



# The effect of upland crop planting on field N<sub>2</sub>O emission from rice-growing seasons: A case study comparing rice-wheat and rice-rapeseed rotations

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## ABSTRACT

Paddy fields, especially under flooded rice-upland crop rotation modes, undergo tremendous changes in soil redox conditions. The effects of different upland crop plantations on field N<sub>2</sub>O emissions from the rice-planting period and the underlying mechanisms are scarce. In this study, two types of flooded rice-upland cropping rotation modes, namely, rice-rapeseed rotation (RR) and rice-wheat rotation (RW), were selected to investigate the effects of different upland crop plantations on field N<sub>2</sub>O emissions during the rice-growing period, in central China. To confirm the effect of upland crop plantations on soil variables and associated functional genes, conventional rice planting (RR-CP and RW-CP) with a part of the non-rice area (RR-NP and RW-NP) during the rice season was implemented. Results revealed that seasonal N<sub>2</sub>O emissions from RR-NP and RW-NP treatments were  $1.83 \pm 0.18$  and  $1.43 \pm 0.25$ , and  $1.41 \pm 0.09$  and  $0.88 \pm 0.08$  kg N ha<sup>-1</sup>, respectively, during two consecutive rice-planting seasons, which were significantly higher than those from RR-CP treatment ( $1.38 \pm 0.16$  and  $0.91 \pm 0.15$  kg N ha<sup>-1</sup>) and RW-CP treatment ( $0.95 \pm 0.08$  and  $0.50 \pm 0.10$  kg N ha<sup>-1</sup>). Higher N<sub>2</sub>O emissions from RR rotation than from RW rotation, regardless of rice planting, indicated that upland cultivation affected soil N<sub>2</sub>O efflux. For rice planting treatments, strong positive relationships between N<sub>2</sub>O fluxes and soil-dissolved organic carbon (DOC), ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and functional genes (*nirS*, *nirK*, and *nosZ* genes) were observed, implying that soil available C and N, and related functional genes are key regulatory factors controlling N<sub>2</sub>O emissions. Compared with the RW-CP treatment, the higher abundance of the *nirK* gene and lower *nosZ*/(*nirS* + *nirK*) ratio from the RR-CP treatment may facilitate greater N<sub>2</sub>O production, while reducing conversion of N<sub>2</sub>O to N<sub>2</sub>, resulting in increased N<sub>2</sub>O emissions. Furthermore, structural equation model (SEM) showed that field flood depth (FD), soil available C and N, and the abundance of *nirS* gene, together, displayed more than 70 % impact effect on N<sub>2</sub>O emission for RR-CP treatment, while, FD, soil available N, and *nirS* and *nirK* genes, together, displayed more than 60 % impact effect on N<sub>2</sub>O emission for RW-CP treatment. Our results suggest that suitable upland crop rice cultivation could be beneficial for mitigating soil N<sub>2</sub>O emissions.

## 1. Introduction

Nitrous oxide (N<sub>2</sub>O), one of the primary greenhouse gases (GHGs) in the troposphere, has received much attention from scientists (Davidson, 2009; Tian et al., 2020; Zheng et al., 2022) because its radiative forcing is considerably higher than that of carbon dioxide (CO<sub>2</sub>) under an

equivalent quality level over a 100-year time scale (IPCC, 2013). Agricultural soils have been suggested to be the main contributor to atmospheric N<sub>2</sub>O, and their contribution will continue to increase in the future (Scheehle et al., 2006; Li et al., 2011; Hu et al., 2015). N<sub>2</sub>O production in the soil is primarily derived from microbial nitrification and denitrification processes, proposed by the “hole-in-the-pipe”

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conceptual model (Firestone and Davidson, 1989).

Paddy fields account for more than 12 % of global croplands. A large amount of N<sub>2</sub>O is released from rice field cultivation (Xing et al., 2002; Carlson et al., 2017; Kritee et al., 2018). China accounts for approximately 20 % of the world's rice-planting area (FAO, 2014). Therein, rice-rapeseed (RR), rice-wheat (RW), and rice-fallow (RF) cropping patterns contributed most of the atmospheric N<sub>2</sub>O. More than 20 % of N<sub>2</sub>O emissions in China are attributed to rice-based cultivation systems, making paddy fields an outstanding N<sub>2</sub>O source (Xing et al., 2002). A characteristic of upland cropping-flooded rice rotation is that the field is flooded during the upland cropping period and the rice-growing season (Kögel-Knabner et al., 2010; Xu et al., 2020).

Paddy fields experience frequent water change events, which are highly pertinent to N<sub>2</sub>O production or reduction processes (Liao et al., 2020). Flooding conditions during the rice-plantation period induce anaerobic microsites in the soil, which favor denitrifier activity to produce N<sub>2</sub>O or facilitate the reduction of N<sub>2</sub>O to N<sub>2</sub> (Shaaban et al., 2018; Congreves et al., 2018; Li et al., 2021). In contrast, upland crop plantations lead to good soil aeration, which supports aerobic microbial activities that favor N<sub>2</sub>O production (Shaaban et al., 2018). Significant changes in soil moisture content can alter microbial community composition and gene abundance. The abundance of nitrifier and denitrifier microbial communities is highly sensitive to fluctuating soil moisture contents (Xu et al., 2016; Hu et al., 2017). Thus, the importance of water regimes has emphasized the investigation of functional genes controlling N<sub>2</sub>O production and consumption during the rice-growing season (Qin et al., 2018). In particular, *nirS*, *nirK*, and *nosZ* genes, which are key functional genes related to denitrification, have been investigated during flooding periods in paddy fields (Shaaban et al., 2018; Zhou et al., 2020). However, the understanding of the effect of the preceding upland plantation on soil N<sub>2</sub>O production-related functional genes during subsequent rice-growing seasons remains limited (Ma et al., 2008; Yang et al., 2017). Therefore, a comparative study of the effects of typical upland cropping patterns on soil N<sub>2</sub>O emissions from subsequent paddy fields is required (Ruser et al., 2001; Drury et al., 2021).

