Rice planting reduced N$_2$O emissions from rice-growing seasons due to increased *nosZ* gene abundance under a rice-wheat rotation system

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A B S T R A C T

Paddy fields experience more frequent events because of redox-induced changes during their growth period, which further govern the processes that influence soil N$_2$O emissions. However, studies on the effects of rice planting on soil N$_2$O emissions and the relevant mechanisms are scarce. Thus, a field employing rice-wheat rotation (RW) in central China, including treatments with rice planting (RW-CC) and without rice planting (RW-NC), was selected to investigate the effects of rice planting on N$_2$O emissions. Several key soil variables and functional genes (*nirS*, *nirK*, and *nosZ*) related to N$_2$O-production and consumption were selected to determine the mechanisms underlying the effects of rice planting on N$_2$O emissions. The results showed that seasonal N$_2$O emissions from RW-NC treatment were 0.88 ± 0.08 and 1.69 ± 0.24 kg N ha^{-1} for the first and subsequent rice cultivation seasons, respectively, and were significantly higher than those from RW-CC treatment (0.51 ± 0.10 and 1.04 ± 0.18 kg N ha^{-1}, respectively). This indicated the mitigation effect of rice planting on field N$_2$O emissions. For both the RW-CC and RW-NC treatments, N$_2$O fluxes were positively correlated with soil ammonium (NH$_4^+$), microbial biomass carbon (MBC), and *nirK* genes, and negatively correlated with the *nosZ* gene, indicating that the production- and consumption-related functional genes of soil available C, N, and N$_2$O are the key factors controlling N$_2$O emissions. Compared to RW-NC treatment, a higher abundance of the *nosZ* gene and lower (*nirS*+*nirK*)/*nosZ* ratios for RW-CC treatment may contribute to a greater reduction of N$_2$O to N$_2$, thus resulting in decreased N$_2$O emissions. Structural equation model (SEM) analysis showed that soil moisture (flood depth), NH$_4^+$, MBC, and functional genes together accounted for more than 85 % of the explanatory effects on N$_2$O emissions for both treatments. In general, our research indicates that promoting rice planting could reduce soil N$_2$O emissions during the rice-growing season, thereby reducing the exposure of soil to N fertilization when adopting field agronomic practices; our results also suggest that crop rotation and intercropping should be encouraged.

1. Introduction

Nitrous oxide (N$_2$O), one of the main greenhouse gases in the troposphere, has garnered considerable attention from scientists (Davidson, 2009; Zheng et al., 2022) because its radiative forcing is significantly higher than that of carbon dioxide (CO$_2$) at an equivalent quality level over a 100-year timescale (IPCC, 2013). Agricultural soils have been identified as the primary source of atmospheric N$_2$O and their contribution is expected to increase in the future (Scheehle et al., 2006; Li et al., 2011a, 2011b; Hu et al., 2015).

Crop soil constitutes the foundation of agricultural ecosystems. Crops

are known to be active contributors to the processing and cycling of soil N$_2$O-related processes; however, the exact mechanisms underlying their interactions with N$_2$O production and emissions are poorly understood (Bakken, 1988; Mahmood et al., 1997; Simojoki and Jaakkola, 2000; Xu et al., 2022b). Additionally, crops in terrestrial ecosystems drive feedback links between plant carbon (C) inputs and soil pores, wherein the latter affect soil N$_2$O emissions. Crop growth can positively, negatively, or neutrally affect soil N$_2$O emissions (Smith and Tiedje, 1979; Ghosh et al., 2002; Li et al., 2011a; Xu et al., 2022b). For instance, Ni et al. (2012) demonstrated that in a corn field, regardless of N fertilization, the N$_2$O emissions of crop-planted soil were higher than those of bare

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soil, indicating that maize growth could increase soil N_2O emissions. Similarly, another study showed that crops boost soil N_2O emissions (Flessa et al., 2002). The positive effects of crop growth on soil N_2O emissions can be ascribed to root-derived organic C stimulating the growth and activity of microorganisms, resulting in O_2 consumption and the occurrence of anaerobic microsites in soil pores, thereby elevating N_2O evolution (Song et al., 2019). Decreased N_2O emissions following crop planting have also observed previously (Sehy et al., 2003; López-Fernández et al., 2007). The mechanisms underlying the negative effect of crop plantations on N_2O emissions may be as follows: 1) N_2O is absorbed and metabolized by plants (Grundmann et al., 2006); 2) crops often retain large amounts of inorganic N during their growth process, especially available C and N in the soil, leading to a lack of N substrates for both nitrification and denitrification processes, thus reducing soil N_2O production (Xing et al., 1998; Davidson, 2009). Hodge et al. (2000) showed decreased soil N content after planting crops; however, higher inorganic N content has been observed in bare soil, providing sufficient substrate to produce N_2O in the soil (Opez-Fernande, 2007; Zhang et al., 2016; Wei et al., 2021). These findings are primarily concerned with the impact of upland crops on soil N_2O emissions, whereas studies on paddy field and their underlying N_2O processes are absent.

Numerous lines of evidence demonstrate that N_2O emissions from paddy fields should not be disregarded because of their high emissions (Xu et al., 2022a; b, 2023), and scholars worldwide have focused on creating a budget for paddy N_2O emissions and determining mitigation measures (Tian et al., 2020). Notably, China manages approximately 20 % of rice areas (FAO, 2022), with rice-wheat rotation (RW) accounting for 16 % of the total rice paddy area of China (IPCC, 2013). Generally, rice absorbs soil moisture and nutrients during its growth, which can change the physical, chemical, and biological states of the soil in paddy fields, thus affecting the related processes of soil N_2O generation and emission (Cai et al., 1997; Ruser et al., 2001; Kögel-Knabner et al., 2010). However, the effects of rice plantations on soil N_2O production-related substrate availability and microbial metabolism are scarcely investigated, which consequently affect soil N_2O emissions (Xu et al., 2022b).

Notably, the dynamics of soil C and N play a significant role in N_2O emissions from paddy fields (Xu et al., 2022a; b, 2023), and are different in areas with and without plants (Bolton et al., 1990). Zhang et al. (2022) proposed that this phenomenon is uncommon, and that the effects of rice growth on soil C and N need to be investigated, particularly on soil dissolved organic carbon (DOC), mineral N, and microbial biomass C and N (MBC and MBN). Active C and N may function as effective substrates for N_2O-production related microorganisms. This could affect microbial activities in soil, such as microbial assimilation and propagation processes, consequently affecting soil N_2O production, consumption, and emissions (Sanchez-Martín et al., 2008; Kader et al., 2013; Xu et al., 2023).

