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Effects of mild alternate wetting and drying irrigation and rice straw application on N_2O emissions in rice cultivation

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Abstract. The shortage of water resources and the decline in soil organic matter (SOM) are important limiting factors affecting the improvement of rice productivity, while alternate wetting and drying (AWD) irrigation and rice straw return are considered favorable mitigation measures.

However, its impact on rice yield and greenhouse gas (GHG) emissions, especially nitrous oxide (N_2O) emissions, needs to be further clarified, which is essential for the development of agronomic measures for water savings, soil fertilization and GHG reduction. Therefore, we explored the effects of mild AWD irrigation combined with rice straw return on N_2O emissions and rice yield through rice pot experiments. This study showed that N_2O emissions were mainly affected by urea application and irrigation methods, and urea application was the main reason. Compared with continuous flooding (CF) irrigation, mild AWD irrigation increased cumulative N_2O emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated N_2O emissions. Compared with CF irrigation, mild AWD irrigation increased the yield-scaled N_2O emissions, and the addition of rice straw further promoted the yield-scaled N_2O emissions under mild AWD irrigation but reduced the global warming potential (GWP) by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation reduced nitrogen uptake by rice in the soil and rice aboveground biomass without reducing rice yield. Therefore, mild AWD irrigation combined with rice straw return is a promising agronomic measure to ensure rice yield, reduce the greenhouse effect and maintain or improve soil fertility.

1 Introduction

Rice is the staple food for more than half of the world's population, and ensuring rice production is crucial to food security (Tang and Cheng, 2018). More than 135 million hectares of rice are cultivated worldwide, and approximately 90% of paddy fields are submerged (Wang et al., 2017a). Feeding a growing population under water scarcity is a major challenge to Asia's food security in the coming decades (Lampayan et al., 2015). In China, more than 60% of freshwater resources are consumed by rice cultivation every year, which not only causes a great waste of freshwater but also causes many environmental problems, such as nonpoint source pollution, eutrophication of water bodies, and greenhouse gas (GHG) emissions (Liao et al., 2020). Therefore, it is urgent to explore paddy field fertilization management measures that can ensure rice yield, reduce water waste, and reduce environmental pollution.

Alternate wetting and drying (AWD) irrigation is an effective water-saving irrigation method that can save approximately 23% of fresh water resources compared with continuous flooding (CF) irrigation (Bouman and Tuong, 2001; Chu et al., 2014). There are usually two types of alternate wetting and drying irrigation: severe AWD irrigation (soil water potential ≥ -30±5 kPa) and mild AWD irrigation (soil water potential ≥ -15±5 kPa). Severe AWD irrigation could reduce rice yield by

22.6%, but under mild AWD irrigation, rice yield was stable or slightly increased (Carrijo et al., 2017; Zhou et al., 2017). Therefore, mild AWD irrigation may be a more promising paddy field management model. Previous studies have shown that AWD irrigation can significantly reduce methane (CH_4) emissions but significantly promote nitrous oxide (N_2O) emissions, while global warming potential (GWP) mitigation is dependent on the magnitude of the increase in N_2O emissions (Lagomarsino et al., 2016; Liang et al., 2017; Kritee et al., 2018; Tan et al., 2018). Therefore, exploring the impact of mild AWD irrigation on GHG emissions, especially N_2O emissions, is conducive to reducing paddy field emissions and maximizing environmental benefits.

Long-term cultivation without organic matter supplementation leads to serious degradation of cultivated land in China, which reduces the soil organic matter (SOM) content (zhou et al., 2021), which is not conducive to rice production and sustainable agricultural development (chen et al., 2016). Straw return to the field is considered to be an important measure for improving SOM (huang et al., 2021), and it is beneficial for reducing the environmental pollution caused by burning straw or discarding it randomly (wang et al., 2018). In addition, rice straw return may also cause changes in paddy field GHG emissions (Naser et al., 2007; Ye et al., 2017; Sun et al., 2018; Yu et al., 2021), nitrogen use efficiency (Liu et al., 2021), and rice yield (Chen et al., 2016; Ku et al., 2019). Although there have been many studies on mild paddy AWD irrigation or rice straw return, few studies have focused on the effects of mild AWD irrigation combined with rice straw return on rice cultivation. Under the conventional fertilization mode, whether mild AWD irrigation combined with rice straw returning can achieve the optimal goals of water saving, yield increase, and reduction of greenhouse gas emissions needs to be further explored. Therefore, the purpose of this study was to investigate the effect of mild AWD irrigation combined with rice straw returning on N_2O emissions and rice yield in rice cultivation, and to explore the nitrogen supply of soil, urea and rice straw to rice growth through ^{15}N labeling technology. We put forward the following hypotheses: 1) Mild AWD irrigation would promote N_2O emission in rice cultivation; 2) Mild AWD irrigation would reduce soil nitrogen uptake by rice; 3) Mild AWD irrigation would maintain or promote rice yield.

2 Materials and methods

2.1 Experimental site and soil properties

The pot experiment was conducted in an open greenhouse at the Shenyang Experimental Station of the Institute of Applied Ecology, Liaoning Province, China (43°32'N, 123°23'E) from June 17th to October 27th, 2020.

