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End water content determines the magnitude of N_2O pulse from nitrifier denitrification after rewetting a fluvo-aquic soil

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ABSTRACT

Large nitrous oxide (N_2O) emissions pulses have been observed after rewetting dry soil. However, few studies have uncoupled the effects of drought severity from the degree to which the soil is saturated. In this study, we conducted three aerobic incubation experiments to investigate the effects of soil rewetting on N_2O emissions from a dryland soil. The results showed that, at constant soil moisture, total N_2O emissions in soil with 90% water-holding capacity (WHC) were significantly higher than those in 30%, 45%, 60% and 75% WHC treatments. In the dry–wet group, the soil moisture content was adjusted from 30%, 45% and 60% WHC to the end content of 75% and 90% WHC, respectively; the cumulative N_2O emissions in the 30–90%, 45–90% and 60–90% WHC nitrogen (N) treatments were significantly higher than those in the 30–75%, 45–75% and 60–75% WHC N treatments. Regarding fertilizer N types, there was no significant difference in N_2O emissions from soil at 90% WHC when $(NH_4)_2SO_4$ or urea was applied. Nitrification inhibitor significantly reduced N_2O emissions in soil applied with NH_4^+-N fertilizer, indicating that nitrification played a major role in N_2O emissions from soils. The contribution of denitrification was negligible, according to the low emission rate of soils with only NO_3^- additions. High N_2O emissions occurred in soil treated with NO_2^-, accounting for about 83.6% of those of the NH_4^+ treatment. Therefore, in this study we concluded that the end water content of soil was more important than the role of drought severity in the dry-wet process and that nitrifier denitrification was probably the main pathway of N_2O production under the condition of 90% WHC moisture after rewetting soil.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas, with a 100-year global warming potential 300 times greater than that of carbon dioxide (CO_2) (Wuebbles, 2009; UNEP, 2013). In addition, N_2O is the single most important depleting substance of the stratospheric ozone layer (Ravishankara et al., 2009; Kanter et al., 2013). The climate change (including global warming and ozone layer depletion) poses threats to ecological system stability and human survival in the coming decades (Tong and Ebi, 2019; Warren et al., 2018) Agricultural activities accounted for about 66% of total anthropogenic N_2O emissions (UNEP, 2013). N_2O from soil is mainly produced

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by biological processes including nitrification and denitrification, depending on the soil's various physical, chemical and biological factors and their interactions (Butterbach-Bahl et al., 2013), which themselves could be affected by the application of nitrogen (N) fertilizer. Since soil moisture content affects the growth and activity of microorganisms, it is another of the most important factors regulating N_2O emissions, especially regarding the microorganisms' responses to soil rewetting (Aguilera et al., 2013; Liu et al., 2017; Barrat et al., 2020). Extensive studies have recorded large N_2O emissions after rewetting dry soil under natural and controlled conditions (Ruser et al., 2006; Cui et al., 2012; Molodovskaya et al., 2012; Harrison-Kirk et al., 2013; Liu et al., 2018). Dryland soil is often in the process of alternating between wet and dry states due to irrigation and rainfall. Unfortunately, global climate change is predicted to increase frequency and intensity of drying-wetting disturbances in soils in the coming decades (IPCC, 2013; Reichstein et al., 2013), which can lead to "hot moments" of N_2O emissions. If they coincide with N fertilizer application, these hot moments can last several days, forming a sizable fraction of total annual N_2O emissions. For example, Cui et al. (2012) showed that N fertilizer application followed by irrigation or rainfall resulted in a huge N_2O emissions pulse, which accounted for about 73% of annual emissions. Molodovskaya et al. (2012) observed that half of the annual N_2O emissions were emitted during the hot moments that occurred over less than 8% of the monitored duration. The soil rewetting will amplify the role of these agricultural ecosystems as sources of N_2O emissions.

Previous studies indicated that the N_2O emission was positively related to frequency and intensity of drying-rewetting cycles (Xu and Luo, 2012; Gao et al., 2018, 2020) which may be a result of soil priming caused by episodic precipitation (Collins et al., 2008). But little is known about the role of drought intensity (soil moisture during drought). According to a recent meta-analysis, the size of N_2O pulse depended on the soil water content after rewetting (Barrat et al., 2020). Barrat et al. (2020) indicated that there was a positive relationship between the change of soil water content and N_2O pulse rate, and that the highest N_2O emissions occurred at about 70% water-holding capacity (WHC) or above. That is, the larger the difference between the dry and wet states of the soil, and the higher the end water content of the soil after rewetting, the larger the N_2O emissions pulse. However, few studies have uncoupled the role of drought severity from the soil saturation level. Therefore, according to this meta-analysis, a large soil moisture change may be just the result of high soil end water content. Exploring the influence of drought severity would provide further insights into the priming effect of alternation between dry and wet states on N_2O emissions in dryland soils.