Variation in soil moisture content considerably influences soil C and N cycling and their stoichiometric ratios, such as soil dissolved organic carbon (DOC) and dissolved inorganic nitrogen (DIN), which are essential substrates for microbial metabolism (Xu et al., 2022a,b). Previous studies have proposed that the ratio of C to nitrate (NO<sub>3</sub>) can be used to evaluate the interactive effects of available C and N on soil N<sub>2</sub>O emissions (Taylor and Townsend, 2010; Lan et al., 2017). However, previous studies have focused on N<sub>2</sub>O emissions during the rice-planting period (Cai et al., 1997; Zou et al., 2005; Zhou et al., 2018). Attention to the influence of prior upland plantations on the stoichiometric ratios of soil available C to N during subsequent rice-planting seasons is lacking.

Therefore, in this study, two rice-rapeseed (RR) and rice-wheat (RW) plantations were selected to investigate the effect of preceding upland plantations on field N<sub>2</sub>O emissions during subsequent rice plantation seasons. Additionally, key soil parameters, environmental factors, and the corresponding functional genes regulating soil N<sub>2</sub>O production and consumption were selected to elucidate the mechanisms underlying this effect. We aimed to provide a basis for the comparison of soil N<sub>2</sub>O emissions under diverse rice-based rotation modes and elucidate the effect of preceding upland plantations on field N<sub>2</sub>O emissions during subsequent rice-growing seasons.

## 2. Materials and methods

### 2.1. Experimental site and design description

The experimental site description of present study and the soil properties here are documented in our previous research (Xu et al., 2022b). The study duration lasted from May 2018 to Sep 2019, including two rice growing seasons. The field experiment with

conventional rice-wheat rotation (RW-CP) and rice-rapeseed rotation (RR-CP) was established in a completely randomized block design. For each rotation, a total of 3 repetitive plots were established. Each plot was designed identically with a size of 4 m × 6 m. During the rice plantation seasons, a 1.5 m × 1.5 m area was left unplanted to obtain a non-rice planted treatment (namely RW-NP and RR-NP) and was separated from the rice plants with a PVC plate. In this study, all rice planted treatments were implemented with the same water management regime. The same fertilization practices conventionally implemented in this region was chosen, with compound fertilizer [the same rate of N, phosphorus (P) and potassium (K) of 16 %] applied as basal fertilizer, and urea added as N dressing during cropping period. Briefly, the N fertilizer with the rate of 225 kg N ha<sup>-1</sup>, P as P<sub>2</sub>O<sub>5</sub> at the rate of 112.5 kg ha<sup>-1</sup> and K as K<sub>2</sub>O at the rate of 225 kg ha<sup>-1</sup>, were arranged during the rice-plantation seasons. While, the N fertilization rate of 192 kg N ha<sup>-1</sup>, P fertilization rate of 112.5 kg ha<sup>-1</sup> and K fertilization rate of 112.5 kg ha<sup>-1</sup> were arranged for the upland crop-growing season. Detailed information of the fertilizer applications and field management practices were shown in Table S1.

### 2.2. N<sub>2</sub>O fluxes measurement

To obtain N<sub>2</sub>O gas samples, the static opaque chamber was chosen, which has been described by Wang et al. (2013). In brief, the chamber consisted of two parts: a base frame, empty in the middle, has an area of 0.25 m<sup>2</sup> and the height of 0.3 m; And the chamber, wrapped with insulating foam, was designed with the bottom area of 0.25 m<sup>2</sup> and the height of 1 m. The base frame was embedded into the soil in each pot after ploughing field. Especially, the upper edge of each base was designed with a groove to be filled with water to keep gas sealing before each gas sampling. The chamber was manually arranged exactly on the groove of the base frame when taking gas samples. Totally, five gas samples were collected from each chamber with 10 min intervals between two gas samplings using 50-mL air-tight syringes (from 08:30–11:30 am). The sample collection frequency was observed twice weekly and continuously adjusted for seven times a week after fertilization and heavy rain. Immediately, the gas sample within each syringe was measured via gas chromatography (GC; 7890 A, Agilent Technologies, California, USA) in the laboratory. The GC equipment was fitted with an electron capture detector (ECD), where N<sub>2</sub> and a mixture of CO<sub>2</sub> and N<sub>2</sub> acting as a carrier and buffering gas, respectively. N<sub>2</sub>O fluxes and cumulative N<sub>2</sub>O emissions were calculated according to the closed-chamber equation and trapezoidal method proposed by Zheng et al. (2008), respectively.

### 2.3. Auxiliary parameter measurements

Accompanying with N<sub>2</sub>O fluxes measurement, other auxiliary variables were measured for both RR-CP and RW-CP treatments. Briefly, the temperature inside each chamber was determined with a portable digital thermometer (JM624, Liwen Electronics LTD, Tianjin, China). During rice-planting period, the field flood water depth (FD) was recorded by a vertical ruler during filed flooding period accompanying with gas samples collection. Soil temperature at 5-cm depth near each base was measured with a temperature probe. Besides, weekly, five topsoil (0–20 cm) samples were randomly collected in each plot and mixed to make up one composited soil sample. Then, the composited soil samples were quickly transported to the laboratory for further analysis (Xu et al., 2022b). Specially, a part of soil samples were stored in a refrigerator at the temperature of – 80 °C for further microbial analyses. Soil bulk density was determined according to the reference documented by Wu et al. (2017). A part of soil samples were used to measure soil water content (SWC), soil DOC, and DIN (including NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) as suggested by Xu et al. (2022a). The daily air temperature and precipitation were documented by a small weather station near to the trial field.