2.2 Experimental design

The pot experiment was designed with a completely random block

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design, including 30 pots (30 cm diameter × 20 cm height). This experiment included two irrigation methods, two nitrogen application levels, two rice straw return levels and three replicates for each combination. The two irrigation methods were CF irrigation and mild AWD irrigation. CF irrigation maintained a water level depth of approximately 3-5 cm throughout the rice-growing season. Mild AWD irrigation water management in the first 7 days was consistent with CF irrigation and then it was naturally pursued. When the soil negative pressure gauge reached -15 kPa, it was subflooded 3-5 cm again and then naturally dried up again. This step was repeated until harvest. CF and mild AWD irrigation were halted 2 weeks before harvest. Nitrogen was applied at 0 kg N ha^{-1} (CK (control check) and ^{15}S (rice straw)) and 225 kg N ha^{-1} (106.13 mg kg^{-1} dry soil) (^{15}U (urea), U^{15}S (urea + rice straw) and ^{15}US (urea + rice straw)). The abundance of urea ^{15}N was 10.20%. Urea was applied three times: base fertilizer 40% (June 17th), tiller topdressing 30% (August 4th) and heading topdressing 30% (August 25th). Rice straw return was applied at 0 kg ha^{-1} (CK and ^{15}U) and 9000 kg ha^{-1} (4.25 g kg^{-1} dry soil) (^{15}S, U^{15}S and ^{15}US). The total nitrogen content of unlabeled rice straw was 0.72%, and the isotope abundance of ^{15}N was 0.59%. The total nitrogen content of labeled rice straw was 0.73%, and the ^{15}N isotope abundance was 22.94%. The rice straw was ground and applied together with the base fertilizer. Phosphate fertilizer was superphosphate (150 kg P_2O_5 ha^{-1}), and potassium fertilizer was potassium chloride (185 kg K_2O ha^{-1}) as a one-time application of basic fertilizer. Every pot was filled with 10.51 kg (9 kg dry soil) of sieved (2 mm) fresh soil. Two hills of rice were planted in each pot. At maturity, the rice yield and aboveground biomass were recorded after being oven dried (105 ℃ for 0.5 h and 60 ℃ for 12 h).

2.3 Soil sample collection and analysis

At the regreening stage, tillering stage, jointing stage, booting stage, filling stage and maturity stage, five points were randomly selected from the 0-10 cm soil layer of each pot and mixed. The soil NH_4^+-N and NO_3^--N were extracted with 2 mol L^{-1} KCl solution (Wu et al., 2019), filtered and analyzed with a continuous flow analyzer (AA3, Bran + Luebbe, Germany). The extraction of soil ^{15}N-NH_4^+-N followed Yu et al. (2020). Soil microbial biomass nitrogen (MBN) was fumigated with chloroform, extracted with 0.5 mol L^{-1} K_2SO_4 (soil: solution = 5 g: 20 ml) (Joergensen et al., 1996), and determined by a TOC analyzer (Elementar vario TOC Analyzer, Germany). The soil ^{15}N-NH_4^+-N content, ^{15}N-MBN and ^{15}N of rice aboveground biomass were determined by a stable isotope ratio mass spectrometer (253 MAT, Thermo Finnigan, Germany).

2.4 Gas sampling and calculation

The static chamber method was used to determine the N_2O flux (Li et al., 2018a). The static chamber with a top seal made of transparent

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plexiglass consisted of two parts, namely, the base and the gas collecting chamber. The base had a diameter of 31 cm, a groove in the middle, and a height of 10 cm. The gas-collecting chamber had a diameter of 30 cm and a height of 70 cm. A small fan and a thermometer were installed in the gas-collecting chamber. N_2O was collected every two days in the first week after fertilization or irrigation and every seven days on the other days. N_2O was sampled at 8:00–11:00 am each sampling day. Every pot was sealed with water when N_2O was collected. Three gas samples were collected at 0, 30 and 60 min after the chamber was airtight, and N_2O was collected with a 50 mL injector and then injected into 200 mL gasbags.

The N_2O concentration was analyzed by a gas chromatograph (Agilent 7890B, Gas Chromatograph, Delaware, USA). The calculation of N_2O fluxes was as follows (Li et al., 2018a):

$$F = \rho \times h \times dc/dt \times 273/(273 + T)$$

where F is the N_2O flux ($\mu g\,m^{-2}\,h^{-1}$); ρ is the N_2O standard-state density (1.964 kg m^{-3}); h is the chamber height above the soil (m); c is the N_2O concentration; dc/dt is the slope of the N_2O concentration curve, estimated using a linear regression model (Vitale et al., 2017); 273 is the gas constant; and T is the average air temperature inside the chamber during N_2O collection (℃).

Cumulative N_2O emissions were calculated using the following formula according to Wang et al. (2011):

$$CE\ (kg\ N_2O\ ha^{-1}) = \sum_{i=1}^{n} (\frac{F_i + F_{i+1}}{2})(t_{i+1} - t_i) \times 24 \times 10^{-2}$$

where *i* is the various sampling times, *t* is the sampling date, *n* is the total measurement time and 10^{-2} is the conversion factor.