Given the huge amounts of N_2O emissions during hot moments after soil rewetting, a better understanding of the underlying N_2O production processes is urgently required to help provide insights into N_2O emissions mitigation. For example, the selection of biological inhibitors or fertilizers could differ based on the sources of N_2O. Generally, N_2O is mainly produced by the nitrification of soil ammonium (NH_4^+) and NH_4^+-producing fertilizers under aerobic conditions, as well as the denitrification of nitrate (NO_3^-) under anaerobic conditions (Bremner, 1997; Butterbach-Bahl et al., 2013). Previous studies have shown that nitrification dominates N_2O production when the soil moisture content is below 60% water-filled pore space (WFPS), whereas denitrification plays a major role when WFPS is above 60% (Linn and Doran, 1984; Bateman and Baggs, 2005). However, the role of denitrification is limited by labile C availability (Ju et al., 2009; Qin et al., 2017) and may be confused with nitrifier denitrification (Wrage-Mönnig et al., 2018). Nitrifier denitrification is an autotrophic process mainly carried out by ammonia oxidizers and could contribute substantially to N_2O emissions from soils applied with NH_4^+-N fertilizer (Wrage-Mönnig et al., 2018). Notably, this process could be enhanced under fluctuating aerobic–anaerobic conditions (Kool et al., 2011), which is a typical trait in rewetted soils. However, there is not currently enough data to address the contribution of nitrifier denitrification to N_2O emissions during soil "wet–dry" cycles.

In-depth study of the mechanism and impact of agricultural soil management measures (mainly fertilization and irrigation) on N_2O emissions is of great significance for reducing dryland soil N_2O emissions. The North China Plain, characterized by fluvo-aquic soil, is one of the main grain-producing areas in China. There have been few studies exploring the effects of N fertilizers on N_2O emissions under different water conditions in this type of soil. Here we used a fluvo-aquic soil from the North China Plain to investigate: (1) the influence of different water content (constant and changed moisture) on soil N_2O emissions; (2) the pathway of N_2O production after soil rewetting.

2. Materials and methods

2.1. Site description and soil sampling

Soil samples (0–20 cm) were collected in March 2013 from a typical field under winter wheat (*T. aestivum L.*) and summer maize (*Z. mays* L.) rotation at Fengqiu Agro-ecological Experimental Station of the Chinese Academy of Sciences, Henan Province, China (35°00′N, 114°24′E). The soil is developed from alluvial sediments of the Yellow River and classified as an aquic inceptisol according to U.S. soil taxonomy (Soil Survey Staff, 2010). The soil (8.2% clay, 34.9% silt and 56.9% sand) contained 885 mg N kg^{-1} as total N, 0.06 mg N kg^{-1} as NO_2^--N, 21.33 mg N kg^{-1} as NO_3^--N, 3.99 mg N kg^{-1} as NH_4^+-N and 13.07 g C kg^{-1} as total C, and had a maximum WHC of 42%, with a pH (0.01 M $CaCl_2$) of 7.65. Soil samples were air-dried, sieved to < 2 mm, and stored at room temperature before use.

2.2. Experiment design

2.2.1. Experiment 1: effects of constant moisture and fluctuating moisture treatments on N_2O emissions

To explore the effect of constant and fluctuating soil moisture on N_2O emissions in dryland soil, 30 g soil samples (on a dry weight basis) were added to a series of 250 mL Erlenmeyer flasks and then moistened to 20% WHC with distilled water. All soils were pre-incubated in an incubator (BSH GZX-300-III, CIMO, Shanghai) at 25 °C for 7 d to stabilize microbial activity and eliminate the drying effect before application of the fertilizer solution. Then the culture flasks were divided into two groups—constant group and dry-

wet group—and each group was further divided into N and no N treatments.

After pre-incubating the soil with constant moisture treatment (~ 1–10 d after incubation, Fig. 1), we added 2 mL of $(NH_4)_2SO_4$ solution to the soil sample in the bottle; the end N content was 250 mg N kg^{-1} dry soil. Subsequently, distilled water was used to adjust the soil moisture of each treatment to 30%, 45%, 60%, 75% and 90% WHC, respectively, with four replicates for each treatment. The flasks were covered with parafilm (PM-996, Parafilm, the USA) with six pin-holes for aeration, and then incubated at 25 °C for 10 d. To maintain a stable water level during the incubation period, distilled water was added every 3 or 4 d.

The gas sample collection was set at 2 h, 1 d, 3 d, 5 d, 7 d and 10 d of the incubation as described in previous literature (Ma et al., 2015). Before each gas sample collection, the sealing film was removed for 30 min. After the N_2O concentration balanced with the atmospheric concentration, a 10 mL disposable syringe with a three-way valve was used to collect the gas sample from the culture flask. This gas sample was used as the initial gas concentration. Then the culture flask was sealed and placed in a constant temperature incubator at 25 °C for 4 h, and the end gas sample was collected. The soil samples were collected after 10 d of incubation, and the extraction method was operated as described in previous literature (Ma et al., 2015).

The dry-wet group was designed on the basis of the constant group (Fig. 1), on day 10 of the incubation we adjusted the water content of the 30%, 45% and 60% WHC treatments to 75% and 90% WHC, respectively, by adding water. The dry-wet group included 30–75% WHC, 45–75% WHC, 60–75% WHC, and 30–90% WHC, 45–90% WHC, 60–75% WHC. The moisture content of the 75% and 90% WHC treatments remained constant throughout the incubation period. The gas samples were collected at 2 h, 1 d, 3 d, 8 d, 13 d and 20 d after the addition of water. The soil samples were collected after cultivation, and the concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in the soil were extracted and measured. The extraction method was described in previous studies (Ma et al., 2015).