## 2.4. Real-time quantitative PCR (qPCR)

In the present study, soil microbial DNA samples were extracted by silica matrix adsorption column method (Kouduka et al., 2012). The abundances of three genes (*nirS* and *nirK* genes in regard to  $N_2O$  production, and the *nosZ* gene responsible for  $N_2O$  consumption) were quantitatively analyzed by quantitative PCR (qPCR) according to previously suggested primer sets (Table S2), which were performed by the SYBR GREEN method. Briefly, the qPCR reaction process with the measurement level varying from 95 % to 110 % was used here. And the numerical value of  $R^2$  for each calibration curve was set higher than 0.98. The total 15  $\mu\text{L}$  qPCR reaction system consisted of 7.5  $\mu\text{L}$  SYBR Green Mix, 1  $\mu\text{L}$  diluted template DNA, 0.7  $\mu\text{L}$  primer and 5.8  $\mu\text{L}$  sterile water. The amplification environments were referred to the previous reported document (Zhou et al., 2020). The copy number of each functional gene was calculated by the equations proposed by Macdonald et al. (2011).

## 2.5. Statistical analyses

Seasonal  $N_2O$  emissions were compared between four treatments by one-way analysis of variance. The abundances of functional genes were compared between RR-CP and RW-CP treatments using independent t-test. Linear or nonlinear regressions were chosen to exhibit the functional relationships between  $N_2O$  fluxes and soil variables and functional genes abundance. Structural equation model (SEM) implemented with AMOS software package (AMOS 21.0, SPSS Inc., Chicago, USA) was used to exhibit the influence effect of environment factors, soil variables and functional genes on  $N_2O$  emission. Figures were prepared with Origin 8.5 software (Origin Lab Corporation, USA). All statistical analysis was performed with SPSS software (SPSS19.0, SPSS Inc., Chicago, USA) and  $p \leq 0.05$  level was chosen to represent statistical significance.

## 3. Results

### 3.1. Climate, environmental and soil variables

The daily air temperature varied from 20.7 °C to 32.5 °C and from 18.3 °C to 33.6 °C. The seasonal precipitation was 496.7 and 559.3 mm for rice-plantation season in 2018 and rice-plantation season in 2019, respectively (Fig. 1). Concomitantly, soil temperature at 5-cm depth varied from 21.6° to 31.4°C and from 21.8° to 32.7°C for the first and subsequent rice plantation seasons respectively, (Fig. 1). The water depth varied from 0 to 4.75 cm and from 0 to 4.65 cm for the RR-CP treatment for the first and subsequent rice planting seasons,

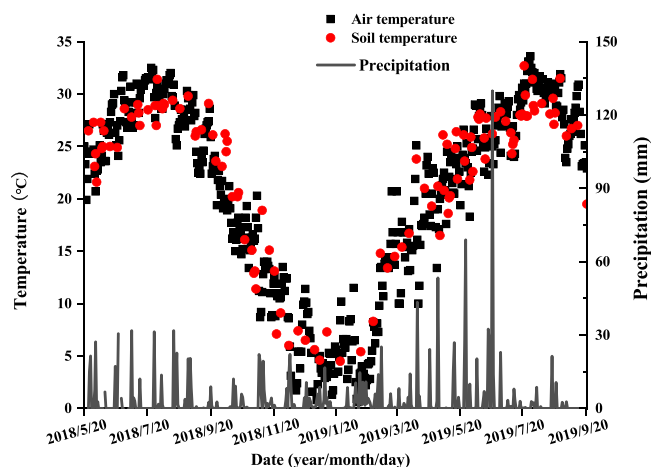


Fig. 1. Dynamics in daily mean air temperature (a), soil temperature at 5-cm depth (b) and Precipitation (c) during the whole experimental period.

respectively, and those varied from 0 to 4.56 cm and from 0 to 4.63 cm from RW-CP treatment (Fig. S1).

Soil ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) contents for both RR-CP and RW-CP treatments increased swiftly after basal N fertilization, and afterwards, it remained at a low level. Soil  $\text{NH}_4^+\text{-N}$  contents varied from 8.14 to 76.01  $\text{mg N kg}^{-1}$  and from 1.48 to 67.57  $\text{mg N kg}^{-1}$  for RR-CP treatment, and those varied from 9.03 to 84.73  $\text{mg N kg}^{-1}$  and from 0.76 to 57.14  $\text{mg N kg}^{-1}$  for RW-CP treatment for the first and subsequent rice seasons, respectively (Fig. 2a). In contrast, a slight elevation was observed in soil nitrate nitrogen ( $\text{NO}_3^+\text{-N}$ ) contents after N fertilizer application, with a range from 2.01 to 22.92  $\text{mg N kg}^{-1}$  and from 0.35 to 26.76  $\text{mg N kg}^{-1}$  for RR-CP, for those rice plantation seasons, respectively (Fig. 2b). These values showed no significant differences as compared to those with RW-CP treatment (varying from 2.24 to 17.04  $\text{mg N kg}^{-1}$  and from 0.36 to 16.61  $\text{mg N kg}^{-1}$ , Fig. 2b). Basically, soil DOC contents kept at a stable level during two rice plantation seasons for both treatments. Soil DOC contents ranged from 25.54 to 52.04  $\text{mg C kg}^{-1}$  and from 21.19 to 58.56  $\text{mg C kg}^{-1}$  for RR-CP treatment, for two rice-plantation seasons, respectively. DOC contents varied from 24.03 to 56.31  $\text{mg C kg}^{-1}$  and 23.92–64.54  $\text{mg C kg}^{-1}$ , for RW-CP treatment (Fig. 2c).

