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Combination of nitrogen and organic fertilizers reduce N₂O emissions while increasing winter wheat grain yields and quality in China

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Wheat grain yields, quality, and nitrous oxide (N₂O) emissions were controlled through the type and application rate of nitrogen (N) fertilizer. Here, we investigated the optimal management of N fertilization by examining the combined effects of organic and N fertilizers on wheat yields, quality, and N₂O emissions. Field trials under six treatments were located on campus farms at Anhui Science and Technology University, including farmer's common practice (270 kg N ha⁻¹, N270), 2/3 reduction in N270 (90 kg N ha⁻¹, N90), organic fertilizer with equal N270 (OF270), 2/3 reduction in OF270 (OF90), 4/5 reduction OF270 + 1/5 reduction N270 (20% OF270 + 80% N270), and 4/5 reduction OF90 + 1/5 reduction N90 (20% OF90 + 80% N90) were applied to winter wheat. The plots were arranged in a randomized complete block experimental design. The N₂O emissions were quantified under different fertilization measures in the peak wheat growing season during sowing, jointing, and grain filling stages, respectively. Compared with N270 and N90 treatments, N₂O emissions were significantly decreased by 18.6% and 27.2%, respectively, under 20% OF270 + 80% N270 and 20% OF90 + 80% N90 ($p < 0.05$). Further, N₂O emissions in N270 were increased by 50.8% relative to N90. Wheat yields increased significantly under 20% OF90 + 80% N90 by 27.6% (N270) and 16.4% (N90), and were considerably enhanced under 20% OF270 + 80% N270 by 40.6% (N270) and 12.7% (N90) in contrast to OF270 and N270 ($p < 0.05$). Compared with N90, the content of wet gluten, protein and starch under 20% OF90 + 80% N90 treatment significantly increased by 7.7%, 13.8% and 7.9%, and enhanced by 7.6%, 4.8%, 8.0% relative to OF90, respectively ($p < 0.05$). The starch content increased significantly by 2.0%, whereas the settlement value decreased considerably by 2.9% under 20% OF270 + 80% N270 ($p < 0.05$), and there was no notable difference in the wet gluten and

protein contents ($p > 0.05$). Our findings indicated that organic fertilizer mixed with N fertilizer can effectively reduce N_2O emissions, increase both the grain yields and quality in wheat field compared with N fertilizer alone.

KEYWORDS

N_2O emission, wheat grain yield, nutritional quality, nitrogen application rate, organic fertilizer

1 Introduction

Nitrous oxide (N_2O) in the ambient atmosphere is the third most abundant greenhouse gas (GHG) contributor to the enhanced greenhouse effect following carbon dioxide (CO_2) and methane (CH_4), which is $\sim 310\times$ and $28\times$ greater than that of CO_2 and CH_4 , respectively, accounting for $\sim 6.2\%$ of GHG-induced warming since the preindustrial era (IPCC, 2013; WMO Greenhouse Gas Bulletin, 2018; Prather et al., 2015). Up to 2012, atmospheric N_2O concentrations increased from 270 ppb to 325 ppb (a $\sim 20\%$ increase) (Qin et al., 2014). Since then, the N_2O emission rate has increased by $\sim 0.26\%$ per year (Forster et al., 2007) primarily as the result of anthropogenic activities (e.g., the application of nitrogen (N) fertilizers on farmland).

Since N is an essential macro-nutrient that supports all organisms, the rational scientific application of N fertilizers can significantly increase crop yields and improve their quality (Liu et al., 2015). China is a major global wheat producer, which cultivation represents some 18.6% of the total national food crop growing area and 18.0% of production in China, respectively, and some 13% of the international wheat cropping acreage. Currently, increased wheat yields are mainly due to enormous N fertilizer inputs (Godfray et al., 2010; Yan et al., 2024). However, the excessive application of N fertilizers has translated to decreased N use efficiencies, which has initiated a cascade of environmental issues such as soil acidification (Chen et al., 2014), ammonia volatilization (Liu et al., 2013), and water eutrophication (Wang et al., 2014). This is particularly the case for increased nitrous oxide (N_2O) emissions from soil (Liu et al., 2015), with $\sim 36\%$ of N_2O being released from agricultural soils on a global scale (Mosier et al., 1998). The excessive application of N fertilizers has resulted in 0.63 MT of N_2O emissions in China, which accounts for 92% of the total national N_2O emissions from agricultural production (Development and Reform Commission, PRC, 2007).