2.2.2. *Experiment 2: effects of different N fertilizer types on soil N_2O emissions*

Soil physicochemical properties can directly influence N_2O emissions. In this experiment, the soil dynamics of soil NH_4^+-N, NO_2^--N, NO_3^--N and pH were investigated in soils treated with different fertilizers. The experiment included six treatment combinations, with three N fertilizer treatments: (1) CK (control): no fertilizer; (2) U treatment: urea, 250 mg N kg^{-1} dry soil; (3) NS treatment: $(NH_4)_2SO_4$, 250 mg N kg^{-1} dry soil; and two moisture treatments: 65% WHC (L) and 90% WHC (H). The soil water content was chosen according to the recent meta-analysis of Barrat et al. (2020), which reported the highest N_2O emissions occurred at about 70% WHC or above and our moisture treatments were chosen to reflect this threshold. There were three replications in each treatment for gas sampling and two replications for the soil sampling group.

The gas samples were collected at 2 h, 1 d, 3 d, 5 d, 7 d, 10 d and 15 d following incubation. After gas collection, two culture bottles in each treatment were randomly chosen as two replications for destructive sampling, and the concentrations of NH_4^+-N, NO_2^--N and

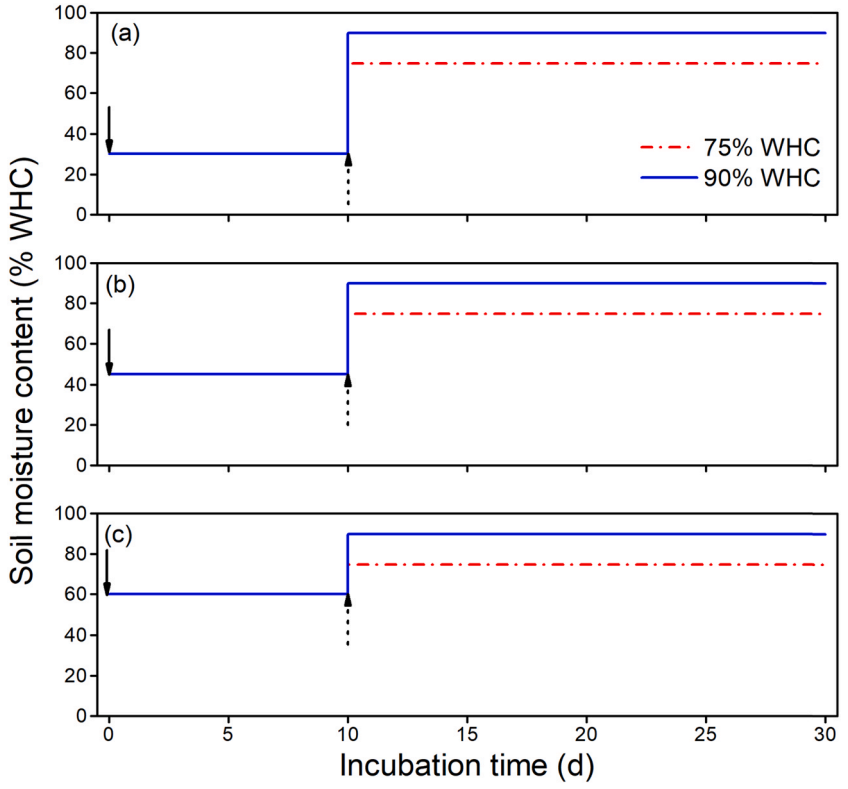

Fig. 1. Schematic diagram of the different moisture treatments. Downward solid arrows indicate fertilizer application events; upward dashed arrows indicate irrigation events.

NO_3^--N in the soil samples were extracted and measured. The methods of gas extraction and soil extraction were identical to Experiment 1.

2.2.3. Experiment 3: exploration of N_2O production pathways in soil

Dicyandiamide (DCD) and glucose was used to explore the N_2O production pathways in soils. DCD is nitrification inhibitor which can inhibit NH_4^+ oxidation (Ma et al., 2015). Denitrification was heterotrophic process which can be stimulated by liable organic carbon (eg. glucose) addition. In this experiment, the soil water content was adjusted with corresponding solution, to achieve 90% WHC for each treatment after the soil samples were pre-incubated. The solution was evenly added to the soil samples in each bottle and to achieve 100 mg N kg^{-1} dry soil and 300 mg C kg^{-1} dry soil according to following treatments: (1) CK (control): no fertilizer; (2) DCD treatment (dicyandiamide, a commercial nitrification inhibitor): adding $(NH_4)_2SO_4$ and DCD (4% of N application); (3) NS treatment: adding $(NH_4)_2SO_4$; (4) NO_3^- treatment: adding potassium nitrate (KNO_3); (5) Glu + NO_3^- treatment: adding glucose and KNO_3; (6) NO_2^- treatment: adding sodium nitrite ($NaNO_2$); (7) Glu + NO_2^- treatment: adding glucose and $NaNO_2$. There were three replications for each treatment. Samples were then incubated at 25 °C for 7 d. The gas samples were collected at 2 h, 1 d, 3 d, 5 d and 7 d after fertilization.

2.3. Analyses and measurements

The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N of the soil filtrates were determined with a Smartchem analyzer (Smartchem200, WESTCO, France). The soil pH was measured in distilled water (CO_2-free), at a soil/water ratio of 1:5, using a pH meter (Sartorius, USA). The N_2O concentrations in the gas samples were simultaneously analyzed using a gas chromatograph (Agilent 7890A, USA) fitted with an electron capture detector (ECD) at 330 °C. The oven was operated at 55 °C and the minimum detection limits of gas fluxes were 2.1 μg N m^{-2} h^{-1} for N_2O.

