

**Effects of mild alternate wetting and drying irrigation and rice straw application on N<sub>2</sub>O emissions in rice cultivation**

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**Abstract.** The shortage of water resources and the decline in soil organic matter (SOM) are critical limiting factors affecting the improvement in rice productivity, while alternate wetting and drying (AWD) irrigation and recycling application of rice straw are considered favourable

mitigation measures. However, the impact of such measures on rice yield and greenhouse gas (GHG) emissions, especially nitrous oxide (N<sub>2</sub>O) emissions, needs to be further clarified to ensure that agronomic practises save water, conserve soil, and reduce GHG. Therefore, we explored the effects of mild AWD irrigation combined with on-site rice straw recycling on N<sub>2</sub>O emissions and rice yield through rice pot experiments. This experiment included two irrigation methods (continuous flooding (CF) irrigation and mild AWD irrigation), two nitrogen (N) application levels (0 and 225 kg N ha<sup>-1</sup>) and two rice straw (S) return levels (0 and 9000 kg ha<sup>-1</sup>), for a total of 10 treatments, and each treatment had three replicates. <sup>15</sup>N-urea and <sup>15</sup>N-S were added to the soil. The results showed that N<sub>2</sub>O emissions were primarily affected by urea application and irrigation methods, with urea application being most important. Compared with CF irrigation, mild AWD irrigation increased cumulative N<sub>2</sub>O emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated N<sub>2</sub>O emissions by 18.1%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation increased the yield-scaled N<sub>2</sub>O emissions by 17.9%, and the addition of rice straw further promoted the yield-scaled N<sub>2</sub>O emissions under mild AWD irrigation by 17.4% but reduced the global warming potential (GWP) (methane (CH<sub>4</sub>) + N<sub>2</sub>O) by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation

reduced the uptake of soil-derived N and aboveground biomass of rice but did not reduce rice yield. Therefore, mild AWD irrigation combined with rice straw return may be a promising agronomic method to maintain rice yield, reduce greenhouse gases, and protect or improve soil fertility.

## **1 Introduction**

Rice is a 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 will be 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 represents a great waste of freshwater and causes many environmental problems, such as nonpoint source pollution, eutrophication, and GHG emissions (Liao et al., 2020). Therefore, it is urgent to explore new methods for managing paddy field fertilization that can ensure high rice yield and reduced water waste and pollution.

AWD irrigation is an effective water-conserving irrigation method that can save approximately 23% of freshwater resources compared with CF irrigation (Bouman and Tuong, 2001; Chu et al., 2014). There are

usually two approaches to alternate wetting and drying irrigation: severe AWD irrigation (soil water potential  $\geq -30 \pm 5$  kPa) and mild AWD irrigation (soil water potential  $\geq -15 \pm 5$  kPa) (Zhou et al., 2017). Severe AWD irrigation could reduce rice yield by 22.6% due to water stress, but under mild AWD irrigation, rice yield can be stable or slightly increased (Carrijo et al., 2017; Zhou et al., 2017). Therefore, mild AWD irrigation may offer a more promising paddy field management model. Previous studies have shown that AWD irrigation can significantly reduce CH<sub>4</sub> emissions but considerably promotes N<sub>2</sub>O emissions, while GWP mitigation is dependent on the magnitude of the increase in the release of N<sub>2</sub>O (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<sub>2</sub>O, is conducive to reducing paddy field emissions and maximizing agricultural and environmental benefits.

Long-term cultivation without organic matter supplementation leads to serious degradation of cultivated land and reduction in 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 a valuable 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 cause changes in paddy field

GHG emissions (Naser et al., 2007; Ye et al., 2017; Sun et al., 2018; Yu et al., 2021), N 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 return can achieve the optimal goals of water saving, yield increase, and reduction in greenhouse gas emissions has remained unclear. Therefore, the purpose of this study was to investigate the effect of mild AWD irrigation combined with rice straw return on N<sub>2</sub>O emissions and rice yield in rice cultivation and to explore the supply of N to rice growth from the soil, urea, and rice straw using <sup>15</sup>N labelling technology. Our initial hypotheses were that: 1) Mild AWD irrigation would promote N<sub>2</sub>O emissions in rice cultivation; and 2) Mild AWD irrigation would maintain or promote rice yield.

## **2 Materials and methods**

### **2.1 Experimental setup**

A 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 17<sup>th</sup> to October 27<sup>th</sup>, 2020. The test soil was an Alfisol with a total C content of 16.01 g/kg and a total N content of 1.36 g/kg.