As described by the "hole-in-the-pipe" conceptual model (Firestone and Davidson, 1989), soil N_2O production is mainly derived from microbial nitrification and denitrification (Hu et al., 2017). For instance, *nirS*, *nirK*, and *nosZ* genes have been suggested as key functional genes affecting N_2O production and consumption during the flooding periods of paddy fields (Shaaban et al., 2018; Zhou et al., 2020; Kong et al., 2021; Xu et al., 2022b, 2023). Notably, long-term flooding conditions support the reduction of N_2O to N_2 with an increased abundance of the *nosZ* gene. However, various soil processes and some key functional genes regulating N_2O production and emissions have garnered increasing recognition (Qin et al., 2018; Maul et al., 2019; Grassmann et al., 2022). Little is known about the effect of rice cultivation on the functional genes involved in nitrification and denitrification processes, which are accompanied by variable soil conditions and could alter N_2O emissions (Ma et al., 2008; Yang et al., 2017; Xu et al., 2022b).

In this study, we primarily focused on the effects of rice cropping on soil N_2O emissions and sought to identify important variables (such as field moisture and soil C and N availability) and essential functional genes that regulate N_2O production and emission. Hence, a field experiment was conducted through treatments with or without rice planting in an RW system to monitor the interannual and seasonal N_2O emissions and investigate the relationships between N_2O efflux and the aforementioned crucial elements. We hypothesized that rice plantations would change soil available C and N fractions due to the absorption and utilization of mineral N and active C secretion. This would consequently affect the abundance of some key functional genes regarding N_2O production and consumption, regardless of rice-based rotations. Thus, the objectives of this study were to: i) determine the differences in soil C and N fractions related to N_2O emissions (i.e., soil DOC, mineral N, MBC, and MBN contents) under various rice-based rotation systems; and ii) explore the potential relationship between N_2O emissions, soil-related C and N fractions, and microbial functional genes.

2. Materials and methods

2.1. Description of the study site and field experimental design

This study was conducted at HuangGang Academy of Agricultural Sciences in Hubei Province, China (30° 63' N, 114° 87' E), which has a subtropical monsoon environment with average annual temperatures and precipitation of 18.1 °C and 1157 mm, respectively. The soil here has a silty loam texture and is classified as fluvo-aquic soil comprising 16.0 % clay, 57.2 % silt, and 26.8 % sand. The initial soil organic carbon (SOC), total nitrogen (TN), bulk density (BD), and pH were, 11.0 g kg^{-1}, 0.80 g kg^{-1}, 1.29 g m^{-3}, and 8.05 (soil:water = 1:2.5), respectively.

The experiment was conducted using a split plot from May 2019 to September 2020. In particular, a plot with an area of 24 m^2 (4 × 6 m) was selected for conventional fertilization (RW-CC). For each plot, an area of 1.5 × 1.5 m was free of rice plantation to act as a non-crop treatment (RW-NC) and was separated from the surrounding rice with a polyethylene plate. Each treatment was repeated thrice. The fertilizer application rate of the flooded rice season consisted of 225 kg N ha^{-1}, 112 kg P_2O_5 ha^{-1}, and 225 kg K_2O ha^{-1}, wherein half of the N and K and all P fertilizers were applied as basal fertilizer, and the other half of the N and K fertilizers were applied as topdressing. In particular, 67 kg N ha^{-1} (urea) was applied 7 days after rice transplanting as tillering fertilizer, 45 kg N ha^{-1} (urea) was applied at the jointing stage, and 112.5 kg K_2O ha^{-1} (potassium chloride) was applied during the heading period. Moreover, the fertilizer application rate during the wheat season consisted of 192 kg N ha^{-1}, 112 kg P_2O_5 ha^{-1}, and 112 kg K_2O ha^{-1}, respectively, wherein 60 % of N and K and all P fertilizers were applied as basal fertilizer, and the remaining N and K fertilizers were applied as topdressing. The detailed information on fertilizer application and management practices is presented in Table S1.

2.2. Measurement of N_2O emission fluxes

A static opaque chamber, extensively discussed by Wang et al. (2013), was selected for collecting N_2O gas samples. In particular, the chamber comprised two components: a base structure with a bottom area and height of 0.25 m^2 and 0.3 m, respectively, and an insulating foam-wrapped chamber cover with a bottom area and height of 0.25 m^2 and 1 m, respectively. After field plowing, the foundation base structure was buried in the ground in each pot. In particular, the upper edge of each base was equipped with a groove filled with water to ensure gas sealing before the start of each gas sampling operation. For each gas sampling event, five gas samples were collected from each chamber using five 50-mL air-tight syringes with an interval of 10 min between two adjacent gas sampling operations. Generally, the frequency of gas collection was twice every week, whereas it was adjusted to seven continuous times per week after fertilization and heavy rain events. Subsequently, gas samples were measured through a gas chromatograph (GC; 7890 A, Agilent Technologies, California, USA) equipped with an electron capture detector (ECD) within 24 h. During the analysis of the

N$_2$O concentrations, N$_2$ and a mixture of CO$_2$ and N$_2$ (10 % CO$_2$ in N$_2$) acted as the carrier and buffering gases, respectively. Finally, N$_2$O emission fluxes and seasonal cumulative N$_2$O emissions were calculated using the closed-chamber equation and trapezoidal method (Zheng et al., 2008).

2.3. Measurements of auxiliary parameters

Synchronous with the N$_2$O flux measurements, several crucial variables were determined (Wu et al., 2017; Xu et al., 2022a). In particular, the air temperature inside each chamber was recorded using a portable digital thermometer (JM624; Liwen Electronics LTD, Tianjin, China). The flood water depth in the field (only for the rice-planting period) was measured using a vertical ruler. Additionally, the soil temperature at a depth of 5 cm was recorded using an electronic probe manually inserted into the soil near each base frame when collecting gas samples. Each week, five topsoil (0–20 cm) samples were randomly drawn from each plot and combined to create a single composite soil sample. Subsequently, the composite sample was immediately brought to the lab and stored at a temperature of 4 °C. A part of the soil samples was used to measure the soil water content (SWC), soil pH, DOC, dissolved inorganic nitrogen (DIN; including NH$_4^+$ and NO$_3^-$), as suggested by Xu et al. (2022b). The soil samples were also used to determine the soil MBC and MBN using the chloroform fumigation method proposed and modified by Brookes et al. (1985). At the start of the experiment, columnar soil samples were collected using cylindrical rings to determine the soil bulk density (BD). The daily air temperature and precipitation were recorded by a small auto-monitoring meteorological station near the experimental site.