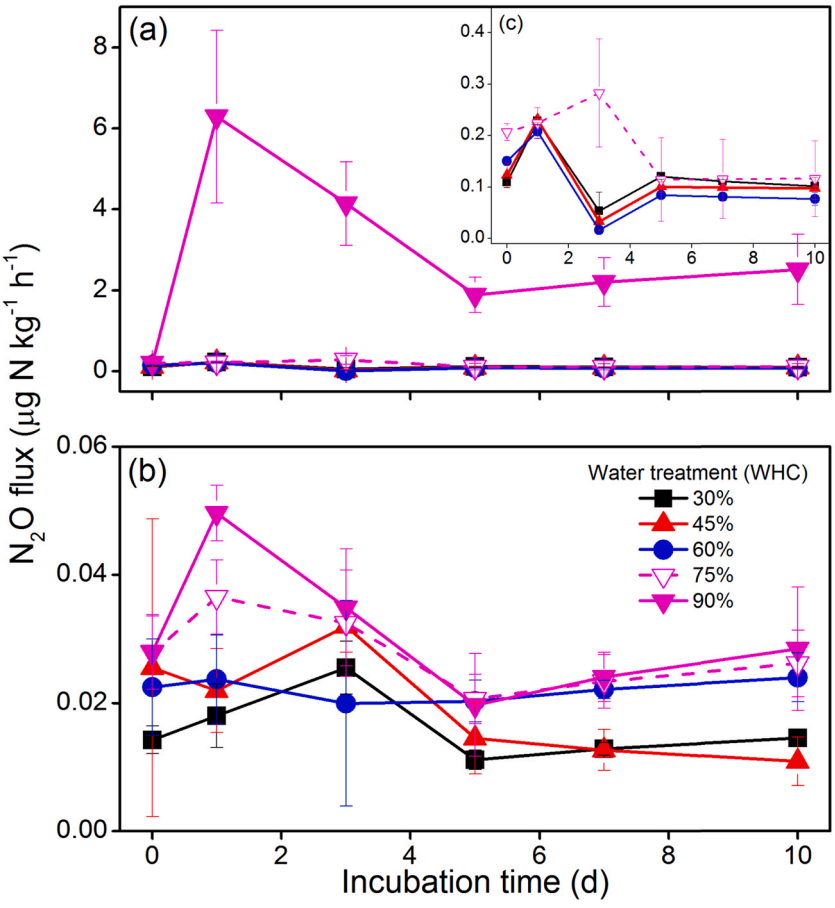

Fig. 2. Dynamics of N_2O emissions under different water treatments (a & c: N addition; b: no N addition).

2.4. Calculations

The N_2O emission factor (*EF*) was calculated according to the following equation:

$$EF = \frac{(S - S_0)/1000}{N} \times 100\% \quad (1)$$

where *S* was the cumulative N_2O emissions in N-fertilized treatments, μg N kg^{-1}; S_0 the cumulative N_2O emission in treatments without N fertilization, μg N kg^{-1}; and *N* was the amount of N addition, mg N kg^{-1}.

Net nitrification rate (N_t) was the average nitrification rate during the incubation period, expressed as:

$$N_t = \frac{\left[NO_3^-\right]_t - \left[NO_3^-\right]_{t_0}}{t - t_0} \quad (2)$$

where N_t was the net nitrification rate, mg N kg^{-1} d^{-1}; $[NO_3^-]$ was NO_3^--N concentration in soil, mg N kg^{-1}; *t* was the d after incubation; and t_0 was the initial time of incubation, 0 d.

2.5. Statistical analyses

We tested for differences in means of N_2O flux and NH_4^+-N, NO_2^--N and NO_3^--N contents among treatments using one-way analysis of variance (ANOVA) and least significant difference (LSD) tests, with a significance level of 5%. Statistical analyses were performed using SPSS (version 16.0, USA), while the graphs were created in Origin 9.0. All results were reported as means (± standard deviation) on a dry soil weight basis.

3. Results

3.1. N_2O emissions from soil under different moisture treatments

In different water treatments, the N_2O fluxes increased significantly at the initial stage of incubation following the application of NH_4^+-N fertilizer, and then gradually decreased with the incubation time (Fig. 2a). The N_2O emission peak for 75% WHC N treatments appeared on day 3 after the incubation, and the N_2O in the 30%, 45%, 60% and 90% WHC N treatments all peaked on day 1 after incubation; the highest N_2O emission peak was 6.29 μg N kg^{-1} h^{-1} in the 90% WHC treatment with N application. In the non-fertilized soil, the change in the N_2O emission rate under different moisture conditions was almost the same as that in the N-fertilized soil, and the largest N_2O emission peak also occurred in the 90% WHC treatment, around 0.05 μg N kg^{-1} h^{-1} (Fig. 2b).

The cumulative N_2O emissions in the N-added soil for each water treatment were significantly higher than those in the non-fertilized soil (Table 1). During the whole incubation period, the cumulative N_2O emissions and emission factor were both highest in the 90% WHC N application treatment, at 644 μg N kg^{-1} and 0.26%, respectively. However, as shown in Table 1, the average nitrification rate within 10 d under 90% WHC water conditions was the lowest among all water treatments with nitrogen, at 19.71 mg N kg^{-1} d^{-1}.

