 4 µl 2.5 mmol l⁻¹ dNTPs, 0.5 µl Ex Taq HS polymerase (5 U µl⁻¹, Takara Biotechnology, Dalian, China), 1 µl of each primer, and 4 µl tenfold-diluted DNA template. The PCR reaction was the same as that for the qPCR assays. PCR productions were gel-purified with the Wizard® SV Gel and PCR Clean-Up Kit (Promega, San Luis Obispo, CA, USA) and then digested with restriction enzyme *FastDigest*® *Mbo* I (Takara Biotechnology, Dalian, China) at 37 °C for 5 min and then at 65 °C for 15 min. The digest products were purified by ethanol precipitation and mixed with deionized formamide and then determined with an Abi Prism® 3130XL Genetic Analyzer (Applied Biosystems). The peak heights were automatically analyzed using GeneScan analysis software 3.7 (Applied Biosystems). The relative abundance of individual terminal restriction fragment (T-RF) was calculated as the percentage of total peak height in a given T-RFLP profile. Only those T-RFs with a relative abundance >1% and fragment lengths in the range of 50–500 bp were considered in further analysis [34].

2.6. Statistical analyses

Repeated measures analysis of variance (ANOVA) with least significant difference (LSD) test was applied to examine the differences of soil CH₄ and N₂O fluxes among the five treatments. Experimental treatment was set as between-subjects factor and measurement date was selected as within-subjects variable. We performed a one-way ANOVA with a LSD test to evaluate the effects of N addition on soil microbial *amoA* gene copy numbers and specific T-RFs. Linear regression analyses were used to examine the relationships between the monthly average of soil CH₄ and N₂O fluxes and the microbial *amoA* gene copy numbers. All statistical analyses were conducted using the SPSS software package (version 16.0), and significant differences were set with *P* values < 0.05 unless otherwise stated. All figures were drawn using the SigmaPlot software package (version 10.0).

3. Results

3.1. Soil CH₄ and N₂O fluxes

Soil CH₄ flux exhibited a significant temporal variation

(*P* < 0.001, Table 1, Fig. 1a–c). Over the measurement period, both soil CH₄ emission and uptake were observed in the control plots and ranged from 4.09 to –33.07 µg C m⁻² h⁻¹, with an average of –5.01 µg C m⁻² h⁻¹ (Fig. 1d). For a certain observation date, neither N levels nor N forms changed soil CH₄ flux (*P*₁ = 0.73, *P*₂ = 0.38, Table 1). Taking all observation data into account, we found that the forms of N addition significantly changed soil CH₄ flux (*P* = 0.033, Table 1). Comparing the control with the high level of NH₄Cl addition there was a significant inhibition on soil CH₄ uptake (Fig. 1b).

Soil N₂O fluxes significantly varied monthly with the maximum occurring in May, 2013 (Fig. 2a–c, Table 1, *P* < 0.001). Soil N₂O fluxes in the control plots ranged from –15.26 to 23.68 µg N m⁻² h⁻¹, with an average of 5.67 µg N m⁻² h⁻¹ (Fig. 2d). For a certain observation date, both N level and N form significantly changed soil N₂O fluxes (*P*₁ = 0.021, *P*₂ = 0.011, Table 1). N addition significantly promoted soil N₂O emissions (Fig. 2d). Relative to the control, low and high levels of NH₄Cl additions increased soil N₂O fluxes by 774% and 1328%, respectively, while the increases in soil N₂O fluxes elicited by low and high levels of NaNO₃ addition were only 546% and 720%, respectively (Fig. 2d).

3.2. Abundance of soil AOA and AOB communities

N addition tended to increase soil AOA *amoA* gene copy numbers (Table 2). The highest AOA *amoA* gene copy numbers occurred in the high-NH₄Cl treatment, which was 15% higher than that of the control (Table 2). Conversely, N additions tended to decrease the abundance of AOB communities in the subtropical plantation forest soils, and the difference between low-NH₄Cl treatment and the control was significant (Table 2). Except low-NaNO₃ treatment, N addition significantly increased the ratio of AOA to AOB abundance (Table 2).

3.3. Structure of soil AOA community

The determination of T-RFLP for the AOB community was not performed due to the low abundance of AOB *amoA* genes. In contrast, four T-RFs of AOA were detected from all soil samples using the enzyme *FastDigest*® *Mbo* I. N levels rather than N forms significantly changed the relative abundance of 329 bp and 421 bp (Table 3). Low levels of NH₄Cl and NaNO₃ additions significantly decreased the relative abundance of 329 bp by 81.82% and 91.02%, respectively (Table 3). High levels of NH₄Cl and NaNO₃ additions significantly decreased the relative abundance of 421 bp by 74.82% and 70.67%, respectively. Furthermore, low and high levels of NH₄Cl additions tended to increase the relative abundance of 443 bp (Table 3).