2.4. Real-time quantitative polymerase chain reaction (qPCR)

In this study, the silica matrix adsorption column technique was used to extract soil microbial DNA samples. According to previously recommended primer sets (Table S2), quantitative polymerase chain reaction (qPCR) was conducted using the SYBR GREEN technique to quantitatively evaluate the abundance of three genes (the nosZ gene responsible for N$_2$O consumption and the nirS and nirK genes involved in N$_2$O production). In particular, we used the qPCR reaction process wherein the measurement levels varied from 95 % to 110 %. The value of R^2 for each calibration curve was set to be higher than 0.98. The qPCR reaction system had a total volume of 15 μL, comprising 7.5 μL of SYBR Green Mix, 1 μL of diluted template DNA, 0.7 μL of the primer, and 5.8 μL of sterile water. The amplification conditions were based on a previous study (Zhou et al., 2020). The formula suggested by Macdonald et al. (2011) was used to determine the copy number of each functional gene.

2.5. Statistical analyses

Seasonal N$_2$O emissions and the abundance of functional genes in the RW-CC and RW-NC treatments were compared using an independent t-test. Linear or nonlinear regression was used to examine the functional relationships between the N$_2$O fluxes, soil variables, and functional gene abundance. A structural equation model (SEM) implemented using the AMOS software package (AMOS 21.0, SPSS Inc., Chicago, IL, USA) was used to examine the influence of environmental factors, soil variables, and functional genes on N$_2$O emissions. Figures were prepared using the Origin 8.5 software (Origin Lab Corporation, USA). All statistical analyses were conducted with the Statistical Package for the Social Sciences (SPSS) software (SPSS19.0, SPSS Inc., Chicago, USA) and $p \leq 0.05$ was set to represent statistical significance.
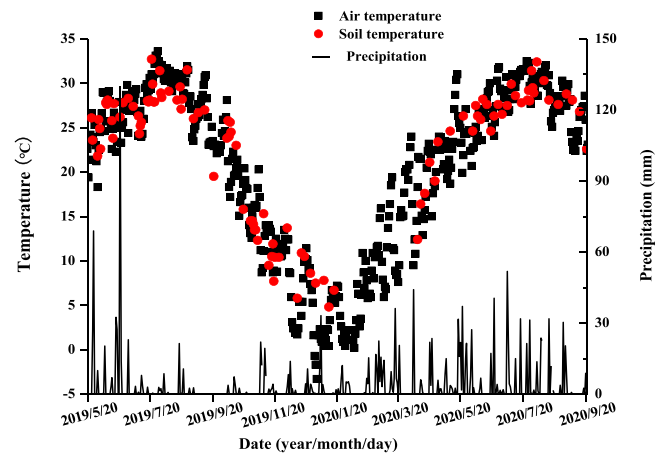

Fig. 1. Dynamics in daily mean air temperature (a), soil temperature at 5-cm depth (b) and Precipitation (c) during the experiment period.

3. Results

3.1. Climatic, environmental, and soil variables

According to our records, the daily air temperature during the experimental period varied from − 3.3 to 33.6 °C. During the trail year of 2019–2020 and the rice season of 2020, the yearly precipitation values were 1103.9 and 559.3 mm, respectively (Fig. 1). Concomitantly over the entire experimental period, the soil temperature at a depth of 5 cm ranged from 4.8 to 32.7 °C for the RW-CC treatment (Fig. 1). The recorded water flood depths varied from 0 to 4.05 cm and 0–4.65 cm, and from 0 to 4.03 cm and 0–4.72 cm for the RW-CC and the RW-NC treatments during the rice planting seasons of 2019 and 2020, respectively (Fig. S1).

In this study, basal N fertilization significantly enhanced the soil ammonium nitrogen (NH$_4^+$-N) levels for both treatments when rice was transplanted, and soil NH$_4^+$-N subsequently decreased to a low level. During the rice planting seasons of 2019 and 2020, the soil NH$_4^+$-N contents ranged from 1.58 to 57.14 mg N kg^{-1} and 0.65–77.14 mg N kg^{-1} for the RW-CC treatment (Fig. 2a), respectively. In contrast, the soil NH$_4^+$-N contents varied from 1.30 to 61.48 mg N kg^{-1} and 0.72–70.15 mg N kg^{-1} for the RW-NC treatment during the rice planting seasons of 2019 and 2020, respectively.