The fluctuations of N_2O flux with incubation time after adding water in different treatments in the dry-wet group on day 10 after incubation were shown in Fig. 3. When the soil water content was adjusted to 90% WHC from 30%, 45% or 60% WHC, the N_2O fluxes increased significantly, and were higher than those within the constant 90% WHC treatment (Fig. 3). A similar pattern was observed in 30–75%, 45–75% and 60–75% WHC N treatments, denoting that N_2O emissions under changing soil moisture were higher than those with constant moisture.

The cumulative N_2O emissions in the fertilized soils were significantly higher than those in the unfertilized soils (Table 2). During the 20 d incubation period, there was no significant difference between the cumulative N_2O emissions of 30–90%, 45–90% and 60–90% WHC N treatments, but these were, respectively, 1.81, 1.98 and 1.86 times higher than the emissions of the constant 90% WHC treatment. The N_2O emission factor in each treatment adjusted to 90% WHC moisture content was significantly higher than that of the treatment adjusted to 75% WHC ($P < 0.05$).

Table 1
The cumulative N_2O emissions, emission factors and net nitrification for each water treatment during the 10 day incubation.

Treatments	Cumulative N_2O emissions (mg N kg^{-1})		N_2O emission factor (%)	Net nitrification rate (mg N kg^{-1} d^{-1})	
	N0	N		N0	N
30% WHC	3.85 ± 0.15c	27.82 ± 2.06b	0.010	0.57 ± 0.04c	24.14 ± 0.19ab
45% WHC	4.44 ± 0.89bc	25.30 ± 1.86b	0.008	0.93 ± 0.17ab	23.56 ± 1.35ab
60% WHC	5.21 ± 0.79b	21.44 ± 1.94b	0.006	0.95 ± 0.15ab	24.87 ± 0.35a
75% WHC	6.49 ± 0.36a	40.35 ± 17.31b	0.014	1.23 ± 0.17a	22.67 ± 0.14b
90% WHC	7.14 ± 0.85a	644.01 ± 108.35a	0.255	0.73 ± 0.07bc	19.71 ± 0.33c

Note: Different lower-case letters within a column denote significant differences at $P < 0.05$ for each treatment during the incubation; N: N addition; N0: no N addition.

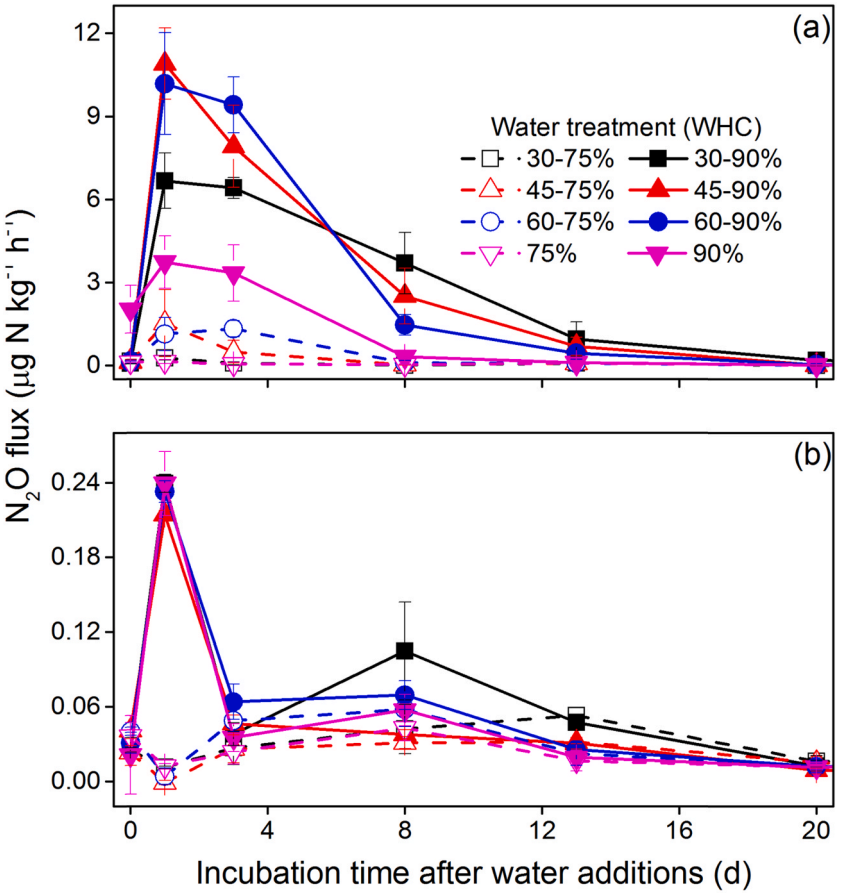

Fig. 3. Dynamics of N_2O emissions under different treatments after water addition (a: N addition; b: no N addition).

Table 2
The cumulative N_2O emissions and emission factors for each treatment after water addition during the 20 day incubation.

Treatments	Cumulative N_2O emissions (mg N kg^{-1})		N_2O emission factor (%)
	N0	N	
30–75% WHC	16.92 ± 1.99cd	30.24 ± 8.70c	0.005
45–75% WHC	11.97 ± 2.15de	111.58 ± 75.59c	0.040
60–75% WHC	15.77 ± 0.70de	175.21 ± 53.75c	0.064
75% WHC	11.27 ± 1.96e	27.28 ± 9.75c	0.006
30–90% WHC	32.03 ± 5.85a	1367.75 ± 301.36a	0.534
45–90% WHC	21.29 ± 0.70bc	1449.41 ± 147.56a	0.571
60–90% WHC	26.56 ± 3.33b	1391.45 ± 95.99a	0.546
90% WHC	21.60 ± 2.47bc	486.89 ± 120.97b	0.186

Note: Different lower-case letters within a column denote significant differences at $P < 0.05$ for each treatment during the incubation; N: N addition; N0: no N addition.