The contribution of ^{15}N markers to NH_4^+-N and MBN and the calculation of the nitrogen source of the aboveground biomass of rice follow Ma et al. (2015). Yield-scaled N_2O was calculated as the ratio between N_2O and rice yield (Li et al., 2018a).

2.5 Statistical analysis

All analyses were performed using SPSS Statistics 16.0 (SPSS, Inc., Chicago, USA). One-way ANOVA was conducted to test the treatment effects with Duncan's test. Significant differences were set at alpha = 0.05. Univariate analysis of variance was used to analyze the response of cumulative N_2O emissions to irrigation method, nitrogen level and rice straw application. Tables and figures were prepared with Excel 2016 (Microsoft Corp., USA) and Origin 8 (Origin Lab Corp., USA), respectively. The data in the figures and tables are the average value ± standard error.

3 Results

3.1 N_2O flux

Three higher N_2O flux peaks appeared after basal fertilizer and two topdressing treatments (Figure 1), and the N_2O flux peak after basal fertilizer application was significantly larger than the last two peaks. After basal fertilizer was applied, the N_2O flux peaks of CF irrigation and mild AWD irrigation were similar. After the first topdressing, the N_2O flux peak of mild AWD irrigation was significantly greater than that of CF irrigation, which was 4 times that of CF on average. In contrast, the N_2O flux peak of CF irrigation after the second topdressing was twice that of mild AWD irrigation. In addition to the above three peaks, the N_2O flux of CF irrigation was close to zero, and mild AWD irrigation had a lower flux peak with alternating wet and dry conditions. The flux of N_2O ranged from -103.93 μg m^{-2} h^{-1} to 2770.50 μg m^{-2} h^{-1}. The N_2O flux appeared negative in the late stage of rice growth. The N_2O fluxes of CF irrigation and mild AWD irrigation were similar in the later stage of rice growth, indicating that drainage had little effect on it. During the entire growth cycle of rice, the N_2O flux of the CK and S treatments was low. There were significant differences in the peak N_2O fluxes between the different treatments. Compared with CK, the application of rice straw alone significantly promoted the N_2O flux on the first day after CF irrigation and mild AWD irrigation. Compared with U, the addition of rice straw in CF irrigation reduced the N_2O flux, while the addition of rice straw in mild AWD irrigation increased the N_2O flux.

3.2 Soil NH_4^+-N, NO_3^--N and MBN concentrations

The soil NH_4^+-N, NO_3^--N, and MBN concentrations varied with the

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growing stage of rice (Fig. 2A, 2C and Fig. 3A). The soil NH_4^+-N concentration first increased and then decreased. The NH_4^+-N concentration under CF irrigation and mild AWD irrigation was low in the late growth period of rice (Fig. 2A). The concentration of NO_3^--N in CF-irrigated soil showed a trend of first increasing and then decreasing and was at a low level in the later growth period of rice, while the concentration of NO_3^--N in mild AWD irrigation showed a trend of first decreasing and then increasing, then decreasing and then increasing (Fig. 2C). The concentration of MBN in CF-irrigated soil first decreased and then increased and then decreased to a lower level, while the concentration of MBN in mild AWD-irrigated soil decreased with the growth stage of rice (Fig. 3A).

There were significant differences in the NH_4^+-N, NO_3^--N, and MBN concentrations between the different treatments. In CF irrigation and mild AWD irrigation, the U treatment had a higher NH_4^+-N concentration in the early stage of rice growth, while the other treatments had no significant difference, but as rice grew, the NH_4^+-N concentration of the US treatment increased, which was significantly greater than that of the U, S and CK treatments (Fig. 2A). In CF irrigation, the NO_3^--N concentration of the U treatment was slightly higher than that of the other treatments, and all treatments had little difference. In mild AWD irrigation, the NO_3^--N concentration of the US treatment was significantly

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higher than that of the U, S and CK treatments. The U treatment had a higher NO_3^--N concentration than the S and CK treatments in the later stage of rice growth (Fig. 2C). The US treatment in CF irrigation and mild AWD irrigation had the highest MBN concentration during the rice growth period (Fig. 3A).

Figure 2B shows that the NH_4^+-N in CF irrigation and mild AWD irrigation mainly came from urea rather than rice straw, and the combined application of urea and rice straw further promoted the release of NH_4^+-N from urea. Regardless of CF irrigation or mild AWD irrigation, rice straw nitrogen was difficult to utilize by microorganisms in the first year under single rice straw application, but rice straw and urea combined application significantly promoted the utilization of rice straw nitrogen by microorganisms. Urea combined with rice straw application was more easily used by microorganisms than urea applied alone (Fig. 3B).

3.3 Sources of aboveground biomass nitrogen in rice

As shown in Fig. 4, under CF irrigation and mild AWD irrigation, compared with CK, a single application of rice straw did not increase the aboveground nitrogen absorption of rice, while the U and US treatments significantly promoted the aboveground nitrogen absorption of rice. Under mild AWD irrigation, the US treatment reduced nitrogen uptake in rice shoots compared with the U treatment. The U and US treatments under CF irrigation promoted the nitrogen uptake of the aboveground rice more than those under mild AWD irrigation.