Preceding studies have indicated that fertilization measures are critical factors that affect soil N_2O emissions from wheat field soils, such as the type and amount of fertilizer, as well as the application strategy (Sun et al., 2017; Shakoore et al., 2018; Liu et al., 2015; Sainju et al., 2020). It has been proven that N fertilizers are important sources of N_2O emissions in wheat field soils, with a positive correlation between the quantity of N fertilizers and N_2O emissions (Peng et al., 2022). For instance, Hou et al. (2018) and Xia et al. (2020) found that N_2O emissions in wheat fields under full N fertilizer application were significantly higher than under reduced N measures. Remarkably, fertilization reduction can effectively decrease the emissions of N_2O from the soil. Recently, special attention has been given to the impacts of mixing dicyandiamide, biochar, and nano-carbon with N fertilizers on N_2O emissions in wheat fields (Liu et al., 2015; Sun et al., 2017). Reduced N_2O

emissions of 45.4%, 33.7%, and 35.0% were observed in wheat fields with the addition of dicyandiamide, nano-carbon, and biochar, respectively, to N fertilizers (Liu et al., 2015; Sun et al., 2017). The addition of biochar or nano-carbon to soil can effectively increase NH_4^+ adsorption, while reducing NH_4^+ and NO_3^- leaching, which reduces the substrates for nitrification and denitrification for N_2O production (Dempster et al., 2012; Jones et al., 2012; Liu et al., 2015).

Apart from the fertilization measures described above, the addition of organic fertilizer also has an important impact on N_2O emissions in wheat fields (Miao et al., 2020). N_2O is an important byproduct of nitrification and denitrification processes mediated by soil microorganisms, which are influenced by the abundance and diversity of functional genes related to nitrifying and denitrifying bacteria (Hu et al., 2015; Harter et al., 2014; Song et al., 2023). The co-application of organic and N fertilizers can significantly enhance soil microbe populations and enzyme activities, while simultaneously increasing the soil humus content and improving the mineralization potential of soil N and N use efficiencies of crops (Li et al., 2016; Yan et al., 2014; Elfstrand et al., 2007; Zhang et al., 2009; Xu et al., 2002). In most cases, compared with the single application of urea, the addition of organic fertilizer decreased N_2O emissions from 15.1% to 33.4% in agricultural soils (Shuai et al., 2018), albeit several studies have reported contrary findings (Frimpong and Baggs, 2010; Pelster et al., 2011). This might have been related to the degree of decomposition, the C/N ratio of organic matter, and the type of organic fertilizer (Zhang, 2012; Frimpong and Baggs, 2010; Feng et al., 2014). However, the above-mentioned studies discussed the differences between N_2O emissions from wheat fields only when different proportions of organic fertilizer were combined with N fertilizers under equal N application rates, and rarely reported the impacts under different N application levels.

Increased wheat grain yields with improved quality have been attributed mostly to the prudent application of fertilizers (Blandino and Reyneri, 2009; Lin et al., 2015; Lv et al., 2020; Zhang et al., 2023), which accounted for $\sim 50\%$ of increased grain production (Wu and Wang, 2002). Liu et al. (2015) reported that the addition of dicyandiamide and nano-carbon to N fertilizers increased wheat yields by 12.3% and 11.9%, respectively, compared with the farmers common N rate. Meanwhile, sensible fertilization can improve grain quality by specifically increasing the protein content from 12.3% to 16.1%, sedimentation value from 28.7% to 47.2%, and wet gluten content from 10.7% to 11.1% (Zhang et al., 2023). Conversely, the irrational application of N fertilizers leads to decreased wheat grain yields, as well as reduced processing and nutritional qualities. Consequently, it is of great significance to logically reduce the application of N fertilizers, while ensuring stable wheat yields and nutritional quality.

Earlier studies investigated wheat fields under various organic fertilizer mixtures and N application levels for their effects on either N₂O emissions or grain yields and quality. Thus, to quantify these parameters in the wheat fields of Huaihe Basin, China under various organic fertilizer conditions we conducted winter wheat growing season experiments using the static chamber and gas chromatograph methods. We aimed to achieve synergistic improvements in wheat yields and quality and decreased N₂O emissions, while ensuring the fertility of farmland, decreasing production costs, and improving N use efficiencies.