However, no prominent elevation was observed in soil NO$_3$-N concentrations after N fertilizer application, which ranged from 0.74 to 16.61 mg N kg^{-1} and 0.24–13.79 mg N kg^{-1} for the RW-CC treatment during the rice planting seasons of 2019 and 2020, respectively. These NO$_3$-N values were comparable to those from the RW-NC treatment, ranging from 0.38 to 17.12 mg N kg^{-1} and 0.18–12.40 mg N kg^{-1} (Fig. 2b). Soil DOC concentrations exhibited seasonal variations in both treatments, with higher values during the rice-growing season than during the wheat-growing period. Soil DOC contents ranged from 19.0 to 64.54 mg C kg^{-1} and 21.64–58.48 mg C kg^{-1} for the RW-CC treatment, whereas they varied from 23.94 to 51.45 mg C kg^{-1} and 14.28–46.31 mg C kg^{-1} for the RW-NC treatment during the first and second rice seasons, respectively (Fig. 2c). Soil MBC concentrations varied from 82.78 to 480.69 mg C kg^{-1} and 99.28–464.25 mg C kg^{-1} for the RW-CC treatment, whereas they varied from 55.43 to 502.99 mg C kg^{-1} and 108.27–457.58 mg C kg^{-1} for the RW-NC treatment during the rice planting seasons of 2019 and 2020, respectively (Fig. 2d). Soil MBN contents ranged from 10.76 to 27.40 mg N kg^{-1} and 10.52–27.37 mg N kg^{-1} for the RW-CC treatment, whereas they ranged from 9.98 to 23.15 mg C kg^{-1} and 6.26–33.25 mg C kg^{-1} for the RW-NC treatment during the rice planting seasons of 2019 and 2020, respectively (Fig. 2e).
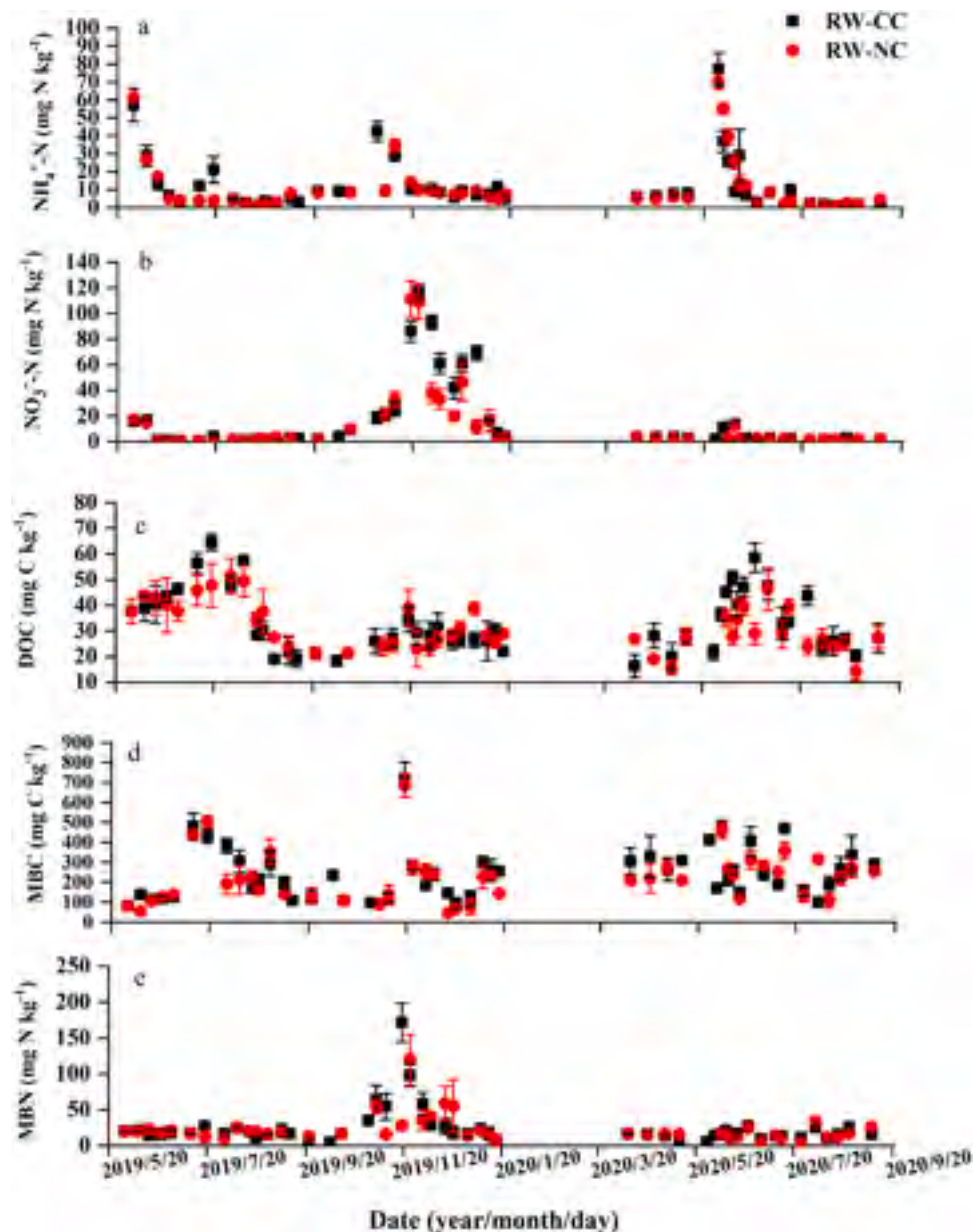
Fig. 2. Dynamics in soil ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic carbon (DOC), soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) during the experiment period.

3.2. N₂O emissions

In this study, the soil N_2O fluxes were markedly altered during the experimental period. The N_2O fluxes varied from -6.20–386.31 ug N m^{-2} h^{-1} and -13.41–822.94 ug N m^{-2} h^{-1} for the RW-NC treatment during the rice growing seasons of 2019 and 2020, respectively, whereas they tended to increase from -24.35–371.96 m^{-2} h^{-1} and -15.85–952.24 ug N m^{-2} h^{-1} for the RW-NC treatment during the first and second rice growing seasons, respectively (Fig. 3). The seasonal cumulative N_2O emissions of the RW-CC treatment amounted to 0.50 ± 0.10 kg N ha^{-1} and 1.04 ± 0.18 kg N ha^{-1} during the rice seasons of 2019 and 2020, respectively, which were significantly lower than those of the RW-NC treatment, with total amounts of 0.88 ± 0.08 and 1.69 ± 0.24 kg N ha^{-1}, respectively ($p < 0.05$, an embedded figure in Fig. 3).

3.3. The relationship between soil N₂O emissions, soil parameters, and gene abundances

Soil N_2O fluxes were found to be correlated with measured soil variables and related functional genes. Correlation analysis showed that N_2O fluxes were positively correlated with soil NH_4^+ and MBC for both treatments during the two rice sowing seasons, whereas they were negatively correlated with the field flood depth (Fig. 4a and b). Moreover, for both treatments, the N_2O fluxes tended to linearly increase with increasing soil NH_4^+ concentrations over the course of the rice growing season, with modest differences in slope between the two treatments (Fig. 5a); conversely, they decreased exponentially with increasing field flood depth (Fig. S2).