### 3.2. Abundance of *nirK*, *nirS* and *nosZ* genes

The abundance of functional genes generally showed a similar pattern for two rice-planting seasons (Fig. 3). The abundance of *nirK*, *nirS* and *nosZ* genes ranged from  $2 \times 10^7$  to  $1.6 \times 10^8$ ,  $1 \times 10^7$  to  $1.3 \times 10^8$ , and  $0.2 \times 10^7$  to  $9 \times 10^7$   $\text{g}^{-1}$  dry soil, for both treatments, respectively. The ratio of *nosZ*/*nirS*+*nirK*) ranged between 0.05 and 0.76  $\text{g}^{-1}$  dry soil, for both treatments. Furthermore, the differences in gene abundance of *nirS*, *nirK* and *nosZ* genes, and the ratio of *nosZ*/*nirS*+*nirK*) were determined for RR-CP and RW-CP treatments during each rice plantation season. Generally, the copy number of *nirK* gene in RR-CP treatment was significantly higher than that in RW-CP treatment during the rice seasons (Fig. 3a). Neither the abundance of *nirS* gene nor *nosZ* gene exhibited significant differences between two treatments

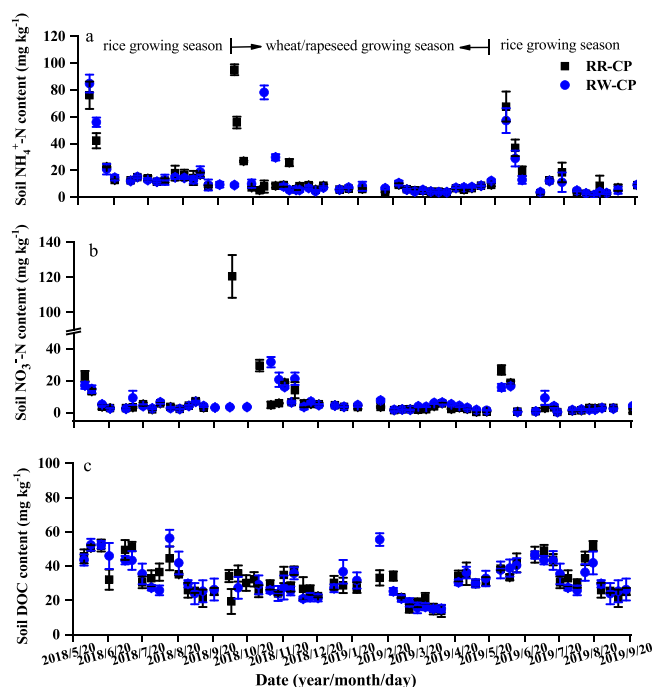
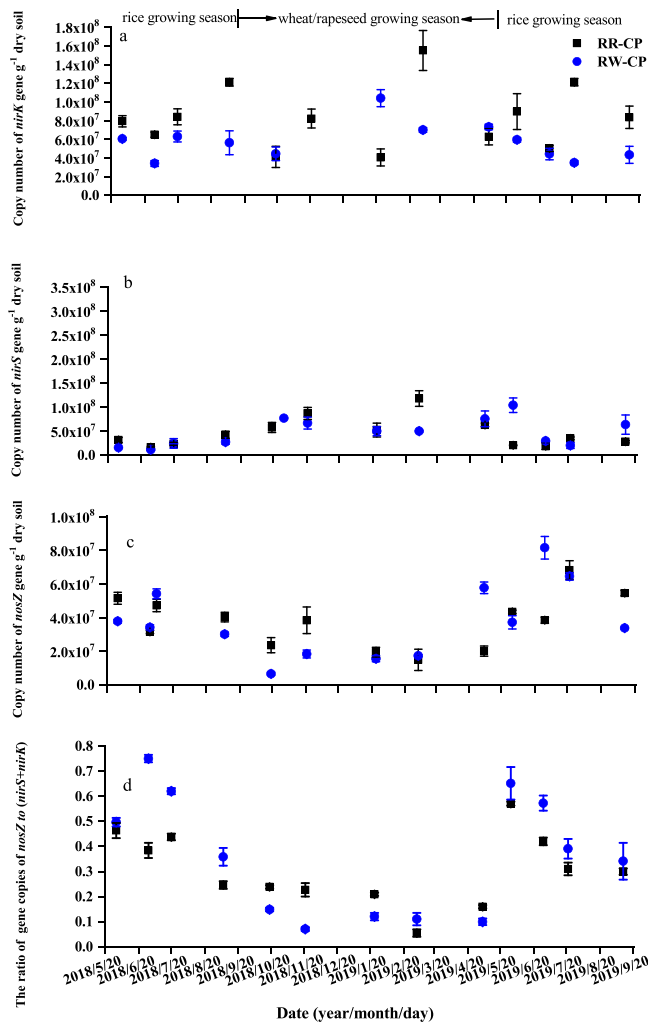


Fig. 2. Dynamics in soil ammonium ( $\text{NH}_4^+\text{-N}$ ), nitrate ( $\text{NO}_3^+\text{-N}$ ) and dissolved organic carbon (DOC) for RR-CP and RW-CP treatments during the whole experimental period.



**Fig. 3.** Dynamics in the abundances of *nirS*, *nirK* and *nosZ* genes and the abundance ratio of *nosZ* to (*nirS*+*nirK*) for RR-CP and RW-CP treatments during the whole experimental period.