2 Materials and methods

2.1 Site description

The experimental sites are located on campus farms at Anhui Science and Technology University in Chuzhou City, Anhui Province, China (32°86'N, 117°04'E, elevation 36 m a.s.l.). The experimental area is home to a humid subtropical monsoon climate with a mean annual temperature of 14.9°C, precipitation of 904.4 mm (mainly concentrated in summer), potential evaporation of 1,609.7 mm, a 212 d frost-free period, and 2,248 h of sunlight. The leading agricultural crops in this region include winter wheat, maize, and rice. This experimental site was established in 2018, where fertilization experiments have been conducted with rice-wheat rotations for 5 years. The soil is classified as moisture soil with key properties that include: pH (8.210), total N (0.492 g kg⁻¹), alkali-hydrolyzable N (68.703 mg kg⁻¹), total phosphorus (0.580 g kg⁻¹), rapidly available phosphorus (3.941 mg kg⁻¹), slowly available potassium (510.633 mg kg⁻¹), and organic matter (12.810 g kg⁻¹).

2.2 Experiment design

This study included six treatments: farmer's common practice (270 kg N ha⁻¹, N270), 2/3 reduction in N270 (90 kg N ha⁻¹, N90), organic fertilizer with equal N270 (OF270), 2/3 reduction in OF270 (OF90), 4/5 reduction OF270 + 1/5 reduction N270 (20% OF270 + 80% N270), and 4/5 reduction OF90 + 1/5 reduction N90 (20%

OF90 + 80% N90) (Table 1). The plots were arranged in a randomized complete block experimental design. Each treatment had three replicates (18 plots in total), each plot was 3.75 m² (3 m × 1.25 m) and 1.2 m depth. To avoid interference through the exchange of water and fertilizer, each plot was isolated using concrete bricks with 1.2 m depth.

The annual application rates of P and K fertilizers in the forms of calcium superphosphate (P₂O₅ content 12%) and potassium sulfate (K₂O content 60%) were 75 and 150 kg ha⁻¹, respectively, which were identical for all treatments. All P and K fertilizers and 40% of N fertilizers (urea with 46% N content) were basally applied, with the remaining 30% of N fertilizers applied as topdressing at the greening-jointing stage and pustulation period, respectively. As the base fertilizer, organic fertilizer was applied into the soil at once. The experiment was conducted from 25 November 2022 to 25 May 2023. The wheat variety was Yannong 999, of which 80 g was sown in each plot.

2.3 N₂O sampling and measurement

N₂O samples were collected at each wheat growth stage using static closed chambers (50 cm × 50 cm × 50 cm) (designed and operated as described in Wu et al. (2020)) with a total of 9 times from 08:00–11:00 a.m. Four stainless steel frames were inserted ~10 cm into the soil 1 week prior to the start of sampling and kept in place until the end of the experiment, which in advance to minimize the disturbance of the soil environment when sampling. The external surface of each chamber was insulated with rubber foam and covered with self-adhesive aluminum foil to minimize solar radiation heating during sampling. In addition, in order to ensure the tightness of the static closed chambers, we add water to the tank at the bottom of the box. The 40 mL gas samples within the chamber were collected at 0, 5, 15, and 30 min after the chamber had been sealed using a 60 mL plastic syringe. Meanwhile, the air temperature in the chamber was measured. The collected gas samples were immediately injected into pre-evacuated vials (12 mL), transported to the laboratory within 24 h, and analyzed using gas chromatography (Agilent 7,890, American).

2.4 N₂O flux calculation

The N₂O flux was calculated according to Liu et al. (2015), using Equation 1:

$$F_{N_2O} = \rho \times h \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + T}$$

where, F_{N_2O} is the N₂O flux (μg m⁻² h⁻¹), ρ is the N₂O gas density in standard state (kg m⁻³), h is the chamber height above the soil (cm), $\Delta c / \Delta t$ is the slope of the linear regression for the gas concentration gradient over time (m³ m⁻³ h⁻¹), and T is the average temperature within the chamber during sampling.

The cumulative N₂O emission flux was calculated according to Liu et al. (2015), using Equation 2:

$$T_{N_2O} = \sum [(F_{i+1} + F_i) / 2] \times (D_{i+1} - D_i) \times 24 / 1000 \times 667 \times 15 \times 10^{-6} \quad (2)$$

TABLE 1 Experimental design with two factors, i.e., organic fertilizer and N fertilizer rate.

Treatment	Factors	
	organic fertilizer rate (kg N ha ⁻¹)	N fertilizer rate (kg N ha ⁻¹)
N90	0	90
N270	0	270
OF90	90	0
OF270	270	0
20% OF90 + 80% N90	72	18
20% OF270 + 80% N270	216	54

where, T_{N_2O} is the cumulative N_2O emissions from a specific growing stage ($kg\ N\ ha^{-1}$), F_i and F_{i+1} denote the N_2O flux of the i and $i + 1$ sub-sampling ($\mu g\ m^{-2}\ h^{-1}$), while D_i and D_{i+1} represent the sampling time (d) (Ji et al., 2011).

