Effects of Nitrogen Fertilizer Levels on Bacterial Communities and N2O Emissions in Paddy Fields: Postprint

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Abstract

As drivers of soil nitrogen transformation, microbial community structure is related to nitrogen utilization and greenhouse gas N2O emissions in paddy fields. This study analyzed changes in paddy field bacterial communities and the abundance of key microbial functional genes for nitrification and denitrification under different nitrogen fertilizer levels [CK (no nitrogen application), N (N application 180 $kg \cdot hm^{-2}$), 2/3N (N application 120 $kg \cdot hm^{-2}$), 1/3N (N application 60 $kg \cdot hm^{-2}$)] using high-throughput sequencing and real-time quantitative PCR techniques. The results showed that increased nitrogen fertilizer levels enhanced the Chao1 index of bacterial species richness and Shannon index of community diversity in paddy fields, and altered bacterial community composition. Specifically, the relative abundance of the nitrification-related phylum Nitrospirae and the acidophilic phylum Acidobacteria increased with nitrogen fertilizer level, whereas the relative abundance of the methanotroph Methylosinus decreased with nitrogen fertilizer level. Nitrogen fertilizer level had a substantial effect on the abundance of ammonia-oxidizing bacterial amoA gene, a key functional gene for nitrification; amoA gene abundance in both the 0–5 cm and 10–20 cm soil layers increased with nitrogen application rate. The abundances of denitrification-related functional genes nirS, qnoB, and nosZ were significantly lower in the no-fertilizer treatment (CK) than in fertilized treatments (1/3N, 2/3N, and N) ($P<0.05$), but nirS gene abundance showed no significant differences among the 1/3N, 2/3N, and N treatments. The abundances of qnoB and nosZ genes in the 0–5 cm soil layer tended to increase with nitrogen fertilizer level, while nosZ gene abundance in the 10–20 cm soil layer was significantly higher under 2/3N and N treatments than under the 1/3N treatment ($P<0.05$). The N2O emission flux from paddy fields under N treatment was significantly higher than that under 2/3N and 1/3N treatments ($P<0.05$), which in turn were significantly higher than that under CK treatment ($P<0.05$). Correlation analysis results indicated that paddy field N2O emission flux was significantly correlated with the relative abundance of phylum Nitrospirae in the 0-5 cm soil layer and amoA gene abundance in the 10-20 cm soil layer ($P<0.05$, n=10). In summary, increased nitrogen fertilizer levels enhanced bacterial community diversity in paddy fields and promoted N2O emissions, and changes in nitrifying microbial communities and their abundance were more closely related to N2O emissions in the paddy fields examined in this study.

Full Text

Preamble

Effect of Nitrogen Fertilizer Level on Bacterial Community and N2O Emission in Paddy Soil

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Abstract: Microbial community structures are critical drivers of nitrogen utilization and nitrous oxide (N2O) emission in paddy soils. Nitrification and denitrification represent the primary pathways for N2O production in soils, mediated by nitrifying and denitrifying bacteria, respectively. This study investigated changes in bacterial communities and the abundance of key microbial functional genes involved in nitrification and denitrification under different nitrogen fertilizer levels [CK (no N fertilization), 1/3N (60 kg $N \cdot hm^{-2}$), 2/3N (120 kg $N \cdot hm^{-2}$), and N (180 kg $N \cdot hm^{-2}$)] using high-throughput sequencing and real-time PCR. The analysis of nitrifying and denitrifying bacteria focused on the ammonia-oxidizing bacterial *amoA* gene and denitrifying bacterial *nirS*, *qnoB*, and *nosZ* genes. Results showed that increased nitrogen application enhanced bacterial species richness (Chao1 index) and community diversity (Shannon index) while altering bacterial community composition. The relative abundances of nitrification-associated *Nitrospirae* and acidophilic *Acidobacteria* increased with nitrogen fertilizer levels, whereas the methanotroph *Methylosinus* decreased. Nitrogen fertilizer level significantly affected *amoA* gene abundance, which increased with nitrogen application in both 0-5 cm and 10-20 cm soil depths. The abundances of *nirS*, *qnoB*, and *nosZ* genes in unfertilized soil (CK) were significantly lower than in fertilized soils (1/3N, 2/3N, and N) ($P < 0.05$), though *nirS* gene abundance showed no significant differences among fertilized treatments. In the 0-5 cm layer, *qnoB* and *nosZ* gene abundances tended to increase with nitrogen level, while in the 10-20 cm layer, *nosZ* abundance was significantly higher under 2/3N and N than under 1/3N ($P < 0.05$). N2O emission flux under N treatment was significantly higher than under 2/3N and 1/3N ($P < 0.05$), which were in turn significantly higher than CK ($P < 0.05$). Correlation analysis revealed significant relationships between N2O emission flux and *Nitrospirae* relative abundance in the 0-5 cm layer and *amoA* gene abundance in the 10-20 cm layer ($P < 0.05$, $n = 10$). In summary, elevated nitrogen application increased bacterial community diversity and promoted N2O emission in paddy soils, with changes in nitrifying microbial communities and abundances showing stronger associations with N2O emissions.