110 The pot experiment used a random block design, including 30 pots  
 111 (30 cm diameter  $\times$  20 cm height). This experiment included two irrigation  
 112 methods, two N application levels, and two rice straw return levels, with  
 113 three replicates of each combination, for a total of 30 rice pots (urea and  
 114 straw were labelled with  $^{15}\text{N}$ , respectively). The two irrigation methods  
 115 were CF irrigation and mild AWD irrigation. CF irrigation maintained a  
 116 water level depth of approximately 3-5 cm throughout the rice-growing  
 117 season. Mild AWD irrigation water management in the first 7 days was  
 118 consistent with CF irrigation and allowed to evaporate under monitoring;  
 119 when the soil negative pressure gauge reached -15 kPa, it was  
 120 sub-flooded to a depth of 3-5 cm again and then naturally allowed to dry  
 121 again. This step was repeated until harvest. CF and mild AWD irrigation  
 122 were halted 2 weeks before harvest. N was applied at 0 kg N ha $^{-1}$  (CK  
 123 (control check) and  $^{15}\text{S}$  (rice straw)) and 225 kg N ha $^{-1}$  (106.13 mg kg $^{-1}$   
 124 dry soil) ( $^{15}\text{U}$  (urea),  $\text{U}^{15}\text{S}$  (urea + rice straw) and  $^{15}\text{US}$  (urea + rice  
 125 straw)). The abundance of urea  $^{15}\text{N}$  was 10.20%. Urea was applied three  
 126 times: base fertilizer 40% (June 17 $^{\text{th}}$ ), tiller topdressing 30% (August 4 $^{\text{th}}$ )  
 127 and heading topdressing 30% (August 25 $^{\text{th}}$ ). Rice straw return was  
 128 applied at 0 kg ha $^{-1}$  (CK and  $^{15}\text{U}$ ) and 9,000 kg ha $^{-1}$  (4.25 g kg $^{-1}$  dry soil)  
 129 ( $^{15}\text{S}$ ,  $\text{U}^{15}\text{S}$  and  $^{15}\text{US}$ ). The total N content of unlabelled rice straw was  
 130 0.72%, and the isotope abundance of  $^{15}\text{N}$  was 0.59%. The total N content  
 131 of labelled rice straw was 0.73%, and the  $^{15}\text{N}$  isotope abundance was

22.94%. The rice straw was ground and applied together with the base fertilizer. Phosphate fertilizer was superphosphate ( $150 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ), and potassium fertilizer was potassium chloride ( $185 \text{ kg K}_2\text{O ha}^{-1}$ ) 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 ( $105^\circ \text{C}$  for 0.5 h and  $60^\circ \text{C}$  for 12 h).

## 2.2 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  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were extracted with  $2 \text{ mol L}^{-1}$  KCl solution (Wu et al., 2019), filtered and analysed with a continuous flow analyser (AA3, Bran + Luebbe, Germany). The extraction of soil  $^{15}\text{N-NH}_4^+\text{-N}$  followed Yu et al. (2020). Soil microbial biomass N (MBN) was fumigated with chloroform, extracted with  $0.5 \text{ mol L}^{-1}$   $\text{K}_2\text{SO}_4$  (soil: solution = 5 g: 20 ml) (Joergensen et al., 1996), and determined by a TOC analyser (Elementar vario TOC Analyzer, Germany). The soil  $^{15}\text{N-NH}_4^+\text{-N}$  content,  $^{15}\text{N-MBN}$  and  $^{15}\text{N}$  of rice aboveground biomass were determined by a stable isotope ratio mass spectrometer (253 MAT, Thermo Finnigan, Germany).

## 2.3 Gas sampling and calculation

The static chamber method was used to determine the  $\text{N}_2\text{O}$  flux (Li

et al., 2018a). The static chamber with a top seal made of transparent 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<sub>2</sub>O was collected every two days in the first week after fertilization or irrigation and every seven days during other periods. N<sub>2</sub>O was sampled at 8:00–11:00 a.m. each sampling day. Every pot was sealed with water when N<sub>2</sub>O was collected. Three gas samples were collected at 0, 30 and 60 min after the chamber was airtight, and N<sub>2</sub>O was collected with a 50 mL injector and then injected into 200 mL gasbags.

The N<sub>2</sub>O concentration was analysed using a gas chromatograph (Agilent 7890B, Gas Chromatograph, Delaware, USA). The calculation of N<sub>2</sub>O fluxes was as follows (Li et al., 2018a):

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

where  $F$  is the N<sub>2</sub>O flux ( $\mu\text{g m}^{-2} \text{ h}^{-1}$ );  $\rho$  is the N<sub>2</sub>O standard-state density ( $1.964 \text{ kg m}^{-3}$ );  $h$  is the chamber height above the soil (m);  $c$  is the N<sub>2</sub>O concentration;  $dc/dt$  is the slope of the N<sub>2</sub>O 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<sub>2</sub>O collection (°C).



Cumulative N<sub>2</sub>O emissions (CE) were calculated using the following formula according to Wang et al. (2011):

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

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 <sup>15</sup>N markers to NH<sub>4</sub><sup>+</sup>-N and MBN and the calculation of the N source of the aboveground biomass of rice followed Ma et al. (2015). Yield-scaled N<sub>2</sub>O was calculated as the ratio between N<sub>2</sub>O and rice yield (Li et al., 2018a).

## 2.4 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 analyse the response of cumulative N<sub>2</sub>O emissions to irrigation method, N level and rice straw application (Table 2). 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  $\pm$  standard error.