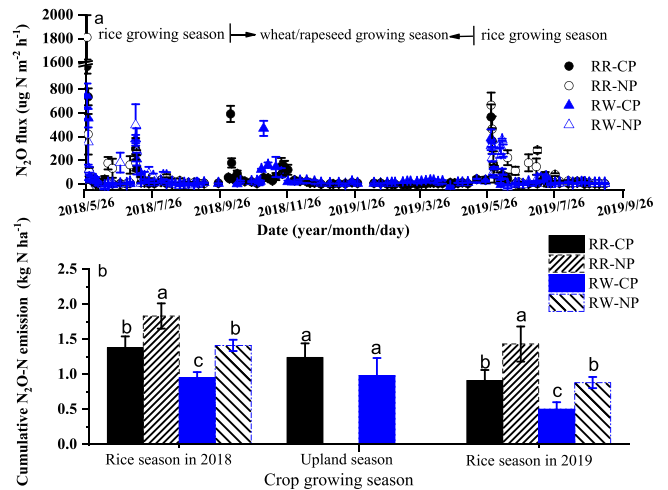
(Fig. 3b, c and Table 1). Besides, the ratio of *nosZ*/*(nirS+nirK)* was obviously influenced by rice-based rotation mode during the first rice-planting season in 2018 (Table 1).

### 3.3. N<sub>2</sub>O emissions

Soil N<sub>2</sub>O fluxes varied from 0.29 to 990.27 μg N m<sup>-2</sup> h<sup>-1</sup> and from -4.06 to 564.65 μg N m<sup>-2</sup> h<sup>-1</sup> in RR-CP treatment during 2018 and 2019, respectively (Fig. 4a). The higher N<sub>2</sub>O emission peak was observed from RR-NP treatment, with a range of -12.18 to 1809.93 μg N m<sup>-2</sup> h<sup>-1</sup> and of 3.32–666.43 μg N m<sup>-2</sup> h<sup>-1</sup> during those two rice-plantation seasons, respectively (Fig. 4a). Comparatively, N<sub>2</sub>O

**Table 1**  
Statistical analysis for the effect of rice paddy planting pattern on the abundance of *nirS*, *nirK* and *nosZ* genes and the ratio of *nosZ* to (*nirS*+*nirK*).

Seasons	<i>nirS</i>		<i>nirK</i>		<i>nosZ</i>		<i>nosZ</i> / ( <i>nirS</i> + <i>nirK</i> )	
	t value	p value	t value	p value	t value	p value	t value	p value
Rice season in 2018	1.363	> 0.05	2.483	< 0.05	0.518	> 0.05	-2.537	< 0.05
Rice season in 2019	-1.486	> 0.05	2.640	< 0.05	-0.234	> 0.05	-0.928	> 0.05



**Fig. 4.** Seasonal patterns in N<sub>2</sub>O fluxes and cumulative N<sub>2</sub>O emissions for RR-CP and RW-CP and RR-NP and RW-NP treatments during the whole experimental period.

fluxes varied from -26.22 to 746.90 μg N m<sup>-2</sup> h<sup>-1</sup> and from -6.20 to 386.92 μg N m<sup>-2</sup> h<sup>-1</sup> from RW-CP treatment, for the first and subsequent rice-growing seasons, respectively. Similarly, the higher N<sub>2</sub>O emission peak was observed for non-rice treatment (RW-NP), which ranged from -14.52 to 1470.75 μg N m<sup>-2</sup> h<sup>-1</sup> and from -24.35 to 371.96 μg N m<sup>-2</sup> h<sup>-1</sup> during two rice-planting seasons (Fig. 4a).

The seasonal N<sub>2</sub>O emissions from RR-CP treatment were 1.38 ± 0.16 and 0.91 ± 0.15 kg N ha<sup>-1</sup> for two rice-plantation seasons, respectively, and were significantly lower than those from RR-NP treatment with the N<sub>2</sub>O emission value of 1.83 ± 0.18 and 1.43 ± 0.25 kg N ha<sup>-1</sup>, from two rice-plantation seasons, respectively (Fig. 4b). Whereas, seasonal cumulative N<sub>2</sub>O emissions were lower for RW rotation, with the amount of 0.95 ± 0.08 kg N ha<sup>-1</sup> and 0.50 ± 0.10 kg N ha<sup>-1</sup> for RW-CP treatment, and 1.41 ± 0.09 kg N ha<sup>-1</sup> and 0.88 ± 0.08 kg N ha<sup>-1</sup> for RW-NP treatment, respectively (Fig. 4b). In total, for both rice rotations, as compared to rice cropping treatments, significantly higher cumulative N<sub>2</sub>O emissions from non-rice treatments were observed.

### 3.4. The relationships between N<sub>2</sub>O fluxes, soil variables and gene abundances

The N<sub>2</sub>O fluxes were found to be interrelated with several soil variables (Fig. 5). Specially, the N<sub>2</sub>O fluxes were linearly positively correlated with soil DOC contents for RW-CP treatment (Fig. 5a), and N<sub>2</sub>O fluxes had a positive linear correlation with soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> for both treatments (Fig. 5b and c), while those were negatively correlated with the ratio of DOC to NO<sub>3</sub> (DOC/NO<sub>3</sub>) for both RR-CP and RW-CP treatments during two rice-planting seasons (Fig. 5d). Besides, a linear relationship between N<sub>2</sub>O fluxes and *nirK* gene was observed for RW-CP treatment (Fig. 6a). For both treatments, positively linear relationships between N<sub>2</sub>O emission fluxes and the abundance of *nirS* genes were observed (Fig. 6b), while a negatively linear relationship between N<sub>2</sub>O fluxes and the ratio of *nosZ*/*(nirS+nirK)* was found only for RW-CP treatment (Fig. 6c).