With different irrigation methods, the effects of urea and rice straw addition on the nitrogen absorption of the aboveground rice were different. Compared with the CK and S treatments, the U and US treatments under CF irrigation significantly promoted the absorption of soil nitrogen by rice, while the difference was not significant except for the S and U treatments under mild AWD irrigation. Compared with mild AWD irrigation, the U and US treatments under CF irrigation significantly promoted the absorption of soil nitrogen by rice. Regardless of the irrigation and fertilization method, the soil was the main source of nitrogen in the aboveground parts of rice, followed by urea and finally rice straw (Fig. 4).

3.4 Cumulative N_2O emissions, rice agronomic properties and yield-scaled N_2O emissions

In addition to the CK treatment, compared with CF irrigation, mild AWD irrigation significantly promoted the accumulation of N_2O during the rice growth period, with an average increase of 28.8% (Table 1). Under CF irrigation, there was no significant difference in the accumulation of N_2O between S and CK or between US and U. However, the addition of rice straw under mild AWD irrigation significantly increased the accumulation of N_2O. Compared with the CK and S treatments, the U and US treatments significantly promoted cumulative

N_2O emissions under the two irrigation modes. As shown in Table 2, both irrigation methods and nitrogen application level affected cumulative N_2O emissions, of which the nitrogen application level had the greatest impact. The interaction between irrigation level and nitrogen fertilizer or rice straw significantly affected cumulative N_2O emissions.

As shown in Table 1, compared with CF irrigation, mild AWD irrigation significantly reduced rice aboveground biomass under US treatment but had no effect on other treatments. Regardless of whether CF irrigation or mild AWD irrigation was applied, there was no significant difference in the rice aboveground biomass between S and CK or between US and U. Compared with the CK and S treatments, the U and US treatments significantly promoted the rice aboveground biomass under the two irrigation modes. Irrigation level had no effect on rice yield under all treatments. Regardless of CF irrigation or mild AWD irrigation, rice yield under the U and US treatments was significantly higher than that under the CK and S treatments, but there was no difference between the former two and the latter two.

In addition to the CK treatment, compared with CF irrigation, mild AWD irrigation significantly promoted yield-scaled N_2O emissions during the rice growth period. Regardless of CF irrigation or mild AWD irrigation, yield-scaled N_2O emissions under the U and US treatments were significantly higher than those under the CK and S treatments. Rice

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straw addition had no effect on the yield-scaled N_2O emissions under CF irrigation but significantly increased the yield-scaled N_2O emissions under mild AWD irrigation.

4 Discussion

4.1 Effects of irrigation methods, nitrogen levels, and rice straw return on N_2O emissions

N_2O emission is significantly affected by soil temperature, water-filled pores and mineral N (NH_4^+-N and NO_3^--N) content (Allen et al., 2010). The N_2O emission peak in CF irrigation occurred only after nitrogen application, while mild AWD irrigation caused other N_2O emission peaks, which might have been caused by the change in soil moisture conditions by mild AWD irrigation (Zhou et al., 2020). The peak of N_2O after fertilization may be due to the fact that a large amount of nitrogen application increases the soil inorganic nitrogen concentration (Fig. 2A and 2C), which in turn promotes the generation of N_2O, which mainly comes from the denitrification process (Wang et al., 2017b; Yano et al., 2014). During the denitrification process, it is easier for microorganisms to use NO_3^--N as an electron acceptor (Fig. 2C), which affects the reduction process of N_2O, resulting in an increase in the ratio of N_2O/N_2 in the denitrification products (Pérez et al., 2000). Our results showed negative N_2O emission fluxex at the later stage of rice growth, which may be due to the decrease of surface soil N_2O concentration due to the strengthening of N_2O reduction process or the weakening of N_2O diffusion process in the soil profile, which allowed atmospheric N_2O to diffuse back into the soil (Chapuis-Lydie et al., 2007). Mild AWD irrigation promoted cumulative N_2O emissions by 28.8% on average, which was the same as hypothesis 1. Similar results were found in previous studies, which may be due to the increased N_2O produced by nitrification and denitrification due to water level alternation (Liang et al., 2017; Zhou et al., 2020) and temperature change (Wu et al., 2019) of mild AWD irrigation. In order to reduce N_2O emissions from paddy fields, researchers generally regulate N_2O production by optimizing N management (Liang et al., 2017), applying inhibitors to control the nitrogen supply rate of nitrogen fertilizers (Wu et al., 2019), etc. In both CF and mild AWD irrigation, there was no obvious N_2O emission peak at the later growth stage of rice, which may have been due to the decrease in soil inorganic nitrogen content (Fig. 2A and 2C) and microbial biomass (Fig. 3A).