2.5 Soil sampling and measurements

Soil samples were collected at a 0–20 cm depth from each plot during the various growth stages of wheat for the analysis of soil properties following the methods described by Sun et al. (2017). Three replicates surrounding each plot were well blended thoroughly as one sample. Repeat sampling 3 times for each of the above mixed soil samples. The samples were dried at room temperature, crushed, and sieved to pass through a 2 mm mesh. The soil organic carbon (SOC) was analyzed using an element analyzer (Vario EI, Elementar, Hanau, Germany). Soil total N (TN) was measured by dry combustion using a Vario Max CNS elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). To measure the ammonium nitrogen (NH_4^+-N) and nitrate nitrogen ($NO_3^- -N$), 10 g of fresh soil was weighed into a 150 mL flask containing 50 mL of 0.0125 M $CaCl_2$, and was subsequently shaken for 60 min at 30 rpm. Then, the suspension was centrifuged at 2,000 rpm for 2 min, and the supernatant was extracted and stored at 4°C for further measurement based on flow-injection analysis (Skalar San++, Netherlands).

The soil temperature and water content were measured using a soil moisture meter (TDR 350, Spectrum Technologies, Inc. USA) at each stage of wheat growth, in unison with the N_2O flux. The soil pH was determined using an electrode meter (FiveEasy FE20, Switzerland) at a soil-water ratio of 1:2.5 (W: V).

2.6 Wheat sampling and measurements

At the wheat ripening stage 10 samples were collected from each plot to determine yields. The numbers of effective panicle and grains per spike and thousand-grain-weight of harvested wheat were quantified following air drying to calculate the theoretical wheat yields.

Theoretical yield were calculated according to (Wang et al., 2018), using Equation 3:

$$Y = P_n \times G_n \times TKW \times 85\% \times 667 \times 15 \quad (3)$$

where, Y is the theoretical wheat yield ($kg\ ha^{-1}$), P_n is the effective panicle number, G_n is the number of effective panicles, TKW is the thousand kernel weight.

A 250 g sample of the fully matured wheat was weighed for quality analysis (e.g., protein, starch, and wet gluten contents) with a near infrared quality analyzer (Pertent IM9500, Sweden)

2.7 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 19.0 (IBM, Armonk, New York, NY, USA). The means and standard errors were calculated for the N_2O flux and environmental

variables. One-way analysis of variance was used to examine the significance of the measured data. Effects were deemed statistically significant if $p < 0.05$. Linear and nonlinear regressions were applied to test the relationships between the N_2O flux and environmental factors.

3 Results

3.1 Soil temperature and moisture

During the entire growth period of wheat, the soil temperature ranged from 11.2°C to 43.0°C ($25.1^\circ C \pm 1.16^\circ C$). Among them, the average soil temperature of N90, N270, OF90, 20%OF90 + 80%N90, and 20%OF270 + 80%N270 were 22.97 ± 2.77 , 26.55 ± 2.99 , 24.39 ± 2.86 , 27.34 ± 3.18 , $22.73^\circ C \pm 2.70^\circ C$, and $26.85^\circ C \pm 2.93^\circ C$, respectively. However, there were no significant differences in soil temperature under the different fertilization treatments. The soil moisture ranged from 2.43% to 25.93% ($15.95\% \pm 0.86\%$), with the highest value in OF270 ($17.36\% \pm 2.24\%$) and the lowest in 20% OF90 + 80%N90 ($14.66\% \pm 2.14\%$). Similarly, the variation trends in soil moisture under the different fertilization treatments were consistent with that of the soil temperature, and there was no significant difference ($P > 0.05$) (Figure 1)

3.2 Soil physical and chemical properties

The physicochemical properties of wheat field soil were significantly influenced by different fertilization practices (Table 2). Compared with the N90, the total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen ($NO_3^- -N$), pH and soil organic carbon (SOC) contents under the N270 increased by 31.7%, 31.1%, 30.2%, 3.4% and 14.8%, respectively ($P < 0.05$). In contrast to OF90, OF270 exhibited significantly increased SOC, ammonium N, nitrate N, and total N contents, while significantly decreased pH ($P < 0.05$). Compared with OF90, 20%OF90 + 80%N90 showed significantly decreased SOC, but significantly increased ammonium N, nitrate N, and total N contents, and revealed a completely opposite trend compared with N90 ($P < 0.05$). Compared with OF270 and N270, the trend of soil physicochemical properties under the 20%OF270 + 80% N270 treatment was consistent with that under the 20%OF90 + 80%N90 treatment.