Additionally, exponential relationships between N_2O fluxes and soil functional genes were observed for both RW-CC and RW-NC treatments (Fig. 7). For both treatments, we found that N_2O fluxes had positive linear correlations with the copy number of the *nirK* gene (Fig. 7a) and negative linear relationships with the copy number of the *nosZ* gene
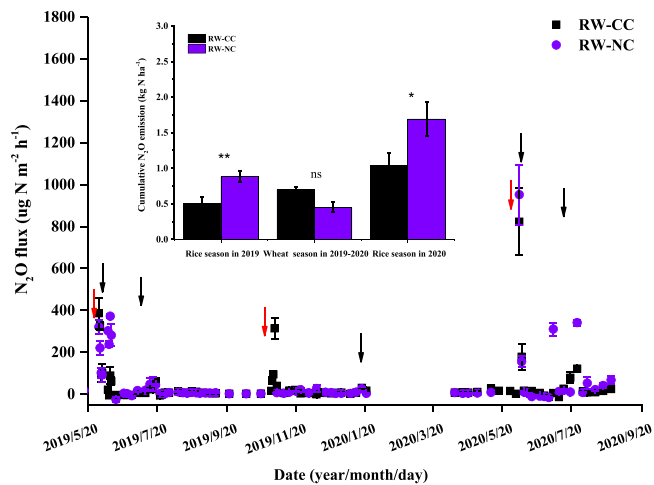
Fig. 3. Seasonal patterns in N$_2$O fluxes and cumulative N$_2$O emission for two treatments during the whole experiment period. The red and black arrows denote applying basal N fertilizer and N dressing, respectively.

(Fig. 7b) and the (*nirS*+*nirK*)/*nosZ* ratio (Fig. 7c). Notably, we observed a significant difference in the linear regression slopes between the two treatments (Fig. 7a and b) ($p < 0.05$).

3.4. Abundances of nirK, nirS, and nosZ genes

In this study, we assessed the abundances of the *nirS*, *nirK*, and *nosZ* genes, as well as the (*nirS*+*nirK*)/*nosZ* ratios for two treatments during each rice growing season. For the two rice sowing seasons, the abundance dynamics of these three genes remained consistent (Fig. 6). No discernible variations in the copy numbers of *nirK* and *nirS* were found between the RW-CC and RW-NC treatments (Fig. 6a and b). Notably, the *nosZ* gene copy number in the RW-CC treatment was considerably higher than that in the RW-NC treatment (Fig. 6c). Additionally, as shown in Fig. 6d and presented in Table S3, rice planting significantly affected the abundance of the *nosZ* gene and the ratio of (*nirS*+*nirK*) to *nosZ* ($p < 0.05$).

3.5. Structural equation model (SEM) describing the effects of various factors on N$_2$O emissions

The structural equation model (SEM) was used to describe the effects of key variables on soil N$_2$O emissions during the rice-planting seasons for the two treatments. Overall, the functional genes (*nirK* and *nosZ*) and soil parameters (NH$_4^+$, MBC, and field flood depth) explained 86 % of soil N$_2$O emissions in the RW-CC treatment (Fig. 8a), whereas in the RW-NC treatment, these factors explained 92 % of soil N$_2$O emissions (Fig. 8b).

4. Discussion

4.1. The impact of rice cropping on N$_2$O emissions

Previous studies demonstrated that rice growth can positively or negatively influence soil N$_2$O emissions (Yu et al., 1997; Kim et al., 2021). In this study, N$_2$O emissions from the RW-CC and RW-NC treatments exhibited similar variations during the experimental period. However, significant differences in N$_2$O emissions during the two rice-growing seasons were observed in the RW system, with higher N$_2$O emissions in the non-rice-planting treatment than in the rice-planting treatment, implying that rice cropping plays a crucial role in soil N$_2$O emissions. Similarly, the average seasonal N$_2$O emission rate of paddy soils with plants was lower than that of soils without plants (López-Fernández et al., 2007), and the decreased N$_2$O fluxes in rice-planted soils could be attributed to the lower denitrification potential (Kim et al., 2021). This could be explained by the competition between rice growth and the N substrate, which supported nitrifying and denitrifying microorganisms after rice transplantation. Thus, soil without rice plantation and with high N content provided adequate substrates for N$_2$O production-related microorganisms and enhanced N$_2$O emissions from the soil (Opez-Fernande, 2007; Davidson, 2009). In addition, the mitigation effects of rice planting on N$_2$O emissions could be expected due to the growing rice roots in the soil that consume oxygen (O$_2$) and favor denitrification, leading to the reduction of N$_2$O to N$_2$ (Yang et al., 2021). Organic C sources derived from rice root excreta (Zhu et al., 2018; Khan et al., 2019) also provide remarkable energy for microbial denitrification and facilitate N$_2$O reduction.

Notably, compared with that in the first rice planting season, N fertilization triggered N$_2$O efflux during the second season. These results were justified by the occurrence of frequent rainfall during the second rice cultivation season, coupled with optimum N availability as a
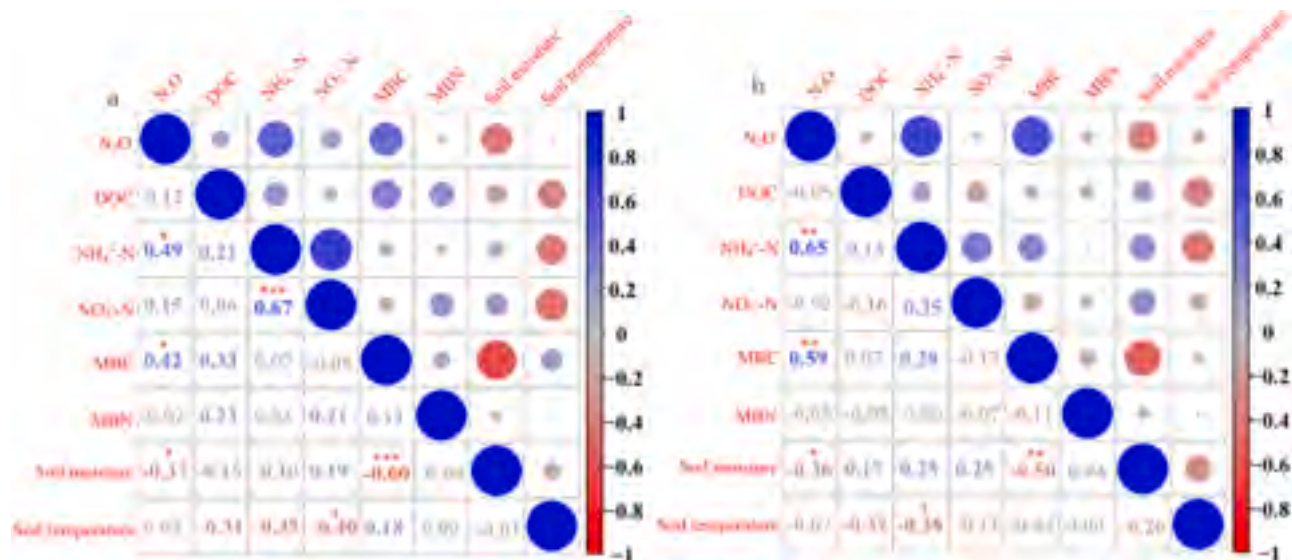