## 3 Results

### 3.1 N<sub>2</sub>O flux

Three higher N<sub>2</sub>O flux peaks appeared after basal fertilizer and two topdressing treatments (Figure 1), and the N<sub>2</sub>O flux peak after basal fertilizer application was significantly larger than the last two peaks. After basal fertilizer was applied, the N<sub>2</sub>O flux peaks of CF irrigation and mild AWD irrigation were similar. After the first topdressing, the N<sub>2</sub>O flux peak of mild AWD irrigation was significantly greater than that of CF irrigation, approximately 1.4 and 9.1 times under the U and US treatments, respectively. In contrast, the N<sub>2</sub>O flux peak of CF irrigation after the second topdressing was 3.5 and 1.6 times higher than that of mild AWD irrigation under the U and US treatments, respectively. In addition to the above three peaks, the N<sub>2</sub>O 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<sub>2</sub>O ranged from -103.93 to 2,770.50  $\mu\text{g m}^{-2} \text{h}^{-1}$ . The N<sub>2</sub>O flux appeared negative in the late stage of rice growth. The N<sub>2</sub>O 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 rice growth cycle, the N<sub>2</sub>O flux of the CK and S treatments was low. There were significant differences in the peak N<sub>2</sub>O fluxes between the different treatments. Compared with CK, the application of rice straw alone significantly promoted the N<sub>2</sub>O 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<sub>2</sub>O flux, while the

addition of rice straw in mild AWD irrigation increased the  $\text{N}_2\text{O}$  flux.

### 3.2 Soil $\text{NH}_4^+\text{-N}$ , $\text{NO}_3^-\text{-N}$ and MBN concentrations

The soil  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and MBN concentrations varied with the growing stage of rice (Fig. 2A, 2C and Fig. 3A). The soil  $\text{NH}_4^+\text{-N}$  concentration first increased and then decreased. The  $\text{NH}_4^+\text{-N}$  concentration under CF irrigation and mild AWD irrigation was low in the late rice growth period (Fig. 2A). The concentration of  $\text{NO}_3^-\text{-N}$  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  $\text{NO}_3^-\text{-N}$  in mild AWD irrigation showed a trend of first decreasing and then increasing, with repeated decreases and increases (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  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and MBN concentrations between the different treatments. In CF irrigation and mild AWD irrigation, the U treatment had a higher  $\text{NH}_4^+\text{-N}$  concentration in the early stage of rice growth, while the other treatments had no significant difference, but as rice grew, the  $\text{NH}_4^+\text{-N}$  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  $\text{NO}_3^-\text{-N}$

concentration of the U treatment was slightly higher than that of the other treatments, and all treatments had little variance. In mild AWD irrigation, the  $\text{NO}_3^-$ -N concentration of the US treatment was significantly higher than that of the U, S and CK treatments. The U treatment had a higher  $\text{NO}_3^-$ -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  $\text{NH}_4^+$ -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  $\text{NH}_4^+$ -N from urea. Regardless of CF irrigation or mild AWD irrigation, rice straw N 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 N by microorganisms. Urea combined with rice straw application may be more easily utilized by microorganisms than urea applied alone (Fig. 3B).

### 3.3 Sources of aboveground biomass N 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 N absorption of rice, while the U and US treatments significantly promoted the aboveground N absorption of rice. Under mild

AWD irrigation, the US treatment reduced N uptake in rice shoots compared with the U treatment. The U and US treatments under CF irrigation promoted the N 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 N absorption of the aboveground rice were varied. Compared with the CK and S treatments, the U and US treatments under CF irrigation significantly promoted the absorption of soil N by rice, while only the S and U treatments had significant differences under mild AWD irrigation. Compared with mild AWD irrigation, the U and US treatments under CF irrigation significantly promoted the absorption of soil N by rice. Regardless of the irrigation and fertilization method, the soil was the main source of N in the aboveground parts of rice, followed by urea and finally rice straw (Fig. 4).

### 3.4 Cumulative N<sub>2</sub>O emissions, rice agronomic properties and yield-scaled N<sub>2</sub>O emissions

In addition to the CK treatment, compared with CF irrigation, mild AWD irrigation significantly promoted the accumulation of N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O by 18.1% (Table 1). Compared with the CK and S treatments, the U and US treatments significantly promoted cumulative N<sub>2</sub>O emissions under the two irrigation modes. As shown in Table 2, irrigation methods, N application level, and rice straw return affected cumulative N<sub>2</sub>O emissions, of which the N application level had the greatest impact. The interaction between irrigation level and N fertilizer or rice straw significantly affected cumulative N<sub>2</sub>O 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<sub>2</sub>O emissions during the rice growth period. Under urea application conditions,

compared with CF irrigation, mild AWD irrigation increased yield-scale  $\text{N}_2\text{O}$  emissions by 17.9%, and the addition of rice straw further promoted yield-scale  $\text{N}_2\text{O}$  emissions by 17.4% under mild AWD irrigation conditions (Table 1). Regardless of CF irrigation or mild AWD irrigation, yield-scaled  $\text{N}_2\text{O}$  emissions under the U and US treatments were significantly higher than those under the CK and S treatments. Rice straw addition had no effect on the yield-scaled  $\text{N}_2\text{O}$  emissions under CF irrigation but significantly increased the yield-scaled  $\text{N}_2\text{O}$  emissions under mild AWD irrigation.