3.2. Dynamics of N_2O emissions in soils treated with different fertilizers

The application of $(NH_4)_2SO_4$ and urea significantly increased N_2O emissions ($P < 0.01$). Under the moisture condition of 65% WHC, the N_2O emissions rate reached its peak on day 5, and the peak of N_2O emissions in the U treatment was 2.26 μg N kg^{-1} h^{-1}, which was 1.24 times higher than that in the NS treatment (Fig. 4). The peak of N_2O emissions in the U treatment appeared on day 5 during incubation, but the peak in the NS treatment appeared on day 7 after cultivation. These emission peaks were 40.84 and 33.56 μg N kg^{-1} h^{-1}, respectively. After 15 d of aerobic incubation, the cumulative emissions of N_2O in each N-applied soil under 65% WHC moisture conditions was U > NS > CK, and the differences were significant. Under 90% WHC moisture conditions, there was no significant difference between U and NS treatments for cumulative N_2O emissions (Table 3).

The correlation analysis of N_2O emissions rate, NH_4^+-N, NO_2^--N, NO_3^--N concentration and pH value in different treatments were

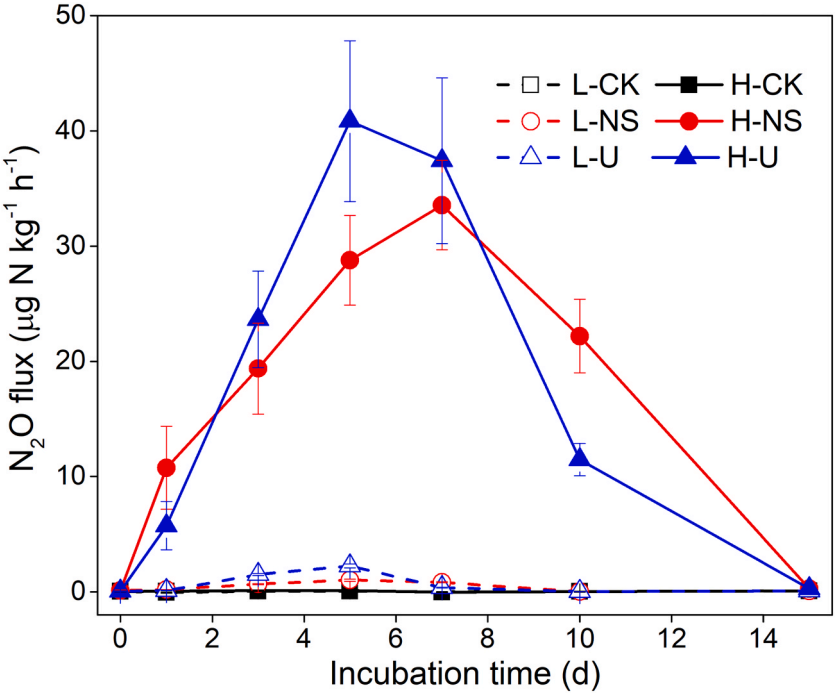

Fig. 4. Dynamics of N_2O emissions under different N fertilization treatments (L: 65%WHC, H: 90%WHC, CK: control, no N addition; NS: $(NH_4)_2SO_4$; U: urea).

Table 3
The cumulative N_2O emissions and emission factors for different fertilizer treatments.

Treatments	Cumulative N_2O emissions (mg N kg^{-1})	N_2O emission factor (%)
L-CK	5.09 ± 3.45c	
L-NS	142.93 ± 6.75b	0.06
L-U	211.54 ± 11.90a	0.08
H-CK	14.88 ± 3.77B	
H-NS	6847.35 ± 954.89A	2.73
H-U	6534.47 ± 1253.59A	2.61

Note: Different lower-case letters within a column denote significant differences at $P < 0.05$ for each N treatment at 65% WHC during the 15 d incubation; different capital letters within a column denote significant differences at $P < 0.05$ for each N treatment at 90% WHC during the incubation; NS: $(NH_4)_2SO_4$; U: urea.

shown in Table 4. Under 90% WHC moisture conditions, there was significantly positive correlation between the N_2O fluxes and the NO_2^--N concentration ($P < 0.01$) in soils applied with urea and $(NH_4)_2SO_4$; under the condition of 65% WHC, the N_2O emissions rate and the NO_2^- concentration had a significantly positive correlation ($P < 0.05$) only in the soil applied with urea. However, the N_2O emissions rate had no significant correlation with NH_4^+-N, NO_3^--N concentrations and pH value in different water and fertilizer combination treatments.

Table 4
Pearson correlation coefficients of the linear correlation between the N_2O flux and NH_4^+, NO_2^-, NO_3^- concentrations and pH under different N fertilization treatments.

Soil moisture content	N fertilizer type	NH_4^+	NO_2^-	NO_3^-	pH
65% WHC	$(NH_4)_2SO_4$	−0.16	0.68	0.12	0.21
	Urea	0.55	0.87*	−0.19	0.76
90% WHC	$(NH_4)_2SO_4$	0.01	0.97**	−0.10	−0.24
	Urea	0.47	0.92**	−0.20	−0.30

* Significant at the 0.05 level.
** Significant at the 0.01 level.