Table 1

Results of repeated ANOVA measurements showing the effects on date, N level, N form, and their interactions on soil CH₄ and N₂O fluxes.

Source of variation	Soil CH ₄ flux		Soil N ₂ O flux	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>Between subjects</i>				
N level	0.94	0.37	4.45	0.045
N form	7.04	0.033	4.09	0.054
N level × N form	0.13	0.73	0.85	0.37
<i>Within subjects</i>				
Date	5.36	<0.001	6.96	<0.001
Date × N level	0.66	0.73	1.56	0.021
Date × N form	1.09	0.38	3.65	0.011
Date × N level × N form	0.65	0.74	0.91	0.53

Fig. 1. Variation of soil CH_4 fluxes under control (a), low and high NH_4Cl (b), and low and high NaNO_3 (c), and arithmetic mean under the five experimental treatments (d). The data is the mean and standard errors. Different letters below the columns mean significant difference among the experimental treatments.

Fig. 2. Variation of soil N_2O fluxes under control (a), low and high NH_4Cl (b), and low and high NaNO_3 (c), and arithmetic mean under the five experimental treatments (d). The data is mean and standard error. Different letters above the columns means significant difference among the experimental treatments.

Table 2Soil AOB and AOA *amoA* gene copy numbers and their ratios under different experimental treatments.

Treatment	Log number of archaeal <i>amoA</i> (copies g ⁻¹ dry soil) ^a	Log number of bacterial <i>amoA</i> (copies g ⁻¹ dry soil) ^a	Log ratio of AOA to AOB ^a
Low-NH ₄ Cl	7.43 ± 0.13 ab	6.51 ± 0.03 b	1.14 ± 0.02 ab
High-NH ₄ Cl	7.79 ± 0.30 a	6.69 ± 0.12 ab	1.16 ± 0.03 a
Low-NaNO ₃	7.02 ± 0.09 ab	6.82 ± 0.05 a	1.03 ± 0.02 bc
High-NaNO ₃	7.43 ± 0.22 ab	6.59 ± 0.08 ab	1.13 ± 0.03 ab
Control	6.77 ± 0.41 b	6.85 ± 0.11 a	0.99 ± 0.07 c

^a Data are mean ± SE. Lowercase letter in the same column represents significant differences among experimental treatments at the level of 0.05.**Table 3**Relative abundances of T-RFs of AOA *amoA* gene restricted by Mbo I under different experimental treatments.

Treatment	T-RFs of AOA <i>amoA</i> genes ^a			
	329 bp	370 bp	421 bp	443 bp
Low-NH ₄ Cl	5.86 ± 1.71 b	49.78 ± 15.28 a	26.45 ± 8.15 ab	17.91 ± 6.58 a
High-NH ₄ Cl	24.24 ± 5.12 a	47.45 ± 5.19 a	9.54 ± 2.46 b	18.77 ± 3.09 a
Low-NaNO ₃	2.25 ± 0.81 b	44.29 ± 16.78 a	37.56 ± 16.30 a	15.91 ± 5.21 ab
High-NaNO ₃	27.61 ± 5.89 a	45.00 ± 11.54 a	11.11 ± 3.35 b	16.28 ± 3.22 ab
Control	32.23 ± 8.11 a	23.38 ± 1.90 a	37.88 ± 10.57 a	6.50 ± 0.56 b

^a Data are mean ± SE. Lowercase letter in the same column presents significant difference among experimental treatments at the level of 0.05.

3.4. Relationships between soil fluxes and ammonia-oxidizer abundances

The monthly average of soil CH₄ fluxes, N₂O fluxes, AOA *amoA* gene copy numbers, and AOB *amoA* gene copy numbers in soils collected in September 2013 were used to perform the regression analysis. Soil AOA *amoA* gene copy numbers were negatively correlated with soil CH₄ uptake fluxes, whereas they were positively correlated with soil N₂O emission fluxes (Fig. 3a and b). The relationships between AOA *amoA* gene copy numbers and soil CH₄ fluxes and between AOA *amoA* gene copy numbers and soil N₂O fluxes were well fitted to linear equations (Fig. 3a and b, Table 4). On the contrary, soil AOB *amoA* gene copy numbers were positively correlated with soil CH₄ fluxes only (Fig. 3c and d, Table 4). Furthermore, the log ratio of AOA to AOB *amoA* gene abundances was negatively correlated with soil CH₄ uptake, whereas a positive relationship was observed for N₂O emissions (Fig. 3e and f, Table 4).