### 3.5. Structural equation model (SEM) describing the effects of various factors on N<sub>2</sub>O emission

In this study, a structural equation model (SEM) was used to evaluate the influence of several key soil variables and functional genes on soil N<sub>2</sub>O emissions during the rice-plantation seasons (Fig. 7). The factors including soil parameters (soil NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub>, DOC and field flood water depth) and the functional genes *nirS* directly and indirectly explained 76



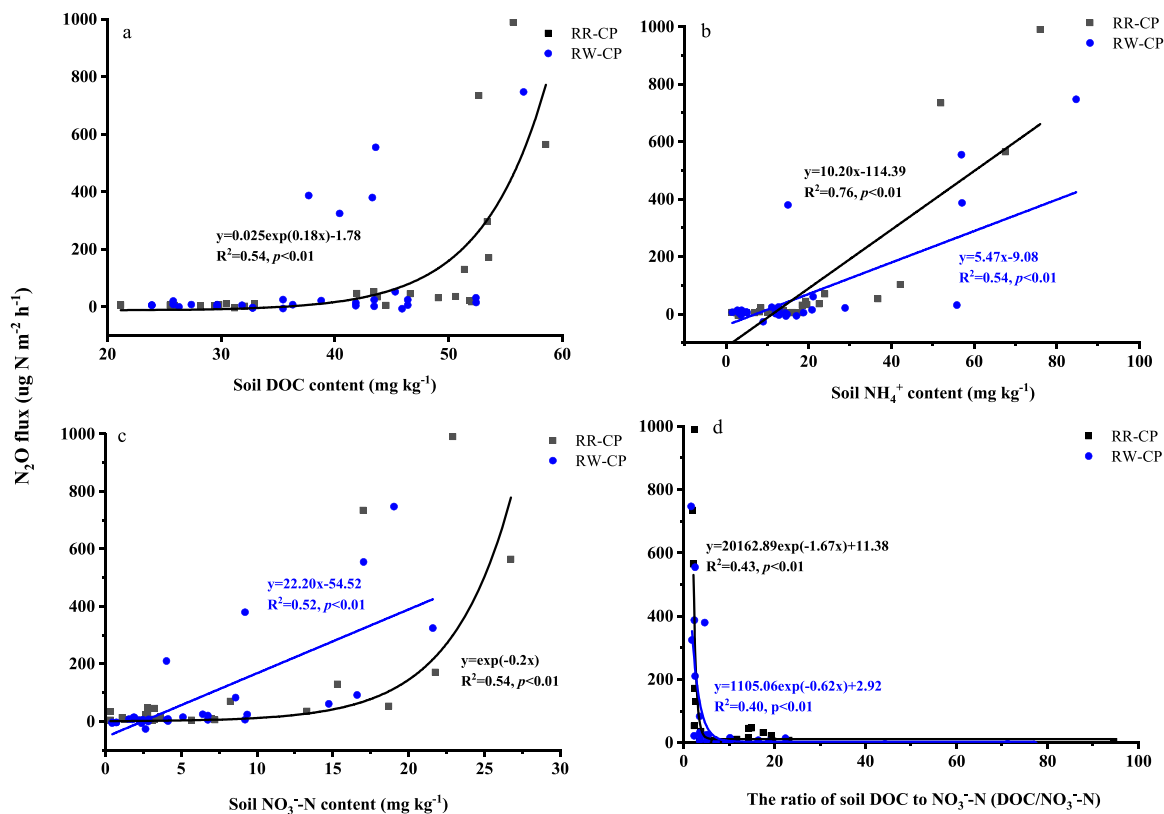


Fig. 5. Relationship between N<sub>2</sub>O fluxes and soil DOC, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N content and the ratio of DOC to NO<sub>3</sub><sup>-</sup>-N for RR-CP and RW-CP treatments during the rice-growing seasons.

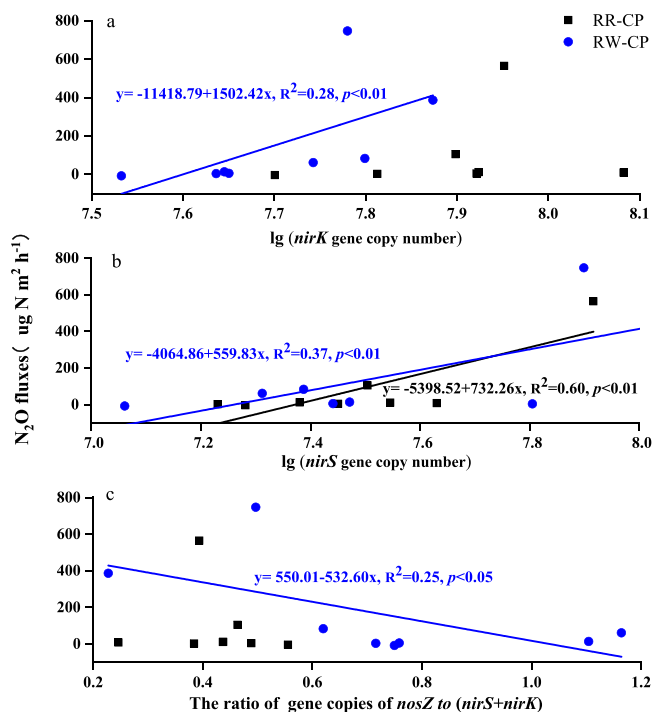


Fig. 6. Relationship between N<sub>2</sub>O fluxes and the abundance of functional genes for RR-CP and RW-CP treatments during the rice-growing seasons.