3.3 N_2O emission flux

During the wheat growing season, N_2O emissions from the wheat field ranged from 5.33 to 88.12 $\mu g\ m^{-2}\ h^{-1}$ (30.40 ± 2.44), with emissions peaks occurring at the sowing, joining, and grain filling stages. Under various fertilization management practices, the N_2O emissions flux trend showed basic consistency (Figure 2A). Compared with N90, the N_2O emission flux of N270 increased significantly by 50.8% ($P < 0.05$), while there was no significant difference between OF90 and OF270 ($P > 0.05$). Relative to N90, the N_2O emission flux decreased significantly by 27.2% under 20%OF90 + 80%N90, while it was enhanced significantly by 100.9% compared with OF90 ($P < 0.05$). Compared with N270 and OF270, the

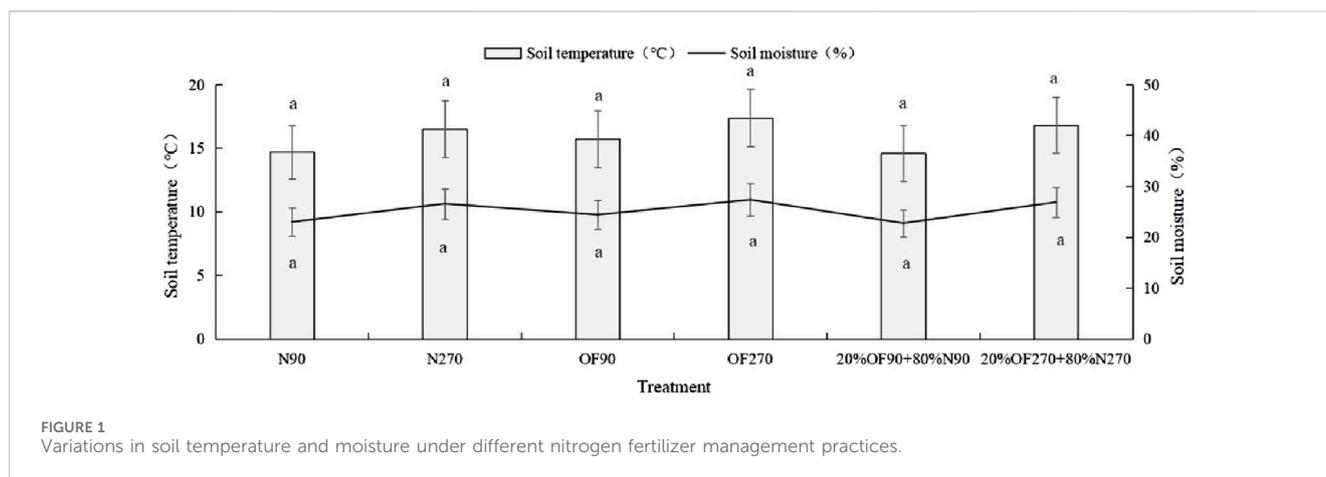


FIGURE 1 Variations in soil temperature and moisture under different nitrogen fertilizer management practices.

TABLE 2 Variations in soil physical and chemical properties under different nitrogen fertilizer management practices.

Treatment	pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)
N90	7.31 ± 0.03 b	10.43 ± 0.28 f	1.45 ± 0.02 c	5.88 ± 0.30 c	25.35 ± 1.01 c
N270	7.56 ± 0.02 a	11.97 ± 0.37 e	1.91 ± 0.02 a	7.71 ± 0.28 a	33.00 ± 1.01 a
OF90	6.25 ± 0.06 d	17.55 ± 0.17 b	0.92 ± 0.02 f	2.76 ± 0.10 f	13.06 ± 0.65 f
OF270	6.04 ± 0.05 e	18.87 ± 0.11 a	1.14 ± 0.03 e	3.83 ± 0.13 e	15.97 ± 0.74 e
20%OF90 + 80%N90	6.34 ± 0.05 d	13.55 ± 0.22 d	1.29 ± 0.02 d	4.96 ± 0.26 d	19.81 ± 0.74 d
20%OF270 + 80%N270	7.03 ± 0.06 c	15.30 ± 0.18 c	1.67 ± 0.02 b	6.68 ± 0.29 b	28.33 ± 2.78 b

variation trend of the N₂O flux under 20%OF270 + 80%N270 was consistent with that of 20%OF90 + 80%N90 (Figure 2B).