Keywords: Nitrogen fertilizer level; Paddy soil; Microbial community; Ammonia-oxidizing bacterial gene; Denitrifying bacterial gene; Nitrous oxide emission; High-throughput sequencing; Real-time PCR

Introduction

Nitrogen fertilizer is not only crucial for increasing crop yields but also a key determinant of N2O emissions from agricultural soils. N2O is a significant agricultural greenhouse gas, with upland and unsaturated paddy soils being major emission sources. Due to increased nitrogen fertilizer inputs, agricultural N2O emissions are projected to rise by 35-60% by 2030 [?]. Microbially-driven nitrification and denitrification are the two most important pathways for soil N2O release [?]. During the ammonia oxidation step of nitrification, N2O is produced as an intermediate via hydroxylamine, with microorganisms containing the ammonia monooxygenase gene *amoA* serving as the primary drivers [?]. Denitrifying bacteria produce N2O during the reduction of NO_3^--N or NO_2^--N to N2, with nitrite reductase (*nirS*), nitric oxide reductase (*qnoB*), and nitrous oxide reductase (*nosZ*) genes commonly used as markers for detecting denitrifiers in the environment [?]. Previous studies have shown that nitrogen application increases *amoA* gene abundance in paddy soils [?], but effects on denitrifying functional gene diversity vary. For instance, long-term nitrogen fertilization significantly increased *nirS* gene diversity in red paddy soils [?], while nitrogen application altered *nosZ* abundance in purple calcareous paddy soil but not in red-yellow clay paddy soil [?]. Given that nitrification and denitrification directly affect N2O emissions, the relationship between functional gene diversity of nitrifying and denitrifying microorganisms and N2O flux warrants further investigation.

High-throughput sequencing technology offers substantial advantages over conventional DNA fingerprinting techniques, providing greater data throughput and information capacity. This technology enables large-scale direct sequencing of soil microbial genes, offering rich insights into microbial species, structure, function, and genetic diversity [?]. High-throughput sequencing has been widely applied in paddy soil bacterial diversity studies. For example, research on biochar effects in red paddy soils revealed increasing trends in plant growth-promoting bacteria [?], while Illumina MiSeq sequencing of bacterial 16S rRNA conserved regions showed that rotation systems had far greater microbial richness (Chao1) than continuous cropping [?]. Application of this technology will undoubtedly advance research on microbial diversity related to N2O release in paddy soils.

Chemical nitrogen fertilizer is a critical factor controlling N2O emissions from croplands. Estimates from 378 sites across China, including single-crop rice, rice-upland rotations, and double-crop rice systems, demonstrate that nitrogen application rate has extremely significant effects on emission outcomes [?], with nitrogen fertilization causing short-term N2O emission spikes [?]. However, the role of soil microorganisms in nitrogen-induced N2O emissions remains unclear, particularly regarding how changes in nitrifying and denitrifying microbial community structures influence emissions. This study employed advanced molecular techniques to analyze paddy soil microbial community changes and their impacts on N2O emissions. Using a field experiment, we applied high-throughput sequencing and real-time PCR to characterize bacterial taxa, community structure, diversity, and functional gene abundances of nitrifying and denitrifying bacteria under different nitrogen levels, evaluating nitrogen fertilizer effects on microbial community structure and function and their relationships with N2O emissions. This research provides a theoretical basis for understanding greenhouse gas emissions in paddy nitrogen biogeochemical cycles and sustainable agricultural development.