3.5. Relationships between soil pH and ammonia-oxidizer abundances

Although low and high levels of NH₄Cl additions tended to decrease soil pH, the difference between N addition treatments and the control was not significant over the short term (Fig. 4a). A significant and negative relationship between the log AOA *amoA* gene copy numbers and soil pH values, and the later could explain 65% of the variation of the former (Fig. 4b). However, no significant correlation was observed between soil pH and AOB *amoA* gene abundance (Fig. 4c).

4. Discussion

4.1. Unamplified AOB population

The relative abundance of soil AOA greatly outnumbered AOB in this study. Numerous studies suggest that AOA is ubiquitous [7,47], and their numerical dominance is over their bacterial counterparts in multiple environments, especially in acidic soils [27,40]. Soil AOB gene sequences were significantly amplified and detected from two of fifteen soil samples, despite using the FastDigest[®] Mbo I enzyme, a procedure that was successfully applied to the typical steppe soils in northern China [34]. Other studies reported that AOB was not

detected in the acidic forest soils, either [33,36]. This is partly attributed to the low soil pH in the subtropical region (Fig. 4). Soil acidification often decreases soil NH₃ concentration, and NH₃ is the main substrate dominating the growth and ecological niche of soil AOA and AOB communities [48]. Also, the affinity of AOA for NH₃ is more than 200 times higher than that of AOB strains, which means AOA has the specific ability to use low-level NH₃ and efficiently compete the limited NH₃ substrate with AOB [48,49].

4.2. Effects of N addition on ammonia-oxidizer communities

High-NH₄Cl additions significantly increased soil AOA abundances, and low-NH₄Cl addition significantly decreased soil AOB abundances; however, neither soil AOA nor AOB abundances were changed by NO₃⁻ – N fertilization (Table 2). These indicate that the effects on soil ammonia-oxidizer communities in the subtropical plantation are stronger by deposited NH₄⁺ – N than by deposited NO₃⁻ – N. First, it is because NH₄⁺ – N fertilization directly increases soil NH₄⁺ – N levels, and NH₄⁺/NH₃ serves as the direct substrate to ammonia monooxygenase in NH₃ oxidation [50]. On the contrary, several studies documented that both NH₄⁺ – N and NO₃⁻ – N fertilizations increased soil AOB abundances in the alpine meadow [35], and the farmlands [32]. These inconsistent results could be attributed to the differences of climatic regions (warm-wet vs. cold-dry), soil pH (acid vs. alkaline), and initial status of soil mineral N (e.g., NH₄⁺/NO₃⁻ < 1 vs. NH₄⁺/NO₃⁻ > 1) [40]. Second, soil acidification could be another reasons leading to the shift of soil ammonia-oxidizer communities. The absorption and assimilation of NH₄⁺ by plants and microorganisms, as well as the leaching of NO₃⁻ and other base cations can release H⁺, which leads to soil acidification; moreover, the promotion to soil acidification by NH₄⁺ – N fertilization is generally greater than by NO₃⁻ – N fertilization due to their contrasting pH, soil nitrification, plant preference for NH₄⁺. Theoretically, the decrease in soil pH caused by N additions reduces soil NH₃ availability because of the formation of ionized NH₄⁺ [51,52]. The concentration of ammonia substrate in soils is considered below the growth threshold of cultured AOB in soil when pH is below 4.5, and this is in turn favorable to the growth of high-affinity AOA community [33,50]. Soil pH at all treatment plots was below 4.5 (Fig. 4a). Therefore, the competition for limited NH₃ substrate between AOA and AOB communities would be intensified under N enrichment, which leads to the

Fig. 3. Relationships between soil CH₄ and N₂O fluxes and AOA, and AOB *amoA* gene copy numbers, as well as between soil CH₄ and N₂O fluxes and the ratios of AOA to AOB abundances.

Table 4

Regression models between the soil CH₄ and N₂O fluxes and the abundances of ammonia-oxidizer communities.

Soil flux	Ammonia-oxidizers	Regression equation	R ²	P Value
CH ₄ flux	AOA (X ₁)	$Y = -3.75X_1 + 32.21$	0.27	0.041
	AOB (X ₂)	$Y = 7.85X_2 - 47.41$	0.35	0.026
	AOA/AOB (X ₃)	$Y = -21.31X_3 + 27.74$	0.25	0.041
N ₂ O flux	AOA (X ₁)	$Y = 32.51X_1 - 228.55$	0.63	<0.001
	AOA/AOB (X ₃)	$Y = 157.93X_3 - 163.08$	0.47	0.003

degeneration of AOB community. The significant relationship between soil ammonia-oxidizer abundances and soil pH values clearly reflected the interaction (Fig. 4b). These results suggest that soil AOA communities play more important roles than AOB communities in N transformation in the subtropical plantation soils and responded differently to N addition.