## **4 Discussion**

### **4.1 Effects of irrigation methods, N levels, and rice straw return on $\text{N}_2\text{O}$ emissions**

$\text{N}_2\text{O}$  emissions are significantly affected by water-filled pores and mineral N ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) content (Allen et al., 2010). The  $\text{N}_2\text{O}$  emission peak in CF irrigation occurred only after N application, while mild AWD irrigation caused other  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  after fertilization may be because a large amount of N application increases the soil inorganic N concentration (Fig. 2A and 2C), which in turn promotes the generation of  $\text{N}_2\text{O}$ , which comes from the denitrification process (Wang et al., 2017b; Yano et al., 2014). During the denitrification process, it is easier for

microorganisms to use  $\text{NO}_3^-$ -N as an electron acceptor (Fig. 2C), which affects the reduction process of  $\text{N}_2\text{O}$ , resulting in an increase in the ratio of  $\text{N}_2\text{O}/\text{N}_2$  in the denitrification products (Pérez et al., 2000). Our results showed a negative  $\text{N}_2\text{O}$  emission flux at the later stage of rice growth, which may be due to the decrease in surface soil  $\text{N}_2\text{O}$  concentration due to the strengthening of the  $\text{N}_2\text{O}$  reduction process or the weakening of the  $\text{N}_2\text{O}$  diffusion process in the soil profile, which allowed atmospheric  $\text{N}_2\text{O}$  to diffuse back into the soil (Chapuis-Lydie et al., 2007). Mild AWD irrigation promoted cumulative  $\text{N}_2\text{O}$  emissions by 28.8% on average, which was as proposed in our Hypothesis 1. Similar results were found in previous studies, which may be due to the increased  $\text{N}_2\text{O}$  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. To reduce  $\text{N}_2\text{O}$  emissions from paddy fields, researchers generally regulate  $\text{N}_2\text{O}$  production by optimizing N management (Liang et al., 2017), applying inhibitors to control the N supply rate of N fertilizers (Wu et al., 2019). In both CF and mild AWD irrigation, there was no obvious  $\text{N}_2\text{O}$  emission peak at the later growth stage of rice, which may have been due to the decrease in soil inorganic N content (Fig. 2A and 2C) and microbial biomass (Fig. 3A).

Compared with CK and S, N fertilizer application (U and US) significantly increased  $\text{N}_2\text{O}$  cumulative emissions and was the most



notable factor in N<sub>2</sub>O generation (Table 2), mainly because N fertilizer application provided sufficient substrates for soil nitrification and denitrification to generate N<sub>2</sub>O (Fiedler et al., 2017; Wu et al., 2021). The peak of N<sub>2</sub>O emissions after base fertilizer application was larger than that after two topdressing, which might have been due to a higher N application rate and simultaneous nitrification and denitrification in initial flooding (Mathieu et al., 2006; Wang et al., 2017b). The peak of N<sub>2</sub>O emissions after the two topdressing treatments with CF irrigation was similar, while the peak of N<sub>2</sub>O emissions after the first topdressing treatment with mild AWD irrigation was significantly larger than that following 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 variations in the soil environment of the two top dressings under mild AWD irrigation changed the utilization of fertilizer N 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 benefit N<sub>2</sub>O reduction (Liao et al., 2020). The negative value of N<sub>2</sub>O emissions appeared at the later stage of rice growth, indicating that the paddy field could also become a sink for N<sub>2</sub>O (van Groenigen et al., 2015), which might be caused by the lack of N supply at the later stage.

Increasing the second topdressing might be beneficial for alleviating N deficiency (Liao et al., 2020).

Unlike the CF irrigation, the addition of rice straw under mild AWD irrigation conditions promoted N<sub>2</sub>O emissions (Table 1), 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), thus promoting the production of N<sub>2</sub>O (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 N (Fig. 3B), which also proved that US treatment was more conducive to the growth of microorganisms.

#### 4.2 Effects of irrigation methods, N levels, and rice straw return on rice production and yield-scaled N<sub>2</sub>O 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 N by rice (Table 1 and Fig. 4), which was consistent with our Hypothesis 2. 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 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 N by rice under AWD irrigation. Urea application was a key factor in improving rice yield (Wang et al., 2017a) but also aggravated soil N uptake by rice, and soil was the largest source of N for rice in all treatments (Fig. 4). Compared with U, under CF irrigation and mild AWD irrigation, US reduced the uptake of soil-derived N by rice, and the trend was more obvious under mild AWD irrigation. Although the trend was not significant, rice straw return may be an effective way to maintain long-term soil fertility (Fig. 4).

In our study, mild AWD irrigation, urea application and rice straw return all increased yield-scaled  $\text{N}_2\text{O}$  emissions (Table 1), mainly due to improved soil aeration and increased inorganic N and rice straw decomposition, resulting in more  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  emissions than CF irrigation, the GWP ( $\text{CH}_4 + \text{N}_2\text{O}$ ) 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 maintains rice yield, slows greenhouse effects ( $\text{CO}_2$  emissions are not considered), and also reduces soil fertility consumption.

## 5 Conclusions

The effects of irrigation methods, N levels and rice straw return on  $\text{N}_2\text{O}$  emissions were explored through pot experiments using rice. We found that  $\text{N}_2\text{O}$  emissions were affected by urea application and irrigation methods, with urea application being the most important. Compared with CF irrigation, mild AWD irrigation increased cumulative  $\text{N}_2\text{O}$  emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated  $\text{N}_2\text{O}$  emissions by 18.1%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation increased the yield-scaled  $\text{N}_2\text{O}$  emissions by 17.9%, and the addition of rice straw further promoted the yield-scaled  $\text{N}_2\text{O}$  emissions under mild AWD irrigation by 17.4% but reduced the GWP ( $\text{CH}_4 + \text{N}_2\text{O}$ ) by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation reduced the uptake of soil-derived N and aboveground biomass of rice but did not reduce rice yield. Therefore, mild AWD irrigation combined with rice straw return may offer a promising agronomic measure to maintain high rice yield, reduce greenhouse effects, and maintain or improve soil fertility.