Among the experimental plots under the six treatments the cumulative emission fluxes of N₂O were highest in N270 and lowest in OF90, with averages of 2.09 ± 0.18 and 0.48 ± 0.03 kg N ha⁻¹, respectively. Increasing the N fertilizer application rate significantly increased the cumulative N₂O emissions, while increasing the application rate of organic fertilizer had no significant impact. Compared with N90, the cumulative N₂O emissions flux in 20% OF90 + 80%N90 decreased substantially by 27.7%, while that of 20% OF270 + 80%N270 was significantly lower (0.43 kg N ha⁻¹) than N270 ($P < 0.05$) (Figure 2C).

3.4 Wheat yield

The kernel number per spikelet, thousand kernel weight, and grain yields under different N management practices showed obvious variations (Table 3). Compared with N90, the kernel number per spikelet, thousand kernel weight, and yield were significantly enhanced by 12.6%, 7.3%, and 25.7%, respectively, under N270 ($P < 0.05$). The thousand kernel weight and yield increased considerably under a higher organic fertilizer application rate ($P < 0.05$), while there was no significant difference in the kernel number per spikelet ($P > 0.05$). Relative to OF90 and N90, the kernel number per spikelet, thousand kernel weight, and yield were significantly increased under 20% OF90 + 80% N90 ($P < 0.05$). The variation trend of wheat quality under 20%

OF270 + 80% N270 was consistent with that of 20% OF90 + 80% N90.

3.5 Wheat quality

The wet gluten, protein, and starch contents, and settlement values were influenced by the various N management practices (Table 4). As can be seen in Table 4, compared with N90 the protein and starch contents and settlement values increased significantly by 5.8%, 2.4%, and 3.3% under the N270 ($P < 0.05$), while there was no notable difference in the wet gluten content ($P > 0.05$). The wet gluten and starch content and settlement value increased significantly with higher organic fertilizer application rates ($P < 0.05$); however, there was no significant change in the protein content ($P > 0.05$). The wet gluten, protein, and starch contents, and settlement value were significantly enhanced by 7.6%, 4.8%, 8.0%, and 10.4%, respectively, relative to OF90 under 20%OF90 + 80%N90, and increased substantially by 7.7%, 13.8%, 7.9%, and 9.5% ($P < 0.05$), respectively, relative to N90. In contrast to N270, the starch content increased significantly by 2.0% and settlement value decreased considerably by 2.9% under 20%OF270 + 80%N270 ($P < 0.05$), and there was no significant difference in the wet gluten and protein contents ($P > 0.05$). However, the wet gluten, protein, and starch contents, and settlement value under 20% OF270 + 80%N270 decreased significantly relative to OF270 ($P < 0.05$).