Fig. 4. Correlation analysis describing the relationships between the factors and N$_2$O emission for two treatment during the rice-growing seasons. a, b represents the RW-CC and RW-NC treatments, respectively.
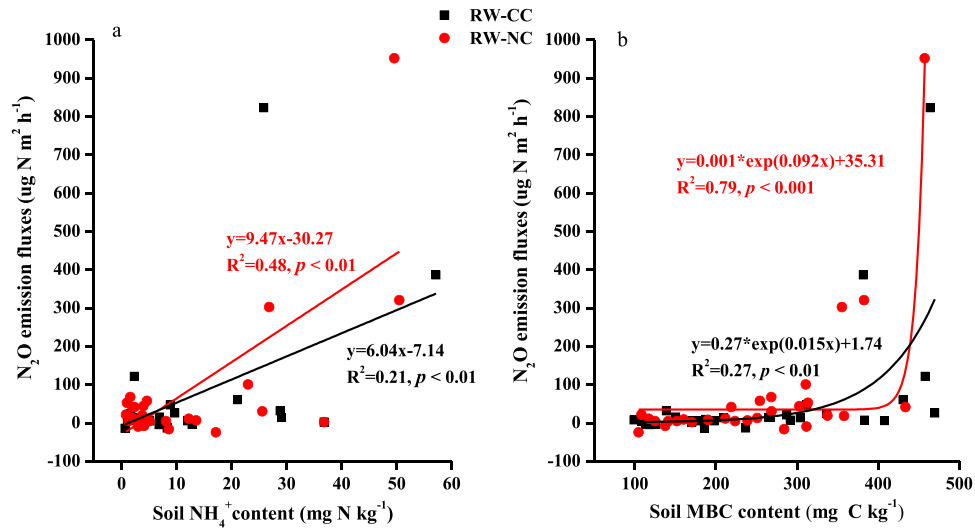
Fig. 5. Relationship between N_2O fluxes and soil NH_4^+-N and MBC content for two treatments during the rice-growing seasons.
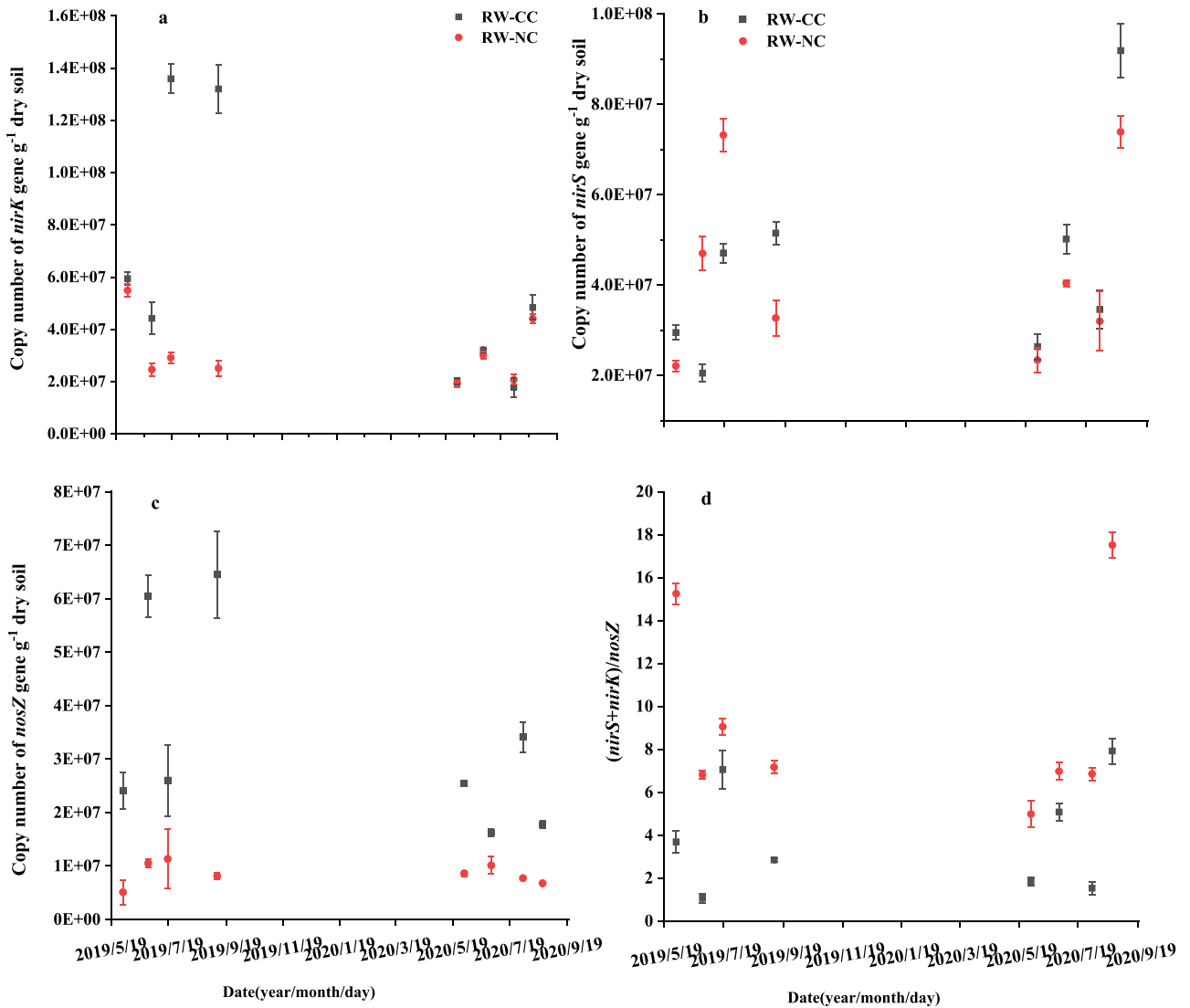

Fig. 6. Dynamics in the abundances of different functional genes for two treatments during the two rice-growing seasons.

substrate. This further enhanced the decomposition of soil organic matter (SOM) and subsequently facilitated microbial N_2O-production, resulting in greater N_2O emissions (Grandy and Robertson, 2006; Zhou et al., 2017). Previous studies have reported greater N_2O emissions occurring during alternating wet and dry cycles (Liao et al., 2020; Xu et al., 2022a, 2022b). Thus, moisture fluctuations play a vital role in microbial N_2O-related activities, and this must be considered when evaluating N_2O emissions from fields (Qin et al., 2020).