438

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

441 **Author contributions.** Kaikuo Wu, Wentao Li, Zhanbo Wei and Zhi  
442 Dong conceived and designed the experiments; Yue Meng and Na Lv  
443 performed the experiments; Kaikuo Wu analyzed the data; Kaikuo Wu  
444 and Lili Zhang wrote the paper and all authors approved submission of  
445 the paper. All authors have read and agreed to the published version of the  
446 manuscript.

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

449 **Acknowledgements.** The authors thank the editors and reviewers for  
450 their constructive comments and suggestions.

451 **Financial support.** This work was Supported by the Strategic Priority  
452 Research Program of the Chinese Academy of Sciences (XDA28090200),  
453 the National Scientific Foundation Project of China (31971531), the  
454 Liaoning "Take the Lead" Project of 2021: Black soil region protection  
455 and farmland soil fertility improvement technology (2021JH1/10400039),  
456 the K.C.Wong Education Foundation (Research and application of  
457 environmental-friendly polymer compound fertilizers 2022-5), and the  
458 Youth Start-up Fund (2022000008).

459 **Review statement.**

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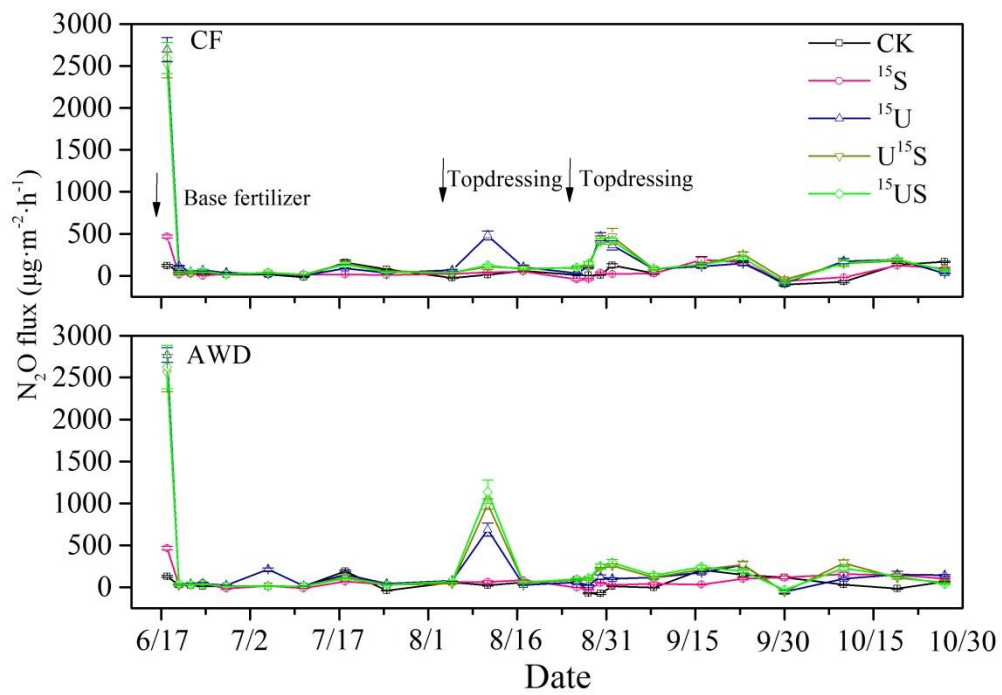
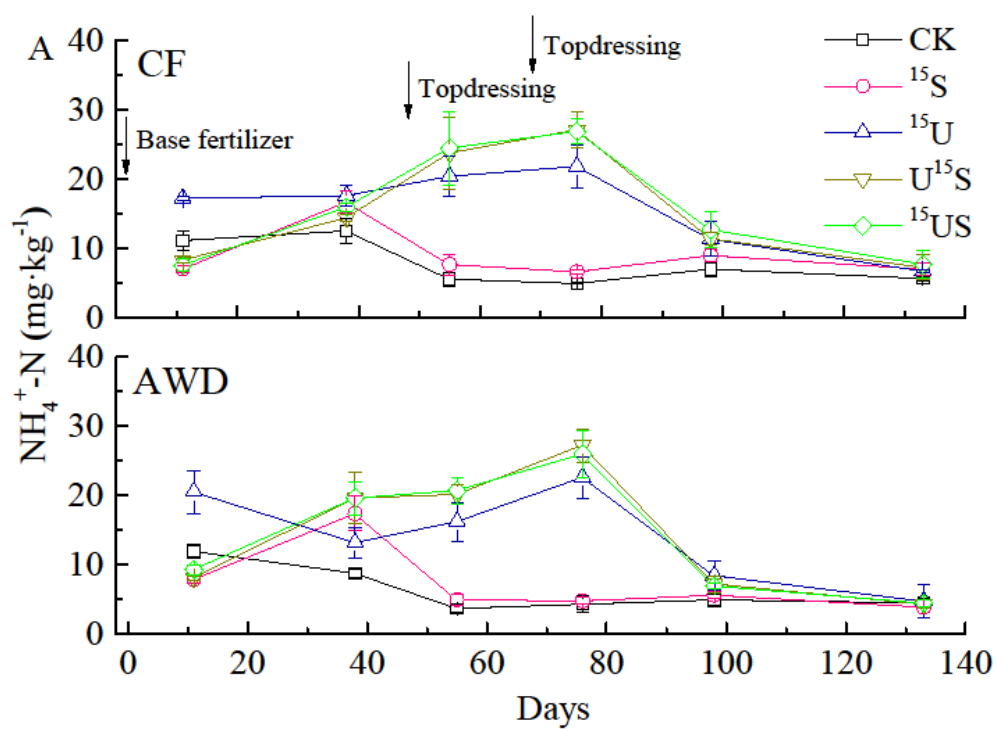
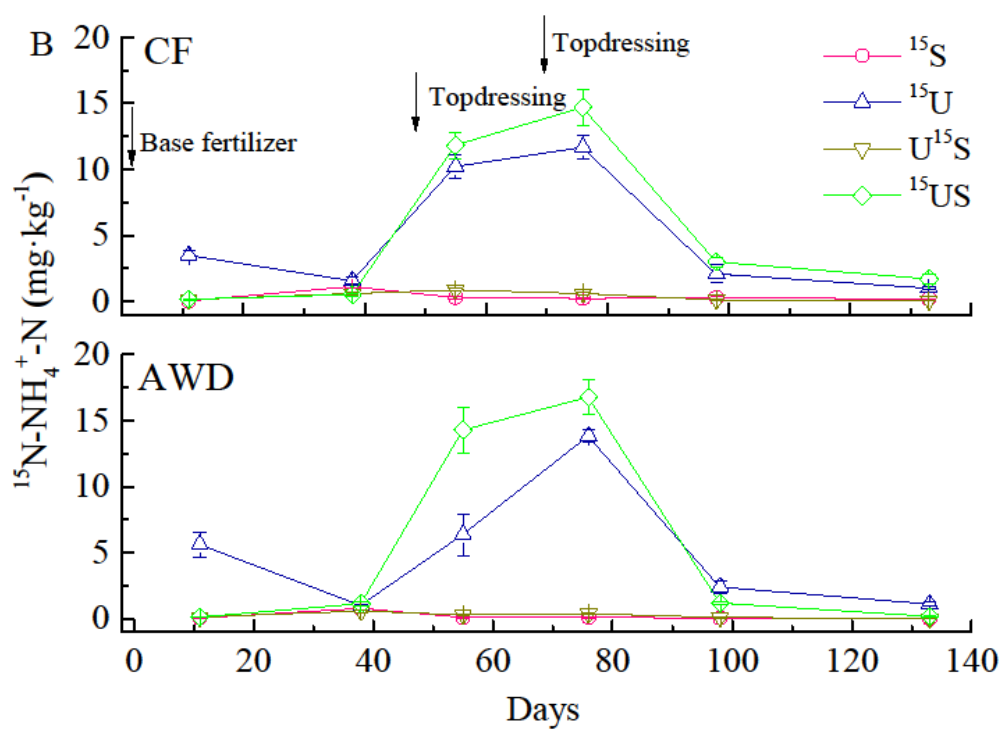