1.1 Field Experiment

The study was conducted in a paddy field at the Fujian Academy of Agricultural Sciences experimental base in Qianyang Village, Fuzhou, Fujian Province (26°11 N, 119°16 E). The tested soil was red soil with the following basic physicochemical properties: organic matter 15.77 $g \cdot kg^{-1}$, pH 5.82, total nitrogen 1.28 $g \cdot kg^{-1}$, total phosphorus 0.27 $g \cdot kg^{-1}$, total potassium 18.35 $g \cdot kg^{-1}$, alkaline hydrolyzable nitrogen 153.6 $mg \cdot kg^{-1}$, available phosphorus 15.67 $mg \cdot kg^{-1}$, and available potassium 77.98 $mg \cdot kg^{-1}$.

Four nitrogen treatments were established based on conventional local nitrogen rates: N [180 $kg(N) \cdot hm^{-2}$], 2/3N [120 $kg(N) \cdot hm^{-2}$], 1/3N [60 $kg(N) \cdot hm^{-2}$], and control CK (no N, P, or K fertilization). Urea served as the nitrogen source, with calcium superphosphate and potassium chloride as phosphorus and potassium fertilizers, respectively. All P and K fertilizers plus half of the nitrogen were applied as basal fertilizer, with the remaining half topdressed at the late tillering stage. Phosphorus and potassium rates were 50 $kg(P_2O_5) \cdot hm^{-2}$ and 100 $kg(K_2O) \cdot hm^{-2}$, respectively. Each treatment had three replicates, with 12 field plots (20 m^2 each) arranged randomly. Rice planting density was 175,000 $plants \cdot hm^{-2}$ using the cultivar 'Huifengyou 3301' (Oryza sativa L.). Water and field management followed local conventional practices.

1.2 Soil Sample Collection

During the flowering and grain-filling stage when rice growth is most vigorous, five random points were selected in each plot to collect soil samples. To distinguish surface and root-zone soils, a soil auger was used to collect samples from 0–5 cm and 10–20 cm depths, which were then mixed to form one composite sample per plot. This yielded 12 samples for each depth (24 total samples). Field-collected samples were stored in ice boxes at 4°C, transported to the laboratory, and after removal of roots, weeds, soil fauna, and stones, were homogenized and stored at -70°C for microbial analysis.

1.3 Soil Microbial DNA Extraction

Total soil microbial DNA was extracted using the FastDNA SPIN Kit for Soil (QBIOgene). Approximately 0.5 g of thawed soil was processed following the manufacturer's protocol, and extracted DNA samples were stored at -20°C until use.

1.4 High-Throughput Sequencing of Soil Microorganisms

Frozen paddy soil DNA samples were shipped on dry ice to Beijing Novogene Bioinformatics Technology Co., Ltd. for microbial community analysis. The V3–V4 region of bacterial 16S rRNA was amplified using primers 338F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTWTCTAAT [**?**]. PCR reactions contained 25 L Phusion High-Fidelity PCR Master Mix with HF Buffer, 3 L DMSO, 3 L each of forward and reverse primers, 10 L DNA template, and sterile water to a final volume of 50 L. Amplification conditions were: 98°C for 30 s; 30 cycles of 98°C for 15 s, 58°C for 15 s, 72°C for 15 s; and final extension at 72°C for 1 min. PCR products were sequenced using the Illumina MiSeq platform. Raw sequences were analyzed using QIIME (version 1.8.0) [**?**], with all samples rarefied to 30,000 sequences for subsequent analyses. At 97% similarity, 16S rRNA gene sequences were classified into taxonomic units at phylum, class, order, family, and genus levels. Alpha diversity indices including Chao1, Shannon, and Simpson were calculated using QIIME, and principal component analysis (PCA) was performed using STAMP [**?**].

1.5 Real-Time Quantitative PCR

Absolute quantification of ammonia-oxidizing bacterial *amoA* genes and denitrifying bacterial *nirS*, *qnoB*, and *nosZ* genes was performed using gene-specific primers [**?**, **?**] (Table 1) on an ABI PRISM 7500 Real-Time PCR System. Each 25 L reaction contained 2 L of 5-fold diluted DNA template plus 23 L reaction mixture comprising 12.5 L SYBR Premix Ex Taq™ (2×), 0.5 L ROX Reference Dye II (50×), 1 L each of forward and reverse primers ($5\ \mathrm{pmol} \cdot \mathrm{L}^{-1}$, Invitrogen, Shanghai), and 8 L ultrapure water.