3.3. Effects of glucose and different N source on the emission of N_2O in the soil

In the first 3 days of culture, the N_2O emissions rate difference between different treatments was significant ($P < 0.05$), but the difference was not significant at the later stage of incubation (Fig. 5). During the entire incubation period, the control group (without fertilization) had almost no N_2O emissions (0.02–0.20 μg N kg^{-1} h^{-1}), whereas N fertilization significantly increased N_2O emissions ($P < 0.05$), and all the emission peaks were observed on day 1 of incubation. The application of glucose significantly promoted N_2O emissions ($P < 0.01$), and the peak of N_2O emissions was largest in the treatment with simultaneous addition of NO_2^- and glucose, reaching 307.93 μg N kg^{-1} h^{-1}.

The cumulative N_2O emissions in the DCD, NH_4^+, NO_3^-, Glu+NO_3^-, NO_2^- and Glu + NO_2^- treatments were significantly higher than in CK (Table 5). Compared with the NH_4^+ treatment, the DCD treatment significantly reduced the cumulative emissions of N_2O by 82.6%. Adding glucose together with NO_3^- significantly promoted the emissions of N_2O, while cumulative emissions were about 489 times greater than for the NO_3^- treatment alone. The application of NO_2^- promoted N_2O emissions, and its total emissions were equivalent to 83.6% of the NH_4^+ treatment. The addition of glucose together with NO_2^- also significantly stimulated N_2O emissions, and cumulative emissions were about 13.26 times greater than for the NO_2^- treatment alone; its N_2O emission factor (15.79%) was the highest among all fertilization treatments.

4. Discussion

4.1. The influence of different water content (constant moisture and changed moisture) on soil N_2O emissions

In experiment 1, after adjusting the soil moisture content from 20% WHC to 30%, 45%, 60%, 75% and 90% WHC, the N_2O emissions flux in each treatment reached a peak at the initial stage of incubation (Fig. 2), indicating that wetting dry soils promoted N_2O emissions, in accordance with results from previous studies (Ruser et al., 2006; Kim et al., 2012). Soil rewetting has a significant simulative effect on N_2O emissions. Wetting dry soils may destroy soil aggregates, promote soil carbon and N mineralization, increase the content of soluble organic matter, and therefore enhance the substrates' availability for nitrification and denitrification processes (Kim et al., 2012). However, the initial 10 day rewetting treatments produced weaker pulses than the second wetting (Figs. 2 and 3), which indicated that reducing the frequency of irrigation maybe a low-cost option to mitigate N_2O emission in cropland.

When the soil moisture was adjusted from 30%, 45% and 60% WHC to the content of 90% WHC, the N_2O emissions from N-fertilized soil were significantly higher than those of the constant 90% WHC treatment. No matter whether the soil moisture was adjusted once or twice, the N_2O emissions in the treatment at 90% WHC were significantly higher than those at 75% WHC (Tables 1 and 2). This result indicated that the amount of N_2O emissions depended mostly on the total amount of water addition with adequate N supply, as also suggested by the recent meta-analysis (Barrat et al., 2020). Moreover, there was no significant difference in the cumulative N_2O emissions of 30–90%, 45–90% and 60–90% WHC N treatments, which indicated that, in the process of changing from

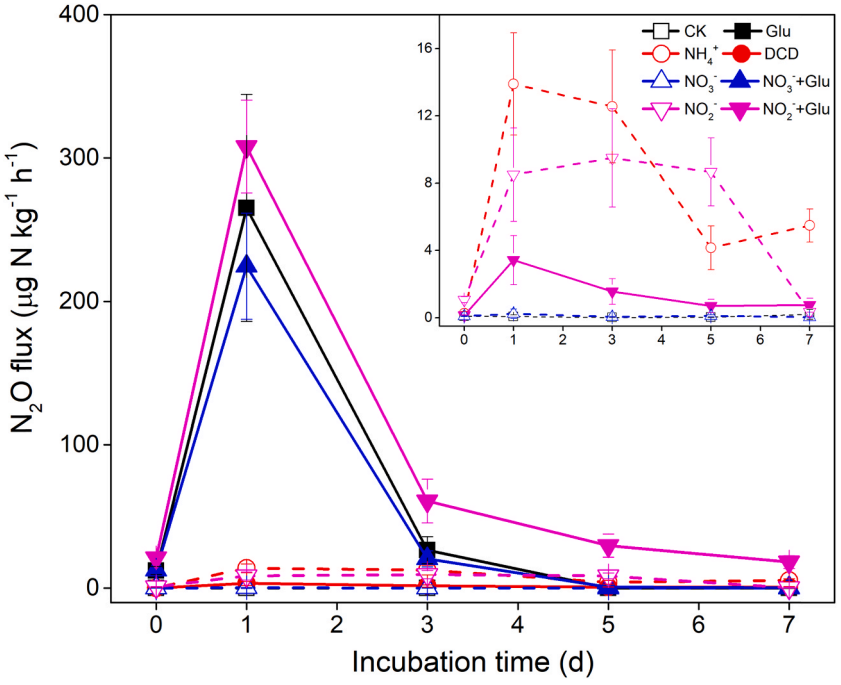

Fig. 5. Dynamics of N_2O emissions under glucose and different N source.

Table 5
The cumulative N_2O emissions and emission factors under different N sources.