Fig. 1 Effects of different treatments on nitrous oxide (N<sub>2</sub>O) flux. Bars represent standard errors (n=3), the same below.



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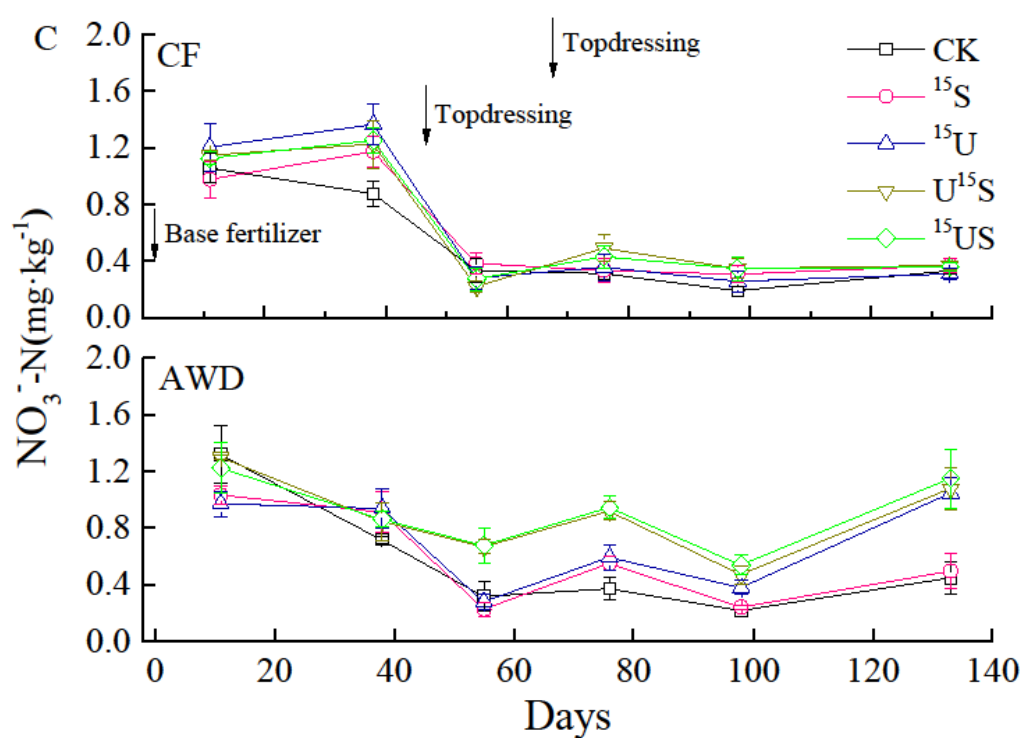


Fig. 2 Changes in soil ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) concentration (A), the contribution of  $^{15}\text{N}$  markers to  $\text{NH}_4^+\text{-N}$  (B) and changes in soil nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) concentration (C) during the growth period of rice (n=3).