Standard curves were established following He et al. [?]. Soil DNA was amplified with each gene-specific primer set, and PCR products were cloned into pMD-18 vectors (TaKaRa, Dalian). Plasmid DNA was extracted from clones with correct inserts, and concentrations were measured using a Nanodrop ND-1000 UV-Vis spectrophotometer to calculate copy numbers. Ten-fold serial dilutions were used to generate standard curves. Standard curve ranges were $9.09\$\times10^{\{8\}-9.09\times10}\{4\}\$$ copies$\cdot$L^{-1} for *amoA*, $3.67\$\times10^{\{9\}-3.67\times10}\{5\}\$$ copies$\cdot$L^{-1} for *nirS*, $5.24\$\times10^{\{9\}-5.24\times10}\{5\}\$$ copies$\cdot$L^{-1} for *qnoB*, and $7.19\$\times10^{\{9\}-7.19\times10}\{5\}\$$ copies$\cdot$L^{-1} for *nosZ*. All standard curves had R^2 values of 0.99, with amplification efficiencies of 1.01, 0.98, 0.99, and 1.01 for *amoA*, *nirS*, *qnoB*, and *nosZ*, respectively.

1.6 N2O Emission Flux Monitoring

On the day of soil sampling, N2O emissions were collected using the static closed chamber method. N2O concentrations were measured by gas chromatography (Agilent Technologies 7890B GC system, USA) equipped with an electron capture detector (ECD) maintained at 55°C. Separation was achieved using a PQ packed column at 55°C. Standard gases were provided by Dalian Date Gas Co., Ltd. The N2O emission flux was calculated using the formula [?]:

$$F = \rho \times h \times \frac{dC}{dt} \times \frac{273}{273 + T} \quad (1)$$

where F is the N2O emission flux [g$\cdot$(m$^2\cdot$h)$^{-1}$]; is the N2O density under standard conditions (1.964 kg$\cdot$m^{-3}); h is the actual height from the chamber top to the water surface after adjusting for field water depth (m); dC/dt is the rate of N2O concentration change in the chamber [L$\cdot$(m$^3\cdot$h)$^{-1}$], calculated as the slope of the linear regression between closure time and gas concentration; and T is the average temperature inside the chamber (°C).

1.7 Data Analysis

Data processing and graphing were performed using Microsoft Excel 2007 and SPSS 13.0. Differences among treatment means were analyzed by one-way ANOVA. Correlation coefficients were calculated using Excel 2007' s CORREL function, with significance levels determined from correlation coefficient tables.

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2 Results and Analysis

Alpha diversity indices are presented in Table 2 . The Chao1 index represents species richness, with higher values indicating greater richness. The Shannon index reflects community diversity, where higher values indicate greater diversity. The Simpson index indicates species dominance, with higher values suggesting lower diversity [?]. Bacterial species richness (Chao1) was lowest under CK, significantly increasing under 1/3N ($P < 0.05$). Although differences between 2/3N and N were not significant, both were significantly higher than 1/3N ($P < 0.05$). Similarly, bacterial Shannon diversity increased significantly with nitrogen level, with N treatment significantly higher than 2/3N and 1/3N ($P < 0.05$), which were significantly higher than CK ($P < 0.05$). Conversely, Simpson indices for the three fertilized treatments were significantly lower than unfertilized CK ($P < 0.05$). These patterns were consistent across both 0–5 cm and 10–20 cm soil depths.

2.1.1 Diversity Changes

High-throughput sequencing of the 16S rRNA V3–V4 region yielded 1,998,211 valid sequences, with an average sequencing depth of 83,258 sequences per sample.

2.1.2 Community Structure Analysis

Dominant bacterial phyla included *Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Firmicutes*, *Chloroflexi*, *Actinobacteria*, and *Acidobacteria*, accounting for 79.60-89.30% of total sequences (average 84.78%). *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* were most abundant, comprising 26.94%, 22.08%, and 18.55% of sequences, respectively, indicating they were the dominant populations.

Figure 1 [Figure 1: see original paper] shows bacterial phylum composition and relative abundances across treatments. *Acidobacteria*, a major phylum, exhibited increasing relative abundance with nitrogen level in both depths, rising from 18.40% and 17.80% under CK to 25.50% and 30.00% under N in the 0-5 cm and 10–20 cm layers, respectively. In contrast, *Proteobacteria* showed the opposite trend, decreasing from 35.50% and 28.20% under CK to 22.80% and 21.40% under N.

Nitrospirae relative abundance also showed clear patterns, increasing from 2.80% and 3.00% under CK to 5.70% and 4.90% under N in the 0–5 cm and 10–20 cm layers, respectively.