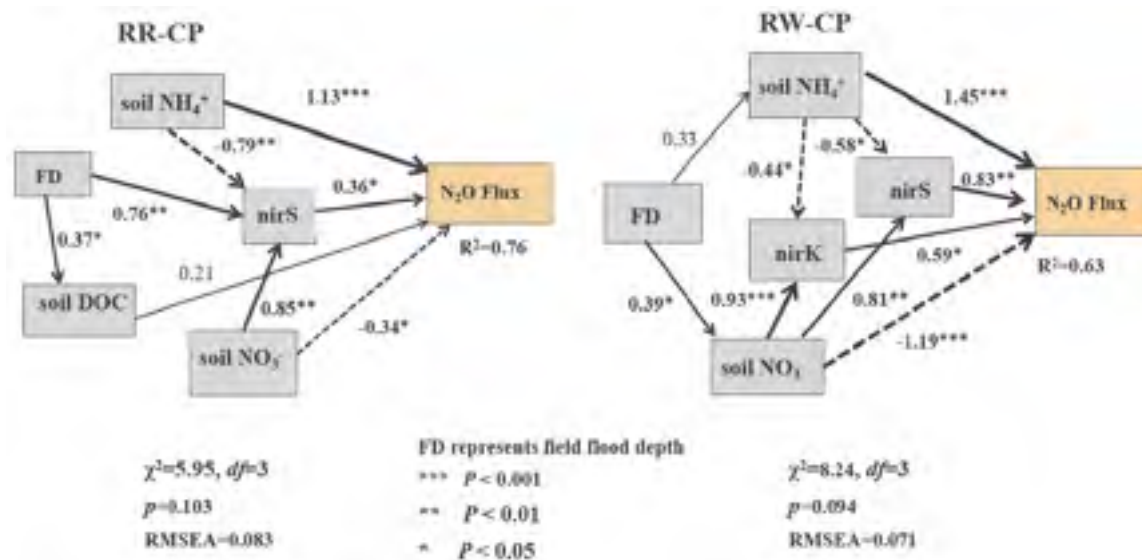
% N<sub>2</sub>O emission for RR-CP treatment (Fig. 7a), while, soil NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, FD and two functional genes i.e. *nirS* and *nirK* exhibited 63 % explanation effect for N<sub>2</sub>O emissions for RW-CP treatment (Fig. 7b).

#### 4. Discussion

##### 4.1. The impact of upland cropping on N<sub>2</sub>O emissions during rice-growing seasons

Although many studies reported field N<sub>2</sub>O emissions from rice-based rotation systems (Cai et al., 1997; Flessa et al., 2002; Zhou et al., 2018; Xu et al., 2022a), the comparative studies under different rotations, especially, how prior upland cropping affects soil N<sub>2</sub>O emission during the subsequent flooded rice plantation season are scarce. In this study, more soil N<sub>2</sub>O emissions during the rice-planting periods under RR rotation compared with RW rotation were observed, regardless of the presence of rice, indicating a crucial role of prior upland cropping in soil N<sub>2</sub>O emissions during the subsequently flooded rice cultivation (Kim et al., 2021). The possible explanations may be as follows: different prior upland crop cultivation certainly influenced soil structure and condition during the subsequent rice cultivation period, consequently affecting the response of N<sub>2</sub>O emissions in soil. In addition, compared with the wheat growth period, rapeseed with higher root length may enhance photosynthesis-derived C and accelerate the exudation of root exudates to the soil during its growth period, which could provide abundant substrates for microbial activities related to N<sub>2</sub>O production during the subsequent rice-planting season, resulting in increased N<sub>2</sub>O emissions (Jiang et al., 2020). Some studies have reported that the emission of soil N<sub>2</sub>O largely depends on the availability of soil available substrates, especially N nutrition (Xu et al., 2022a). Furthermore, prior upland crop planting could play a significant role in the activities of microorganisms responsible for N<sub>2</sub>O production and consumption (Kader et al., 2013).

The RR and RW rotation modes exhibited similar variations in N<sub>2</sub>O emissions, which showed that N<sub>2</sub>O emissions from non-rice planting treatments were significantly higher than those from rice planting treatments, indicating that rice plantations emitted less N<sub>2</sub>O. This can be explained by the higher soil DIN concentration under the non-rice



**Fig. 7.** Structural equation model (SEM) describing the effects of soil variables (FD, soil DOC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) and the functional genes (*nirS* and *nirK*) on  $\text{N}_2\text{O}$  emissions 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.  $\chi^2$  stands for Chi-square,  $df$  for degrees of freedom and RMSEA for root mean square error of approximation.

treatment than under crop planting, which supported  $\text{N}_2\text{O}$  production. Similar results were observed in a previous study in which decreased mineral N content in soil was observed after planting crops, but the content of DIN in bare soil was higher (Hodge et al., 2000). High mineral N content in bare soil provides a sufficient substrate for nitrification and denitrification microorganisms and enhances  $\text{N}_2\text{O}$  emissions (Davidson, 2009; Zhang et al., 2016). Additionally, flooding during rice plantation contributes to oxygen depletion and creates an anaerobic soil environment, which may ultimately favor complete denitrification, reducing  $\text{N}_2\text{O}$  emissions (Yang et al., 2021). We also assumed that organic C compound exudates from rice roots acted as energy sources for microorganisms responsible for  $\text{N}_2\text{O}$  reduction (Zhu et al., 2018; Khan et al., 2019).