Compared with CK and S, nitrogen fertilizer application (U and US) significantly increased N_2O cumulative emissions and was the most important factor of N_2O generation, mainly because nitrogen fertilizer application provided sufficient substrates for soil nitrification and denitrification to generate N_2O (Fiedler et al., 2017; Wu et al., 2021). The peak of N_2O emissions after base fertilizer application was larger than that after two topdressing, which might have been due to a higher nitrogen application rate and simultaneous nitrification and denitrification in initial flooding (Mathieu et al., 2006; Wang et al., 2017b). The peak of N_2O emissions after the two topdressing treatments with CF irrigation was similar, while the peak of N_2O emissions of the first topdressing treatment with mild AWD irrigation was significantly larger than that of the second topdressing treatment. This may be because the soil environment (temperature, moisture, etc.) changed little in long-term flooding under CF irrigation (Lagomarsino et al., 2016; Verhoeven et al., 2018; Congreves et al., 2019), while the difference in the soil environment of the two top dressings under mild AWD irrigation changed the utilization of fertilizer nitrogen by microorganisms (Fig. 3B). Therefore, reducing the amount of the first top dressing under mild AWD irrigation and maintaining flooding for approximately one week after fertilization may be beneficial to N_2O reduction (Liao et al., 2020). The negative value of N_2O emissions appeared at the later stage of rice growth, indicating that the paddy field could also become a sink for N_2O (van Groenigen et al., 2015), which might be caused by the lack of nitrogen supply at the later stage. Increasing the second topdressing might be beneficial for alleviating nitrogen deficiency (Liao et al., 2020).

Different from CF irrigation, the addition of rice straw under mild AWD irrigation conditions promoted N_2O emissions, probably because the alternation of wet and dry conditions promoted the decomposition of rice straw (Andren et al., 1993; Buchen et al., 2016; Chen et al., 2016), which was beneficial to the growth of microorganisms (Fig. 3A) and thus promoted the production of N_2O (Said-Pullicino et al., 2014; Wang et al., 2018; Wu et al., 2021). In addition, compared with U and S, US promoted microbial absorption of urea and rice straw nitrogen (Fig. 3B), which also proved that US treatment was more conducive to the growth of microorganisms.

4.2 Rice production and yield-scaled N_2O emissions

The change in irrigation method did not cause differences in rice yield (Table 1), but under the US treatment, mild AWD irrigation significantly reduced the aboveground biomass of rice and the uptake of soil nitrogen by rice (Table 1 and Fig. 4), which was consistent with our hypothesis 2 and 3. Previous studies have also shown that mild AWD irrigation can stabilize or increase rice yield. This may be because mild AWD irrigation can promote the transport of nutrients from stems and leaves to grains during the reproductive growth stage of rice, while inhibiting ineffective tillering and increasing the number of effective panicles, thereby reducing the excessive vegetative growth of rice (Carrijo et al., 2017; Li et al., 2018b; Liao et al., 2020; Zhang et al., 2009). This may also be an important reason for the decrease in the uptake of soil nitrogen by rice under AWD irrigation. Urea application was a key

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factor in improving rice yield (Wang et al., 2017a) but also aggravated soil nitrogen uptake by rice, and soil was the largest source of nitrogen for rice in all treatments (Fig. 4). Compared with U, under CF irrigation and mild AWD irrigation, US reduced the uptake of soil-derived nitrogen by rice (the difference was not significant), and the trend was more obvious under mild AWD irrigation, which may be another reason for rice straw return to maintain soil fertility.

In our study, mild AWD irrigation, urea application and rice straw return under mild AWD irrigation all increased yield-scaled N_2O emissions (Table 1), mainly due to improved soil aeration and increased inorganic nitrogen and rice straw decomposition, resulting in more N_2O production (Andren et al., 1993; Buchen et al., 2016; Chen et al., 2016; Lagomarsino et al., 2016; Fiedler et al., 2017; Verhoeven et al., 2018; Congreves et al., 2019; Wu et al., 2021). Although mild AWD irrigation had higher yield-scaled N_2O emissions than CF irrigation, the GWP (CH_4 + N_2O) under mild AWD irrigation was significantly lower than that under CF irrigation and decreased by 8.1%, 57.9%, 11.8% and 62.9% under CK, S, U and US, respectively (Table S1). Therefore, mild AWD irrigation combined with rice straw return may be a promising agronomic measure that not only ensures rice yield and slows the greenhouse effect but also reduces soil fertility consumption.

5 Conclusions

The effects of irrigation methods, nitrogen levels and rice straw return on N_2O emissions were explored through pot experiments with rice. We found that N_2O emissions were mainly affected by urea application and irrigation methods, and urea application was the main reason. Compared with CF irrigation, mild AWD irrigation increased cumulative N_2O emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated N_2O emissions. Compared with CF irrigation, mild AWD irrigation increased the yield-scaled N_2O emissions, and the addition of rice straw further promoted the yield-scaled N_2O emissions under mild AWD irrigation but reduced the GWP by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation reduced nitrogen uptake by rice in the soil and rice aboveground biomass without reducing rice yield. Therefore, mild AWD irrigation combined with rice straw return is a promising agronomic measure to ensure rice yield, reduce the greenhouse effect and maintain or improve soil fertility.

Data availability. Original data are available upon request. Material necessary for this study’s findings is presented in the paper.