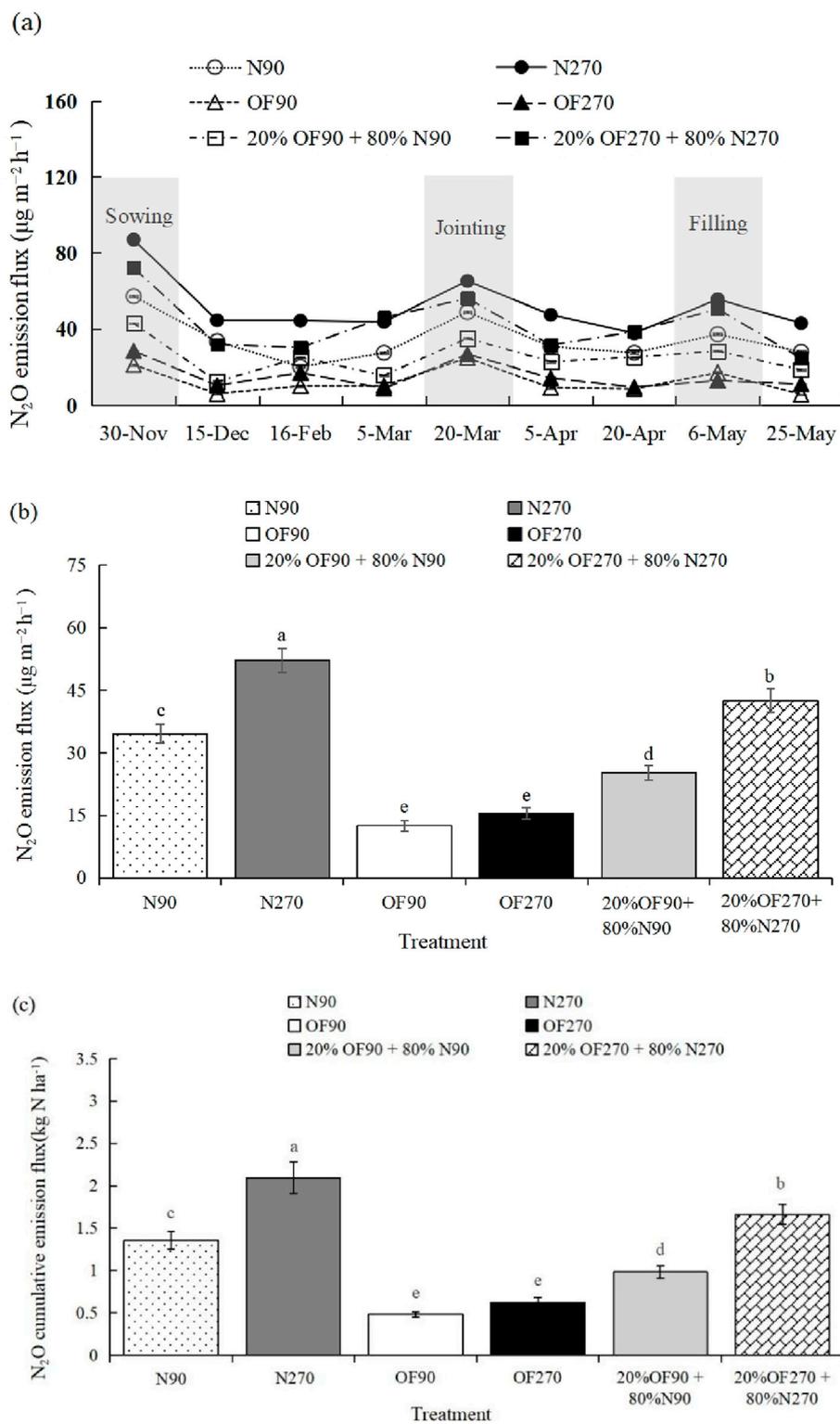


FIGURE 2 Variations of emission flux (A, B) and cumulative emission flux (C) of N_2O in soil under different nitrogen fertilizer management practices.