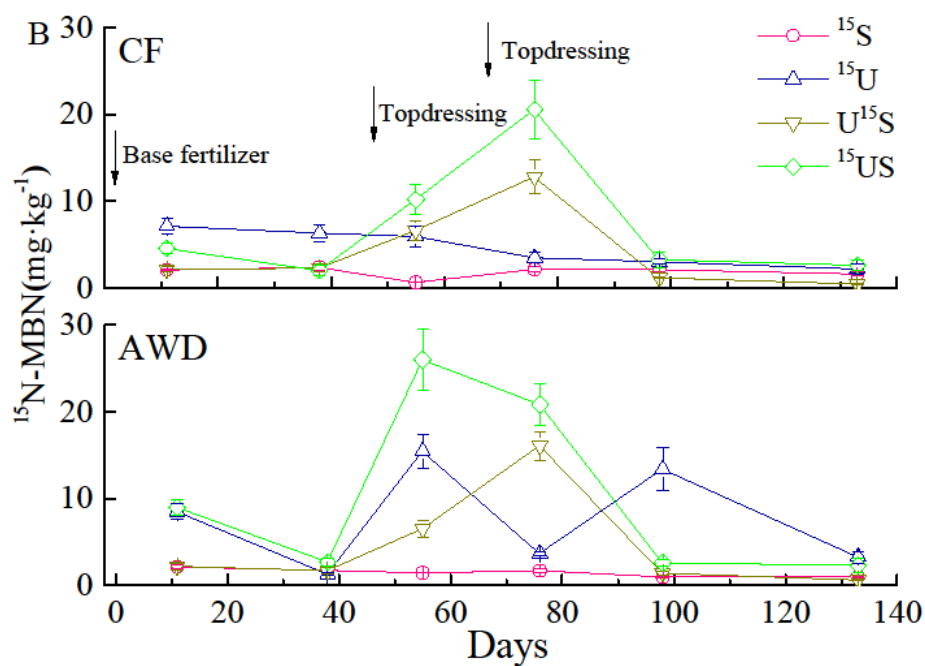
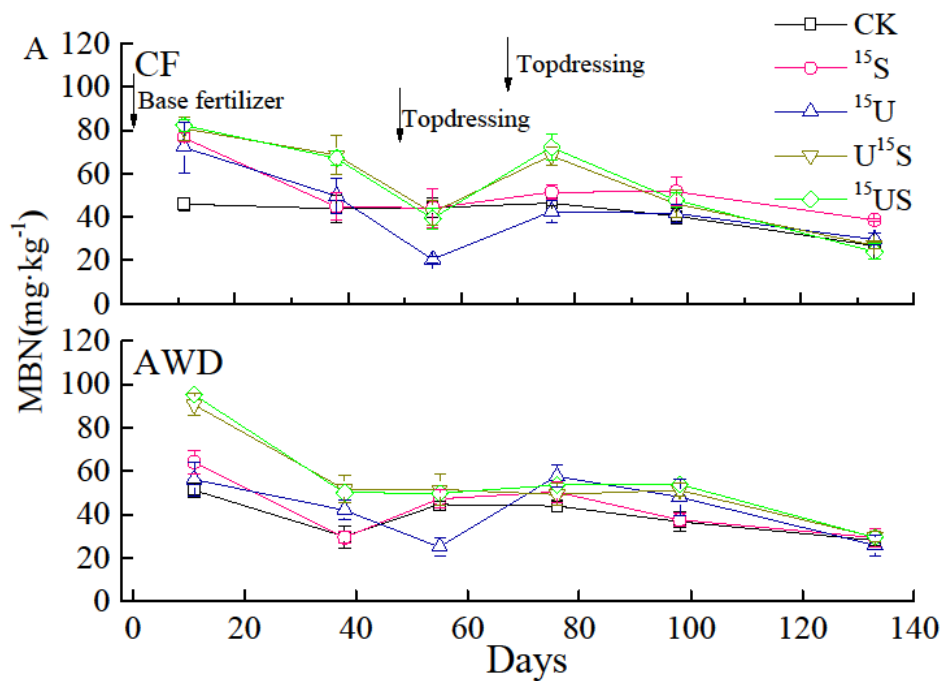


Fig. 3 Changes in the concentration of microbial biomass N (MBN) (A) in the soil during the rice growth period and the contribution of <sup>15</sup>N markers to MBN (B) (n=3).