Author contributions. Kaikuo Wu, Wentao Li, Zhanbo Wei and Zhi Dong conceived and designed the experiments; Yue Meng and Na Lv performed the experiments; Kaikuo Wu analyzed the data; Kaikuo Wu

and Lili Zhang wrote the paper and all authors approved submission of the paper. All authors have read and agreed to the published version of the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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Review statement.

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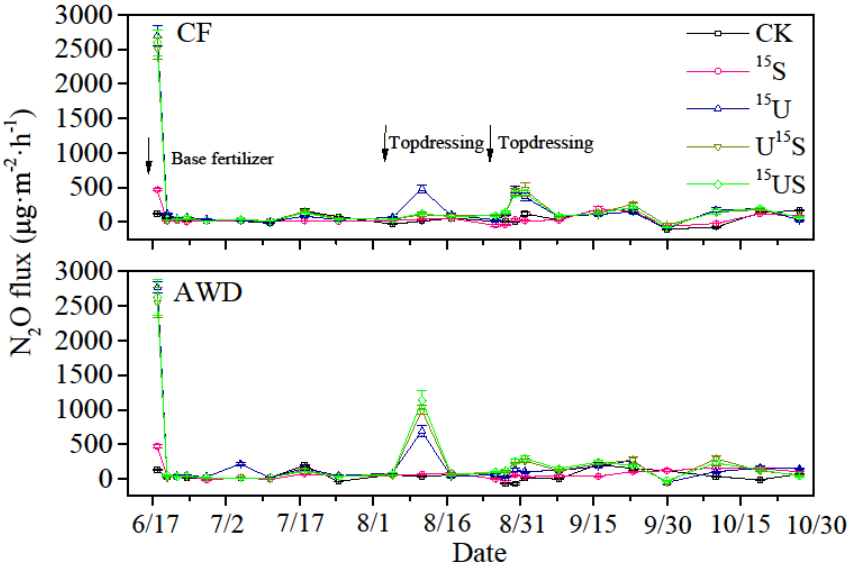
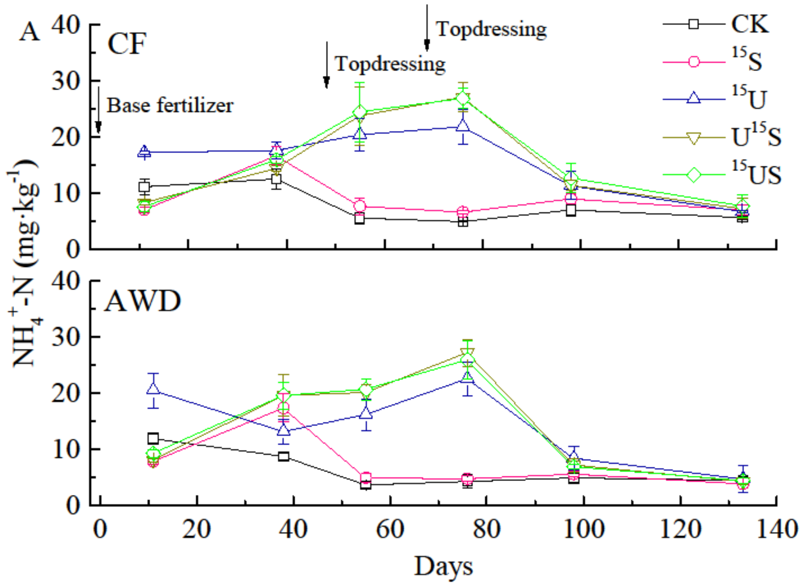


Fig. 1 Effects of different treatments on N_2O flux. Bars represent standard errors (n=3), the same below.