4.2. The influence of soil available C and N on N_2O emissions

Soil available C and N, such as DOC, NH_4^+, NO_3^-, MBC, and MBN, are often recognized as essential substrates for microbial nitrification and denitrification processes (Shaaban et al., 2019; Xu et al., 2022b). During the rice cultivation season, the highest N_2O emissions were accompanied by N fertilization, especially after basal N fertilization. Such large N_2O emissions indicated the higher soil mineral N content after N fertilization, implying the stimulation of N_2O production-related microorganism activity when sufficient mineral N was available (You et al., 2022). This was further confirmed by the positive correlation between N_2O fluxes and soil NH_4^+ content for both treatments (Fig. 7). Furthermore, for both treatments, soil MBC concentrations were positively and negatively correlated with N_2O fluxes and *nosZ*, respectively, indicating that soil MBC could directly and indirectly facilitate N_2O emissions. Similarly, a positive correlation between soil MBC and N_2O fluxes was previously observed in a paddy-based field (Xu et al., 2022a, 2022b). These results indicate that the available C and N may act as substrates to support microbial N_2O production (Meijide et al., 2007; Xu et al., 2022a; b, 2023). Our results also corroborate those of Xu et al. (2022a, 2022b), who summarized that soil N_2O fluxes increased with increasing available soil C concentrations. SEM analysis also revealed that soil-available C and N had positive or negative effects on *nirK* or *nosZ*, consequently influencing N_2O emissions. Zhou et al. (2020) suggested that the available soil C and N and their ratios play key roles in regulating soil N_2O production and emissions.

4.3. The relationship between N_2O production-related genes and N_2O emission

Microbial functional genes have been known to affect N_2O production and emission (Zhou et al., 2020; Lin et al., 2023). Whether plant growth could affect N_2O emissions by affecting functional genes related to N_2O production and consumption needs to be investigated further (Lenhart et al., 2019). In this study, three functional genes (*nirS*, *nirK*, and *nosZ*) involved in denitrification were selected to investigate the effect of the rice cropping system on their abundances and explore the relationships between these genes and N_2O emissions. Therefore, the abundances of *nirK* and *nirS* genes could reflect the intensity of denitrification, as they encode nitrite reductase, which catalyzes the reduction of NO_2^- to gaseous oxynitride and has been recognized as a key limiting step in N_2O production (Zhou et al., 2020; Qin et al., 2021). Under certain conditions, N_2O can be further reduced to N_2 by nitrous oxide reductases encoded by the *nosZ* gene during denitrification. However, no discernible differences were observed in the abundances of the *nirS* or *nirK* genes between the RW-CC and RW-NC treatments. Regression and SEM analyses only showed a strong relationship between the *nirK* gene and N_2O emission flux for both treatments (Figs. 7 and 8), indicating that the *nirK* gene may play a primary role in regulating the reduction of NO_2^- to NO, consequently influencing the field N_2O emissions. Our results agree with those of Zhou et al. (2020), who found that both *nirK* and *nirS* genes exhibit positive relationships with soil N_2O emissions. Notably, as previously proposed, during the field flooding period, the reduction of NO_2^- to NO (further reduction to N_2O) through the nitrifier denitrification pathway, which is catalyzed by copper-containing nitrite reductase encoded by the *nirK* gene, can occur easily (Chandran et al., 2011; Kozlowski et al., 2014). In contrast, some
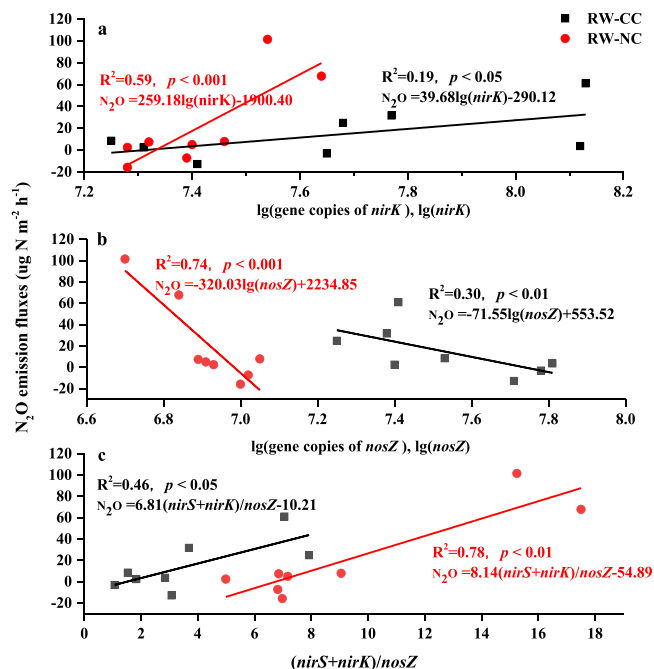

Fig. 7. Relationships between N_2O fluxes and abundances of different functional genes for two treatments during the rice-growing season.

studies have emphasized that the *nirS* gene has a stronger effect on N_2O production and emission in soil than the *nirK* gene (Qin et al., 2020; Zhou et al., 2020). Based on these results, we suggest that the roles of *nirS* and *nirK* genes in N_2O emissions need to be further explored in paddy fields in future studies.