Figure 2 [Figure 2: see original paper] displays the top 10 most abundant bacterial genera in 0–5 cm (Fig. 2a) and 10–20 cm (Fig. 2b) layers. Three genera [*Desulfobacca*, *Clostridium*, and the methanotroph *Methylosinus*] decreased with nitrogen level, with *Methylosinus* showing a clear trend of $N \leq 2/3N \leq 1/3N < CK$ in the 0–5 cm layer. The remaining seven genera [*4-29*, *Mycobacterium*, *Anaeromyxobacter*, *GOUTA19*, *Geobacter*, *Candidatus*, and *Rhodoplanes*] generally increased with nitrogen level. Notably, *4-29* and *GOUTA19* (both belonging to *Nitrospirae*) showed significantly higher relative abundances under medium and high nitrogen (N or 2/3N) compared to low nitrogen (1/3N) or CK ($P < 0.05$).

Principal component analysis (PCA) based on high-throughput sequencing revealed distinct clustering patterns (Figure 3 [Figure 3: see original paper]). CK bacterial communities separated clearly from fertilized treatments along PC1. The 1/3N treatment also differed from 2/3N and N along PC1, while 2/3N and N showed separation along PC2. Additionally, CK and 1/3N treatments exhibited depth-related differences along PC2, whereas 2/3N and N showed minimal depth effects. These results indicate progressive community changes with nitrogen level, with the largest difference between unfertilized and fertilized soils, and moderate divergence among fertilized treatments.

2.2 Abundance of Nitrification and Denitrification Functional Genes

Real-time PCR analysis quantified key functional genes related to nitrogen transformation and N2O emission. The abundance of *amoA* genes, which drive the critical ammonia oxidation step in nitrification, increased with nitrogen application in both soil depths (Figure 4a [Figure 4: see original paper]). In the 0-5 cm layer, all treatment differences were significant ($P < 0.05$).

Denitrification involves three key enzyme genes: *nirS*, *qnoB*, and *nosZ*. *nirS* gene abundance in CK was significantly lower than in fertilized treatments in both depths ($P < 0.05$), but showed no differences among 1/3N, 2/3N, and N (Figure 4b), indicating that while nitrogen fertilization significantly increased *nirS* abundance, the nitrogen rate had little effect.

qnoB gene abundance varied substantially in the 0-5 cm layer, showing an increasing trend of $N \geq 2/3N \geq 1/3N > CK$, with N significantly higher than 1/3N and CK ($P < 0.05$), and 2/3N and 1/3N significantly higher than CK ($P < 0.05$) (Figure 4c). In the 10-20 cm layer, no significant differences occurred among fertilized treatments, though all were significantly higher than CK ($P < 0.05$). All treatments showed significantly higher *qnoB* abundance in 0-5 cm than in 10-20 cm ($P < 0.05$), indicating more active and abundant *qnoB* genes in surface soil.

nosZ gene abundance also showed significant trends (Figure 4d): $N > 1/3N > CK$ in both depths ($P < 0.05$). In the 0-5 cm layer, 2/3N was intermediate between N and 1/3N, not differing significantly from either. In the 10-20 cm layer, 2/3N was significantly higher than 1/3N ($P < 0.05$) but similar to N, demonstrating active *nosZ* gene responses to nitrogen.

2.3 N2O Emission from Paddy Soils

During the flowering and grain-filling stage, N2O emissions were monitored concurrently with microbial community analysis. Increased nitrogen levels enhanced N2O emissions, with N treatment showing significantly higher flux than other treatments ($P < 0.05$) (Figure 5 [Figure 5: see original paper]). The 1/3N and 2/3N treatments did not differ significantly but were both significantly higher than CK ($P < 0.05$).

Correlation analysis between N2O emission flux and functional gene copy numbers or *Nitrospirae* relative abundance (including genera *4-29* and *GOUTA19* among the top 10 genera) revealed significant correlations (Table 3). *Nitrospirae* relative abundance in the 0–5 cm layer and *amoA* gene copy number in the 10–20 cm layer showed the strongest significant correlations with N2O flux ($P < 0.05$, $n = 10$). Other indices were not significantly correlated.