A
CF
AWD
Base fertilizer
Topdressing
Topdressing
CK
^{15}S
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U^{15}S
^{15}US
NH_4^+-N (mg·kg^{-1})
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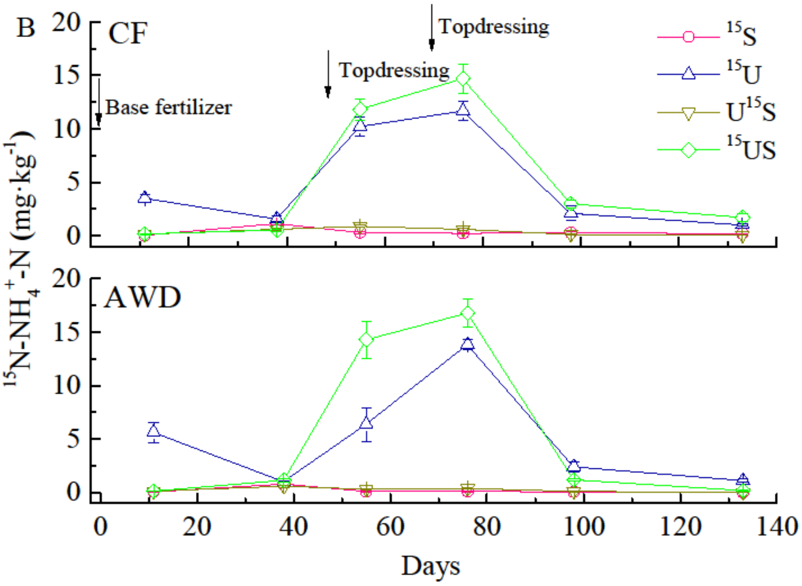
30
40
0
20
40
60
80
100
120
140
Days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Base fertilizer
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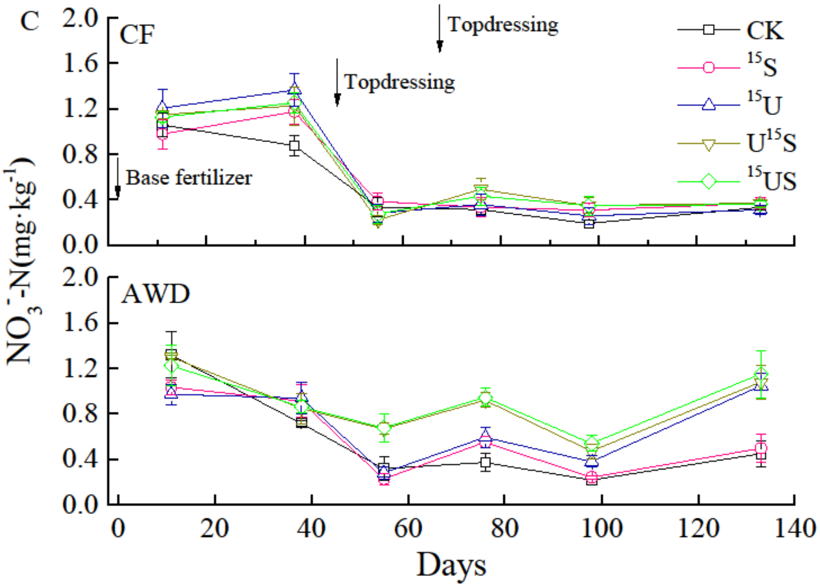

Fig. 2 Changes in soil NH_4^+-N concentration (A), the contribution of ^{15}N markers to NH_4^+-N (B) and changes in soil NO_3^--N concentration (C) during the growth period of rice (n=3).

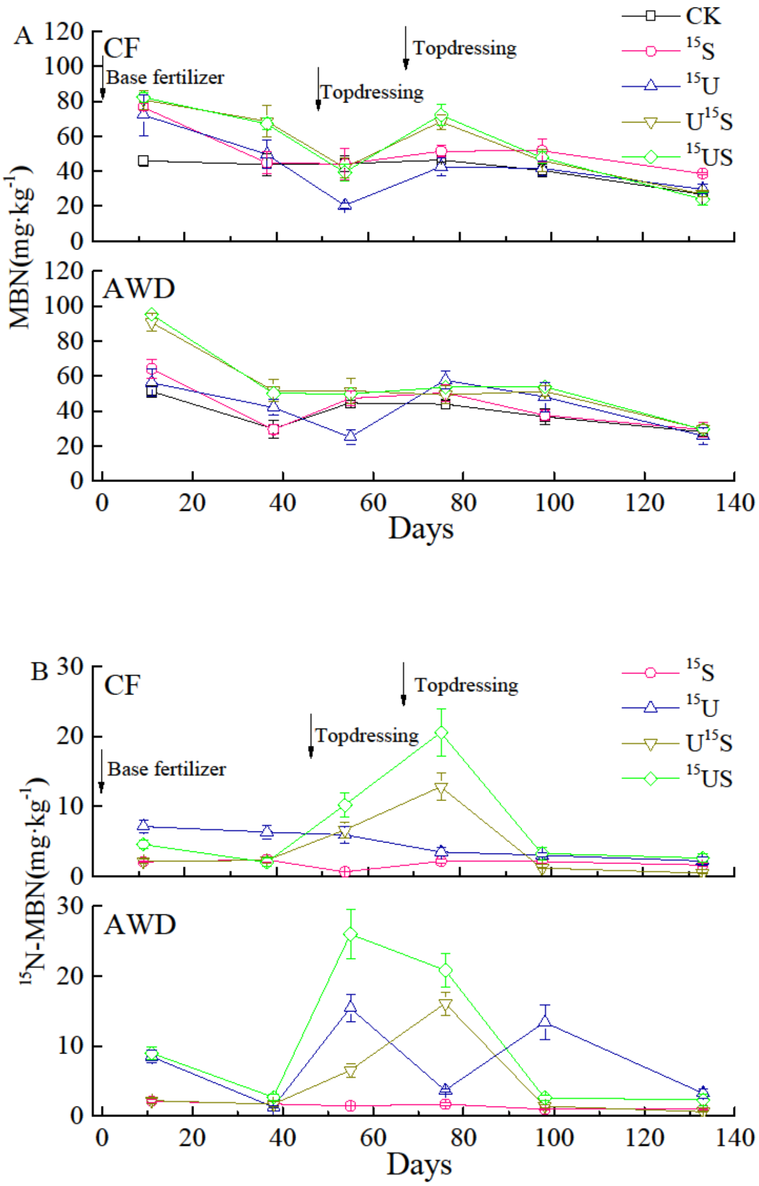

Fig. 3 Changes in the concentration of MBN (A) in the soil during the rice growth period and the contribution of ^{15}N markers to MBN (B) (n=3).