#### 4.2. The relationship between soil variables and $\text{N}_2\text{O}$ emission

Soil available C and N, such as DOC,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , microbial biomass C (MBC), and microbial biomass N (MBN), are recognized as essential substrates for microbial nitrification and denitrification processes (Meijide et al., 2007; Shaaban et al., 2019; Xu et al., 2022a,b). In this study, basal N fertilization triggered soil  $\text{N}_2\text{O}$  emissions during the rice-planting period. This is because the soil mineral N content was high after N fertilization, thus providing sufficient N substrate for  $\text{N}_2\text{O}$  production-related microorganisms (Simojoki et al., 2000; Ni et al., 2012; Zhang et al., 2016). This can be further confirmed by the positive correlations between  $\text{N}_2\text{O}$  emissions and soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents for RR-CP and RW-CP treatments (Fig. 4b and c). Similarly, previous reports have indicated that soil DOC is an active substrate that can be utilized by microorganisms, affecting  $\text{N}_2\text{O}$  production and emissions (Shaaban et al., 2019). The results of this study showed that a greater DOC content could promote  $\text{N}_2\text{O}$  emissions for the RW-CP treatment (Fig. 4a). In addition, we observed that  $\text{N}_2\text{O}$  emissions during the rice-growing period had a good relationship with the ratio of soil DOC to  $\text{NO}_3^-$ , which is similar to previous studies (Hu et al., 2015; Zhou et al., 2017). For example, Lan et al. (2017) found that  $\text{N}_2\text{O}$  emissions peaked when the  $\text{DOC}/\text{NO}_3^-$  stoichiometric ratio was between 0.69 and 0.84. Zhou et al. (2017) also reported a relationship between soil  $\text{DOC}/(\text{NH}_4^+ + \text{NO}_3^-)$  and  $\text{N}_2\text{O}$  fluxes. The results showed that the active soil C/N ratio was also an important factor in regulating  $\text{N}_2\text{O}$  emissions from paddy fields.

#### 4.3. The effect of prior upland cropping on functional genes in subsequent rice seasons

It has been suggested that microbial functional genes can affect  $\text{N}_2\text{O}$  production and consumption (Zhou et al., 2020). However, little is known about whether prior upland cropping can affect key functional genes regarding  $\text{N}_2\text{O}$  production and consumption (Lenhart et al., 2019), which was investigated in our study. Regarding *nirS* and *nirK* genes, controlling the reduction of nitrite ( $\text{NO}_2^-$ ) to nitric oxide (NO) has been proposed as a key limiting step for  $\text{N}_2\text{O}$  production (Lenhart et al., 2019; Zhou et al., 2020). Indeed, we found that compared with the RW-CP treatment, RR-CP had a higher copy number of the *nirK* functional gene (Table 1). The higher gene abundance may be expressed more and produce greater nitrite reductase to promote the conversion of nitrite ( $\text{NO}_2^-$ ) to nitric oxide (NO), which can be easily reduced to produce  $\text{N}_2\text{O}$ , consequently contributing to greater  $\text{N}_2\text{O}$  emissions from the RR-CP treatment. Similarly, higher *nirK* gene abundance accompanied by greater  $\text{N}_2\text{O}$  emissions has been found in previous studies (Hai et al., 2009; Yang et al., 2022). Additionally, Zhou et al. (2020) found that after straw addition, the soil  $\text{N}_2\text{O}$  fluxes were significantly positively correlated with the abundance of the *nirK* gene. SEM analyses also showed the contribution of the abundance of the *nirK* gene to soil  $\text{N}_2\text{O}$  emissions.

Denitrifying *nosZ* regulates the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$ . However, the differences in the abundance of these two genes (*nirK* and *nosZ*) between the RR-CP and RW-CP treatments were not significant. Similarly, Xu et al. (2022b) reported no difference in the abundance of the *nosZ* gene between rice and non-rice plantation treatments under a double rice-fallow cultivation mode. Additionally, we found that  $\text{N}_2\text{O}$  fluxes were negatively correlated with the ratio of *nosZ*/*(nirS+nirK)* for the RW-CP treatment. A significant difference in the gene abundance ratio between the RR-CP and RW-CP treatments was observed during the first rice plantation season (Table 1). As suggested, a higher *nosZ*/*(nirS+nirK)* ratio indicated a greater reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Zhou et al., 2020), which may further explain the lower  $\text{N}_2\text{O}$  emissions during the rice-planting period under RW rotation. Thus, our results imply that the ratio of *nosZ*/*(nirS+nirK)* in soil  $\text{N}_2\text{O}$  emissions should be considered in a rice-based field system.

#### 4.4. The implication for future research

Generally, our study indicated that rice-based rotation system played a greater role in regulating soil N<sub>2</sub>O emissions, especially, the effect of upland crop planting on field N<sub>2</sub>O emissions from rice-growing seasons was obvious. However, N<sub>2</sub>O production and consumption pathways possibly co-occur during the rice-growing period with fluctuating field water level and substrates conditions. Taken together, our study also revealed the environmental parameters, soil variables and N<sub>2</sub>O related microorganisms may control N<sub>2</sub>O production and consumption and subsequently affecting soil N<sub>2</sub>O emissions. Thus, we suggest that the difference in characteristics of N<sub>2</sub>O-production related microorganisms and functional genes, which could be affected by the prior upland planting cultivation, should be further considered in the future research.

#### 5. Conclusions

The present study investigated the effect of upland crop planting on field N<sub>2</sub>O emissions from rice-growing seasons and observed higher N<sub>2</sub>O emissions from RR rotation than those from RW rotation regardless of rice planting, indicating that upland cultivation could affect soil N<sub>2</sub>O efflux. Our findings also revealed that N<sub>2</sub>O emissions from paddy field could be collectively regulated by several key soil variables and functional genes, like, soil NH<sub>4</sub><sup>+</sup>, DOC and *nirS* and *nirK* genes and the gene abundance ratio of *nosZ* to (*nirS* + *nirK*). Generally, our results suggested upland cropping could affect soil N<sub>2</sub>O emissions during the subsequent rice planting season, thus, for the mitigation of field N<sub>2</sub>O emissions, suitable upland cropping should be selected and encouraged.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108365](https://doi.org/10.1016/j.agee.2023.108365).

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