3 Discussion and Conclusion

Advances in DNA sequencing technology have established high-throughput sequencing as an essential tool for studying soil microbial diversity and assessing soil quality. This study employed high-throughput sequencing to examine bacterial community changes under different nitrogen levels in paddy soils. During the rice flowering and grain-filling period, fertilized paddies exhibited significantly higher bacterial species richness and diversity than unfertilized paddies, with nitrogen level increasing bacterial richness, though the effect plateaued at higher nitrogen rates. Compared to unfertilized soil, fertilized paddy soils have increased nutrient (available N, P, K) and organic carbon contents, providing more favorable microenvironments for bacterial survival and growth, thereby enhancing diversity [**?**]. The dominant phyla in this study—*Proteobacteria*, *Acidobacteria*, and *Chloroflexi*—comprised the majority of soil bacteria. *Acidobacteria* abundance was most responsive to nitrogen level, increasing with nitrogen rate. As acidophilic bacteria, *Acidobacteria* benefit from soil acidification associated with nitrogen fertilization [**?**].

Although *Nitrospirae* represented a relatively small proportion of the bacterial community, it responded strongly to nitrogen level, with its relative abundance increasing significantly with nitrogen application. Among the top 10 most abundant genera, *4-29* and *GOUTA19* belong to *Nitrospirae*, and both increased with nitrogen level. *Nitrospirae* drives soil nitrification, and its increased abundance may enhance nitrification activity, which produces N2O as a byproduct. Correlation analysis confirmed a significant relationship between *Nitrospirae* relative abundance in surface soil and N2O emissions ($P < 0.05$, $n = 10$), suggesting that increased *Nitrospirae* contributed to N2O release in this study. Additionally, nitrogen fertilization suppressed *Methylosinus* methanotrophs, particularly in the 0–5 cm layer where abundance decreased significantly with nitrogen level, providing a microbial community-level explanation for nitrogen inhibition of methane oxidation [**?**].

Real-time PCR analysis revealed that *amoA* gene abundance, which encodes the key enzyme in the rate-limiting ammonia oxidation step of nitrification, was closely related to nitrogen rate, increasing significantly with nitrogen level in both depths, especially in the 10–20 cm layer where all treatment differences were significant ($P < 0.05$). Denitrification involves four sequential enzymes: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. Analysis of three key enzyme genes showed that unfertilized soil had significantly lower *nirS*, *qnoB*, and *nosZ* abundances than fertilized soils. Among fertilized treatments, *qnoB* and *nosZ* showed stronger responses to nitrogen rate than *nirS*, with both genes increasing with nitrogen level in the 0–5 cm layer and *nosZ* increasing significantly under medium and high nitrogen in the 10–20 cm layer. In contrast, *nirS* abundance showed no clear response to nitrogen rate, with no differences among high, medium, and low nitrogen treatments. These results indicate that *qnoB* and *nosZ* genes driving denitrification were more sensitive to nitrogen application than *nirS* in this paddy soil.

Nitrification and denitrification are considered major pathways for soil N2O production. Correlation analysis with microbial relative abundances and functional gene copy numbers revealed stronger associations between N2O emissions and nitrification-related microorganisms, as evidenced by significant correlations with *Nitrospirae* abundance in the 0–5 cm layer and *amoA* gene abundance in the 10–20 cm layer ($P < 0.05$, $n = 10$). Many studies have found no clear relationship between denitrifying functional gene abundance and N2O emissions, such as in potato fields where denitrifying gene (*nirK*, *nosZ*, *cnorB*) abundances were unrelated to N2O release across seasons [**?**], in cropland soils where short-term (72 h) denitrifying gene (*nosZ*, *nirS*) abundance changes showed no correlation with N2O emissions [**?**], and in wetlands where *nosZ* abundance was not clearly related to N2O release at 70% water-filled pore space [**?**]. Similarly, this study found no correlation between increased denitrifying gene (*nirS*, *qnoB*, *nosZ*) abundances due to nitrogen fertilization and N2O emissions.

In summary, this study demonstrated that higher nitrogen rates, compared to zero or low nitrogen, enhanced bacterial community diversity and altered community composition in paddy soils during the rice flowering and grain-filling period, while increasing greenhouse gas N2O emissions. Changes in microbial community composition and abundance involved in nitrification showed closer associations with increased N2O emissions. However, since soil N2O production involves multiple pathways and enzymes with complex interactions, systematic analysis of key microbial functional gene diversity and their roles in N2O production throughout rice growth is needed. Furthermore, DNA-level studies of nitrifying and denitrifying microbial genes only represent potential physiological functions, and RNA-level investigations are required to further elucidate relationships between functional gene diversity and N2O emissions.

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Note: Figure translations are in progress. See original paper for figures.