Treatments	Cumulative N_2O emissions (mg N kg^{-1})	N_2O emission factor (%)
CK	10.22 ± 5.51	
DCD + NH_4^+	248.19 ± 115.52	0.24
NH_4^+	1424.22 ± 350.37	1.41
NO_3^-	18.43 ± 7.12	0.01
NO_3^- + Glu	9025.18 ± 1577.82	9.01
NO_2^-	1190.27 ± 342.67	1.18
NO_2^- + Glu	15,787.43 ± 2326.05	15.78

dry to wet, the end water content of the soil was more important than the role of drought severity. A different pattern was observed in the 30–75%, 45–75% and 60–75% WHC N treatments, and the high variability among these may be due to the complexity of N_2O microbial production processes under this soil moisture condition (Linn and Doran, 1984; Bateman and Baggs, 2005).

4.2. Effects of soil characteristics and N fertilizer types on N_2O emissions

In this study, as shown in Table 4, there was stronger relationship between soil N_2O emissions and NO_2^- concentration than other soil characteristics (NH_4^+, NO_3^- concentrations and pH). Although the correlation became weak under the condition of 65% WHC in the treatment of $(NH_4)_2SO_4$. These results indicated that the underlying N_2O pathways may be related to soil NO_2^- and would be independent of fertilizer type in high water level and the sources of N_2O emissions are worth further pursuing.

Previous studies indicated that urea addition produced higher N_2O emissions in cropland soils than those soils with $(NH_4)_2SO_4$ addition (Cai et al., 1997; Zhu et al., 2013; Tierling and Kuhlmann, 2018; Castellano-Hinojosa et al., 2020), which was also observed in our study. Under the condition of 65% WHC, the N_2O emissions in the urea treatment were significantly higher than those in the $(NH_4)_2SO_4$ treatment, which was attributed to the following reasons. Urea hydrolysis increases soil pH, while the nitrification process in the soil with $(NH_4)_2SO_4$ additions releases H^+ and results in soil acidification. Notably, NO_2^- is unstable under acidic conditions and is easily chemically decomposed (Van Cleemput and Samater, 1995). In addition, soil solution-phase ammonia (NH_3) could inhibit NO_2^- oxidization (Venterea et al., 2015), yet the soil solution-phase ammonia (NH_3) could convert into ammonium (NH_4^+) as a result of soil acidification. A previous study also reported that high NH_4^+ and high pH benefited soil NO_2^- accumulation (Alexander, 1978). Consequently, the cumulative peak of NO_2^- in the $(NH_4)_2SO_4$ treatment was significantly lower than that in the urea treatment.

4.3. Sources of N_2O emissions during hot moments

Regardless of the fertilizer type, N_2O emissions in 90% WHC fertilized soil were the highest from the results of experiments 1 and 2. It has been reported that denitrification was the dominant way to produce N_2O under such anaerobic water conditions (Davidson, 1992). However, previous studies have also shown that nitrifier denitrification is the main process of N_2O production in soil under low oxygen conditions, accounting for almost up to 100% of emissions (Kool et al., 2010, 2011).

In experiments 1 and 2, the concentration of NH_4^+ fertilizer applied to the soil was reduced to almost 0 mg N kg^{-1} in the later period of incubation, while the NO_3^- concentration in each treatment reached its maximum value and stabilized at 300 mg N kg^{-1}. This indicated that nitrification significantly occurred in soils not tightly coupled with denitrification. As shown in experiment 3, the DCD treatment can significantly reduce the cumulative N_2O emissions by 82.6% (Table 5) compared with the NH_4^+ treatment, suggesting that the contribution of nitrification to N_2O production was far greater than that of denitrification. The negligible contribution of denitrification was also verified by the low emissions rate of soils with only NO_3^- additions (Fig. 5). Insufficient available carbon may be a limiting factor affecting the denitrification process in the soil, which can be verified by the larger N_2O emissions in the soil after adding glucose to the NO_3^- treatment (as well as Glu + NO_2^- treatment), a similar result which was also observed in previous studies (Ju et al., 2011).

In the soil applied with NO_2^-, total N_2O emissions were equivalent to 83.6% of the NH_4^+ treatment, indicating that pathways derived from NO_2^- made a great contribution to N_2O emissions. In our previous study, the contribution of chemical denitrification to N_2O emissions was eliminated by sterilization experiments (Ma et al., 2015). A recent review by Wrage-Mönnig et al. (2018) showed that nitrifier denitrification would be facilitated under environments with O_2 limitation or variable O_2 concentrations, typical characteristics in rewetting soils. Given the limited role of denitrification and chemical denitrification as stated above, the higher N_2O production after rewetting soils in this study was probably from nitrifier denitrification. Further investigation on magnitude of nitrifier denitrification under drying-wetting disturbances in soils is needed against the background of future global climate change.

5. Conclusion

In this study, when soil moisture content increased from 30%, 45% and 60% WHC to 75% and 90% WHC, soil N_2O emissions significantly increased; soil N_2O emissions remained the highest under 90% WHC moisture conditions regardless of whether it was a constant moisture treatment or a fluctuating moisture treatment. Therefore, final soil water content rather than drought severity determined the magnitude of N_2O pulse in fluvo-aquic soil from the North China Plain.

We found that the denitrification process made a negligible contribution to N_2O emissions, mainly due to the low soil organic

matter content of the selected soil, whereas nitrifier denitrification may be the primary pathway of N_2O emissions after rewetting fluvo-aquic soils.

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.

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