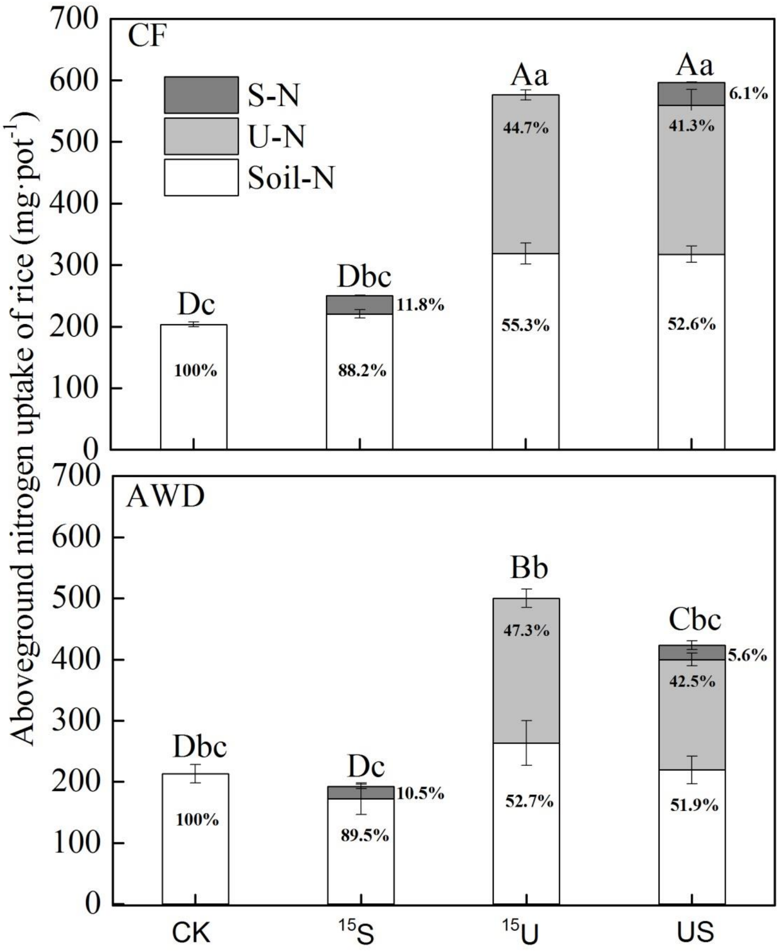

Fig. 4 The source of nitrogen in the aboveground biomass of rice at the maturity stage (n=3). Different capital letters indicate significant differences in total nitrogen uptake of rice above ground (P < 0.05), and different lower case letters indicate significant differences in soil nitrogen supply (P < 0.05).

Table 1 Effects of different treatments on cumulative N_2O emissions, rice aboveground biomass, rice yield and yield-scaled N_2O emission. The values denote means ± standard errors (n=3). Different lowercase letters indicate significant differences ($P < 0.05$).

Treaments		Cumulative N_2O emissions	Rice aboveground biomass	Rice yield	Yield-scaled N_2O emission
		kg ha^{-1}	g pot^{-1}	g pot^{-1}	g kg^{-1}
CF	CK	1.48 ±0.06 ef	37.11 ±2.53 f	21.63 ±1.81 b	0.24 ±0.01 d
	^{15}S	1.24 ±0.05 f	37.99 ±2.69 f	20.91 ±1.63 b	0.21 ±0.03 d
	^{15}U	4.02 ±0.30 c	82.54 ±10.39 ab	36.56 ±2.75 a	0.39 ±0.04 c
	U+^{15}S	3.89 ±0.09 c	87.58 ±7.70 a	35.98 ±1.72 a	0.38 ±0.02 c
	^{15}U+S	3.76 ±0.02 c	80.99 ±19.54 abc	35.85 ±2.50 a	0.37 ±0.03 c
AWD	CK	1.64 ±0.15 e	45.31 ±3.07 ef	22.92 ±1.07 b	0.25 ±0.02 d
	^{15}S	2.07 ±0.17 d	34.78 ±4.86 f	20.42 ±0.46 b	0.36 ±0.02 c
	^{15}U	4.48 ±0.12 b	64.44 ±7.33 bcd	34.81 ±1.64 a	0.46 ±0.03 b
	U+^{15}S	5.05 ±0.28 a	62.92 ±2.49 cde	34.50 ±1.76 a	0.53 ±0.01 a
	^{15}U+S	5.29 ±0.25 a	60.44 ±6.74 de	34.85 ±1.18 a	0.54 ±0.03 a

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Table 2 Cumulative N_2O emissions in response to irrigation method, nitrogen level, and straw returning. ** indicated significant treatment effects within a main category ($P < 0.01$).

Factors	Cumulative N_2O emissions
Irrigation method (I)	124.13**
Nitrogen level (N)	1903.3**
Straw (S)	7.98
I×N	11.74**
I×S	40.39**
N×S	1.78
I×N×S	1.21