However, significant differences were observed in the abundances of the denitrifying *nosZ* gene between the RW-CC and RW-NC treatments (Fig. 6c and Table S3). Furthermore, SEM revealed a strong negative relationship between *nosZ* gene abundance and N_2O emissions (Figs. 7 and 8). We speculate that the higher abundance of the *nosZ* gene contributed to a greater reduction in N_2O to N_2, resulting in lower N_2O emissions in the RW-CC treatment. Consistent with our results, a previous study revealed that a higher abundance of *nosZ* gene facilitates the reduction of N_2O to N_2, resulting in lower N_2O emissions (Liu et al., 2010; Shaaban et al., 2018). N_2O is the electron receptor for nitrous oxide reductase; therefore, the abundance of *nosZ* containing microbes may be relevant to soil N_2O emissions (Qin et al., 2020). In contrast, Li et al. (2021) reported that N_2O emissions did not decrease significantly and were accompanied by a greater *nosZ* gene abundance. Additionally, Xu et al. (2022b) reported no differences in the abundances of *nosZ* between rice and non-rice plantations under a double rice-fallow cultivation mode. These results indicate that the role of *nosZ* gene abundance in N_2O emissions from field conditions in rice-based cultivation modes is complex and requires further study.

Additionally, N_2O fluxes were positively correlated with the (*nirS*+*nirK*)/*nosZ* ratio, indicating that the abundance ratio of functional genes involved in denitrification could regulate N_2O emissions. Consistent with our results, previous studies have attributed greater N_2O emissions to higher (*nirS*+*nirK*)/*nosZ* ratios (Kong et al., 2021; You et al., 2022). As proposed previously, the (*nirS*+*nirK*)/*nosZ* ratio represents the ability of denitrifying genes to determine whether N_2O is emitted into the atmosphere as a greenhouse gas or reduced to N_2 (Chen et al., 2020). Thus, the (*nirS*+*nirK*)/*nosZ* ratio should be considered when investigating soil N_2O emissions in rice-based field systems in the future.

4.4. Implications for future research

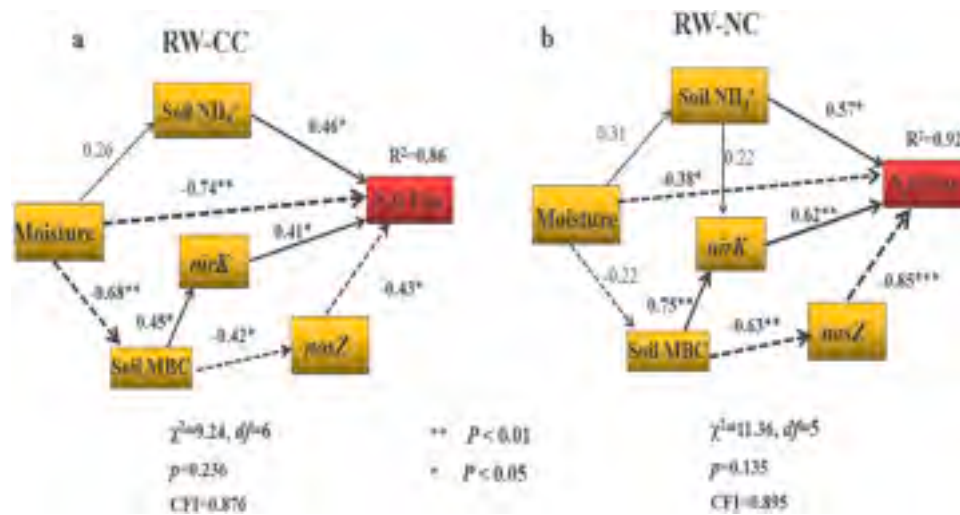The results of this study showed that the rice cropping system plays a

Fig. 8. Structural equation model (SEM) describing the effects of soil parameters (soil NH_4^+ and MBC, moisture reflects field flood depth) and the functional genes (*nirK* and *nosZ*) on N2O emission for each treatment during the rice-growing seasons. Continuous or dashed lines indicate positive or negative effects, respectively. Width of the arrow indicates strength of the effect, numbers close to lines are standardized direct influence coefficients and R^2 stands for the proportion of variables explained by these drivers. χ^2 stands for Chi-square, *df* for degrees of freedom, CFI for comparative fit index, and RMSEA for root mean square error of approximation.

significant role in regulating soil N_2O emissions. However, the frequent co-occurrence of N_2O production and consumption pathways in paddy fields, especially during the rice growing period with fluctuating moisture (i.e., redox-induced) and substrate conditions, may control N_2O production and consumption and subsequently affect N_2O emissions. Considered together, environmental factors, along with soil parameters and N_2O-production related microorganisms might be important parameters regulating N_2O production, consumption, and emission. Therefore, we suggest that studies should focus on the differences in the characteristics of N_2O-production related microorganisms, which could be affected by changing substrate concentrations and habitats, when investigating soil N_2O emissions in a paddy-based field. We suggest that several key functional genes related to N_2O production and reduction should be investigated further in future studies.

5. Conclusions

This study showed that seasonal cumulative N_2O emissions from non-rice cropping treatments were significantly higher than those from rice plantation treatments, indicating a mitigating effect of rice cropping on field N_2O emissions under a typical rice-wheat rotation system. Compared with those in the non-rice cropping treatment, the higher abundance of *nosZ* gene and lower (*nirS*+*nirK*)/*nosZ* ratio in the rice-growing treatment may facilitate greater N_2O reduction to N_2, resulting in decreased N_2O emissions. Therefore, theoretically, our results also revealed that N_2O emissions are primarily regulated by the interaction of key soil parameters and nitrification and denitrification functional genes, such as soil NH_4^+, MBC, *nirK*, and *nosZ*. Thus, studies must consider the differences in the characteristics of available C and N substrates and N_2O-production related microorganisms, which could be affected by changing soil conditions and habitats, when investigating soil N_2O emissions in a rice-based field. In summary, our findings suggest that rice planting is beneficial for the mitigation of soil N_2O emissions during the rice cultivation period; thus, soil N fertilization must be reduced when implementing field agronomic practices.

CRediT authorship contribution statement

Peng Xu did the experiment and wrote the original manuscript; Mengdie Jiang supported to do the experiment and helped to prepare the pictures; Imran Khan assisted to take samples; Jinsong Zhao helped improve the manuscript and Ronggui Hu gave the idea.

Declaration of Competing Interest

The authors declare no conflict of interest in the paper entitled "Rice planting reduced N_2O emissions from rice-growing seasons due to increased *nosZ* gene abundance under a rice-wheat rotation system".

Data Availability

Data will be made available on request.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2023.127025.

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