Besides altering the abundances of ammonia-oxidizer communities, N additions significantly changed the structure of the soil AOA community. Similar results were found in the subtropical acidic forest and agricultural soils [27,53], and in the temperate alkaline grassland and agricultural soils [31,34]. Although we did

not determine the changes in soil ammonia-oxidizer community composition caused by N additions using clone libraries and phylogenetic tree, the *Nitrososphaera* cluster could dominate the AOA community referring to the results from both acidic and alkaline soils [31,34,53,54]. Tourba et al. [55] also documented that *Nitrososphaera* can utilize ammonia or urea as an energy source and grow well in a high-ammonia environment. In order to clarify the coupling between soil methanotrophs and N₂O-producing bacteria communities, further study is needed to examine their activities and compositions using cloning and sequence analysis.

4.3. Linkages between soil CH₄ and N₂O fluxes and ammonia-oxidizer communities

Nitrogen additions significantly promoted soil N₂O emissions and tended to inhibit soil CH₄ uptake in the subtropical plantation; furthermore, significant relationships between soil N₂O and CH₄ fluxes and ammonia-oxidizer abundances were observed. Taking all treatments into account, we found that soil CH₄ uptake decreased with the increase in AOA *amoA* gene abundances, whereas increased with the AOB *amoA* gene abundances (Fig. 3a

Fig. 4. Variation of soil pH at different experimental treatments, and relationships between soil *amoA* gene copy numbers and soil pH values.

and b); however, an opposite relationship was found between soil N₂O emission and AOA abundance (Fig. 3d). These indicate that the increase in AOA *amoA* gene abundance might increase the activities of soil ammonia-oxidizing microorganisms and the decrease in AOB *amoA* gene abundance perhaps decrease methane-oxidizing microorganisms, thereby indirectly promoted soil N₂O emission and inhibited soil CH₄ uptake under N enrichment. Therefore, we could modify soil CH₄ and N₂O emission by regulating the abundance and composition of ammonia-oxidizer communities (e.g., using nitrification inhibitors) [29,38,56].

The potential mechanisms involved in the above responses are complicated. First, soil CH₄ can only be oxidized by bacterial AMO rather than archaeal AMO [57], and the decrease in AOB abundance under N enrichment would directly decrease soil CH₄ uptake. Second, O₂ availability in the soil profile constrains CH₄ and NH₃ oxidation and the competition of soil ammonia-oxidizers is stronger than that of soil methanotrophs [11]. Soil ammonia oxidation is dominated by archaeal AMO. NH₃ oxidation by soil AOA consumes a large number of O₂ in soils, which in turn inhibits soil CH₄ oxidation and favors soil denitrification [9,50]. One of our previous results demonstrated that a high level of NH₄Cl addition significantly led to soil NO₃⁻ accumulation, which indirectly indicates a high O₂ consumption caused by a high nitrification rate [23]. Using ¹⁵N-tracing experiment and ¹⁵N-tracing models, denitrification was believed to be the largest source of N₂O flux relative to nitrification and other N transformations in the subtropical forest soils [58,59]. On the other hand, accelerated nitrification by NH₄⁺ additions gradually decreased soil pH, which would result in higher N₂O/N₂ ratio during soil denitrification [60]. However, other microorganisms (e.g., methane oxidizers, methanogens and denitrifiers) were also involved in the production and consumption of soil CH₄ and N₂O. The significant correlations between ammonia-oxidizer communities and soil CH₄ and N₂O fluxes only indicated their close linkages. Other microorganisms, especially methanotrophs, also have the capability to oxidize CH₄ and NH₃ in soils. To obtain a clear picture of the interaction between soil CH₄ uptake and N₂O emission under N enrichment, further study is required to address the *pmoA* genes of methanotrophs.

5. Conclusions

This study investigated the effects of NH₄⁺ – N and NO₃⁻ – N fertilization on the abundance and structure of soil ammonia-oxidizer communities, soil CH₄ and N₂O fluxes, and their linkages in the subtropical plantation of southern China. We found that N addition increased AOA abundance, decreased AOB abundance, and altered composition of ammonia-oxidizer communities, which could explain to a certain extent the inhabitation to CH₄ uptake and the promotion to N₂O emission from the subtropical plantation soil. Moreover, NH₄⁺ – N fertilization had stronger effects than NO₃⁻ – N fertilizer additions. Our study confirmed the close linkages between soil ammonia-oxidizers and soil CH₄ and N₂O fluxes, as well as the different roles of soil AOA and AOB communities in regulating soil CH₄ uptake and N₂O emission. Unfortunately, the measured soil CH₄ and N₂O exchange fluxes are combined results of several production and consumption processes, which unable to establish direct links between reaction rates and functional microbial activities. Further study is necessary to measure the gross N transformation rates, CH₄ production and oxidation rates, and the abundance and composition of related functional microbial community in ambient and N enrichment soils.

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