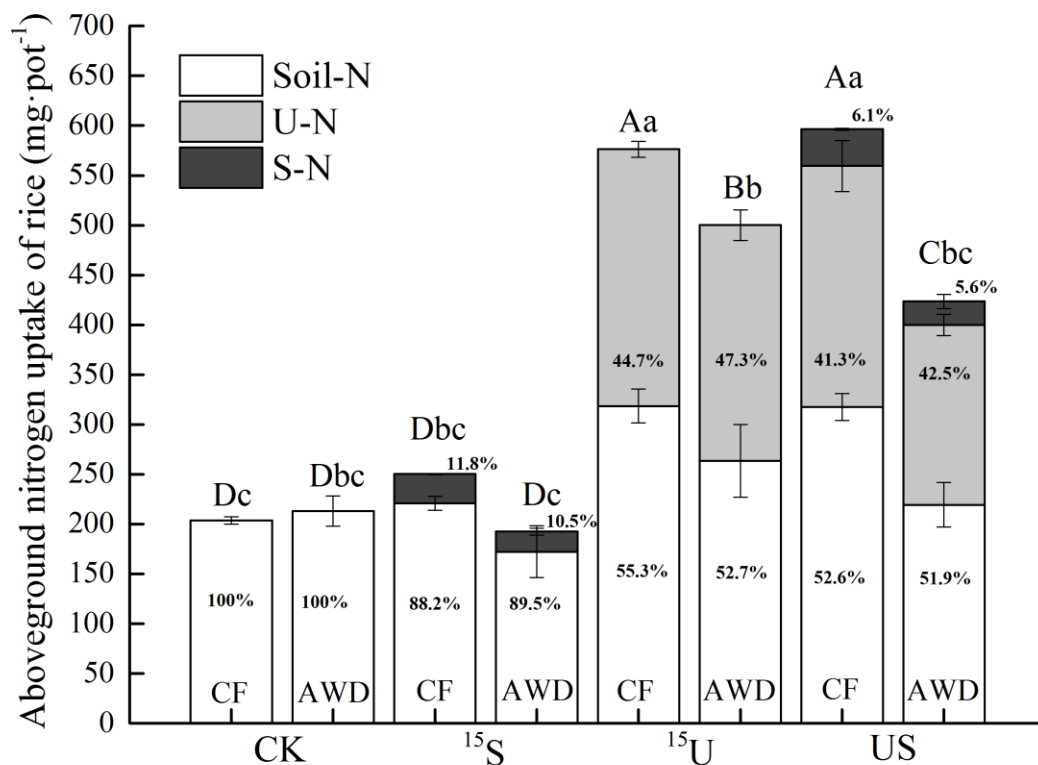


Fig. 4 The source of nitrogen in the aboveground biomass of rice at the maturity stage (n=3). CF: continuous flooding irrigation, AWD: mild alternate wetting and drying irrigation, Soil-N: Soil derived nitrogen, U-N: Urea derived nitrogen, S-N: Rice straw derived nitrogen. Different capital letters indicate 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 nitrous oxide (N<sub>2</sub>O) emissions, rice aboveground biomass, rice yield and yield-scaled N<sub>2</sub>O emission. The values denote means  $\pm$  standard errors (n=3). Different lowercase letters indicate significant differences ( $P < 0.05$ ).

Treatments		Cumulative N <sub>2</sub> O emissions kg ha <sup>-1</sup>	Rice aboveground biomass g pot <sup>-1</sup>	Rice yield g pot <sup>-1</sup>	Yield-scaled N <sub>2</sub> O emission g kg <sup>-1</sup>
CF	CK	1.48 $\pm$ 0.06 ef	37.11 $\pm$ 2.53 f	21.63 $\pm$ 1.81 b	0.24 $\pm$ 0.01 d
	<sup>15</sup> S	1.24 $\pm$ 0.05 f	37.99 $\pm$ 2.69 f	20.91 $\pm$ 1.63 b	0.21 $\pm$ 0.03 d
	<sup>15</sup> U	4.02 $\pm$ 0.30 c	82.54 $\pm$ 10.39 ab	36.56 $\pm$ 2.75 a	0.39 $\pm$ 0.04 c
	U+ <sup>15</sup> S	3.89 $\pm$ 0.09 c	87.58 $\pm$ 7.70 a	35.98 $\pm$ 1.72 a	0.38 $\pm$ 0.02 c
	<sup>15</sup> U+S	3.76 $\pm$ 0.02 c	80.99 $\pm$ 19.54 abc	35.85 $\pm$ 2.50 a	0.37 $\pm$ 0.03 c
AWD	CK	1.64 $\pm$ 0.15 e	45.31 $\pm$ 3.07 ef	22.92 $\pm$ 1.07 b	0.25 $\pm$ 0.02 d
	<sup>15</sup> S	2.07 $\pm$ 0.17 d	34.78 $\pm$ 4.86 f	20.42 $\pm$ 0.46 b	0.36 $\pm$ 0.02 c
	<sup>15</sup> U	4.48 $\pm$ 0.12 b	64.44 $\pm$ 7.33 bcd	34.81 $\pm$ 1.64 a	0.46 $\pm$ 0.03 b
	U+ <sup>15</sup> S	5.05 $\pm$ 0.28 a	62.92 $\pm$ 2.49 cde	34.50 $\pm$ 1.76 a	0.53 $\pm$ 0.01 a
	<sup>15</sup> U+S	5.29 $\pm$ 0.25 a	60.44 $\pm$ 6.74 de	34.85 $\pm$ 1.18 a	0.54 $\pm$ 0.03 a

Table 2 Cumulative nitrous oxide (N<sub>2</sub>O) emissions in response to irrigation method, nitrogen level, and straw returning. \* indicated significant treatment effects within a main category ( $P < 0.05$ ), \*\*\* indicated significant treatment effects within a main category ( $P < 0.001$ ).

Factors	Cumulative N <sub>2</sub> O emissions
Irrigation method (I)	88.576***
Nitrogen level (N)	1525***
Straw (S)	6.393*
I×N	6.275*
I×S	26.288***
N×S	1.426
I×N×S	0.178