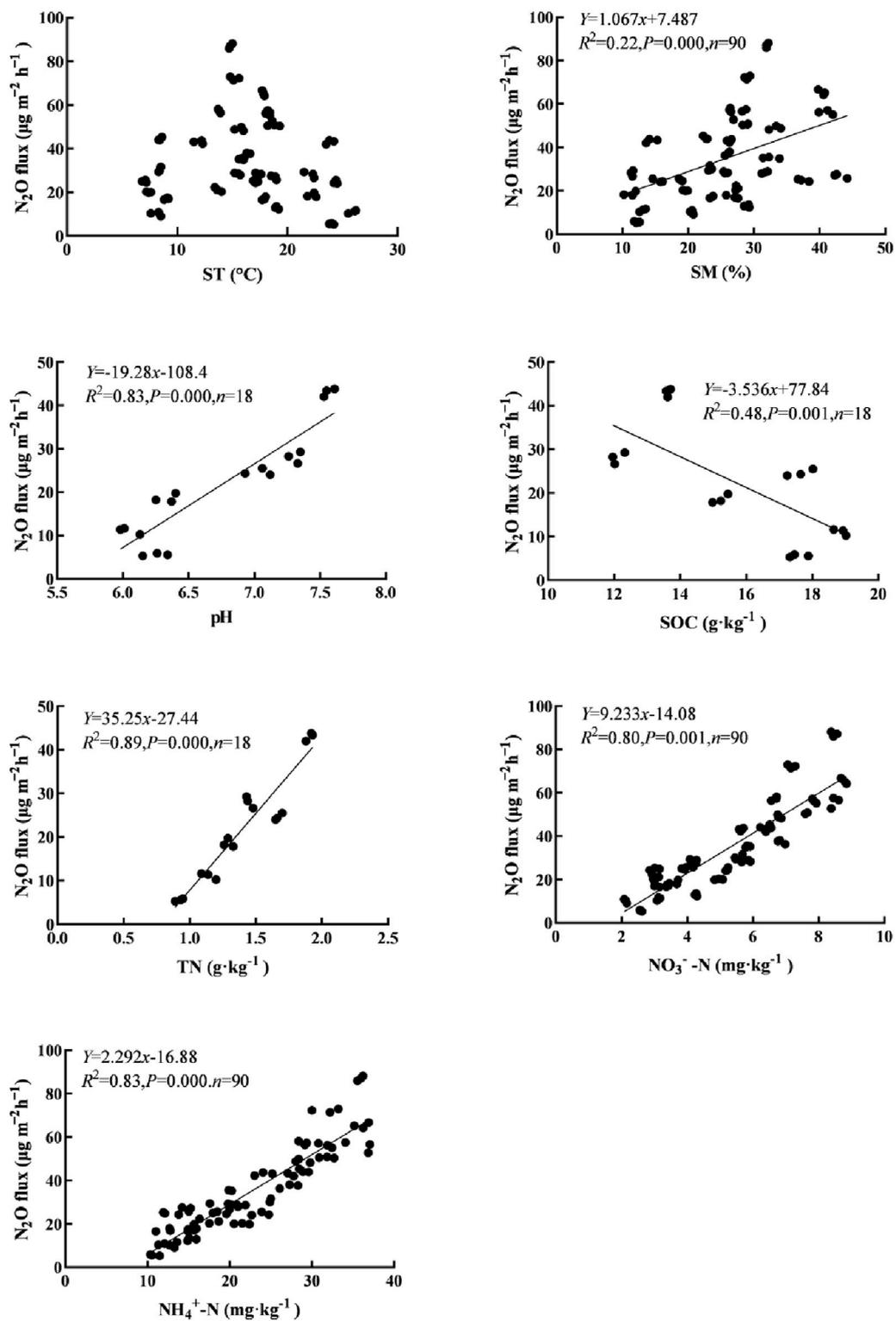


FIGURE 3 Analysis of correlations between nitrous oxide emissions and soil nutrients.

TABLE 3 Variations in grain yields under different nitrogen fertilizer management practices.

Treatment	GNS (grain)	TKW (g)	Spike number per hectare (10 ⁴ stems·hm ⁻²)	Yield (kg hm ⁻²)
N90	33.80 ± 0.29 d	40.37 ± 1.00 de	390.20 ± 1.74 d	4,525.44 ± 54.64 d
N270	38.07 ± 0.09 b	43.33 ± 0.12 b	405.85 ± 0.71 b	5,690.54 ± 21.59 b
OF90	32.87 ± 0.41 d	37.23 ± 0.41 f	396.73 ± 1.81 c	4,126.70 ± 70.05 e
OF270	33.37 ± 0.65 d	39.43 ± 0.60 e	407.60 ± 1.25 b	4,561.78 ± 166.12 d
20% OF90 + 80% N90	36.90 ± 0.12 c	41.46 ± 0.41 c	404.93 ± 2.00 b	5,266.85 ± 73.17 c
20% OF270 + 80% N270	39.73 ± 0.24 a	45.83 ± 0.12 a	414.47 ± 1.75 a	6,415.30 ± 6.15 a

Note: "GNS" represented "the kernel number per spikelet" and "TKW" represented "thousand kernel weight".

TABLE 4 Variations in grain quality under different nitrogen fertilizer management practices.

Treatment	Wet gluten (wet base)%	Protein (dry base)%	Starch (dry base)%	Settlement value Fixed value = 14 mL
N90	36.90 ± 0.45 c	17.87 ± 0.89 d	68.53 ± 0.20 d	67.10 ± 0.15 c
N270	37.20 ± 0.25 bc	18.90 ± 0.06 c	70.20 ± 0.17 c	69.33 ± 0.20 b
OF90	36.93 ± 0.55 c	19.40 ± 0.17 b	68.50 ± 0.23 d	66.57 ± 0.18 c
OF270	38.30 ± 0.15 b	19.63 ± 0.15 b	70.77 ± 0.20 c	68.53 ± 0.37 b
20% OF90 + 80% N90	39.73 ± 0.22 a	20.33 ± 0.18 a	73.97 ± 0.12 a	73.50 ± 0.67 a
20% OF270 + 80% N270	37.00 ± 0.15 c	18.80 ± 0.12 c	71.57 ± 0.19 b	67.30 ± 0.17 c

3.6 Relationships between N₂O emission fluxes and soil nutrients

To investigate the effects of soil nutrients on N₂O emission fluxes in wheat field, multiple statistical analyses were employed to identify the relationships between the soil total N, SOC, NO₃⁻-N, NH₄⁺-N, pH, soil moisture, soil temperature, and N₂O flux (Figure 3). In this study, it was found that the N₂O emission flux from the wheat field was positively correlated with the soil TN ($R^2 = 0.89$, $P < 0.001$), NO₃⁻-N ($R^2 = 0.80$, $P < 0.001$), NH₄⁺-N ($R^2 = 0.83$, $P < 0.001$), soil moisture ($R^2 = 0.22$, $P < 0.001$), and soil pH ($R^2 = 0.83$, $P < 0.001$), while it was negatively correlated with the SOC ($R^2 = 0.48$, $P < 0.01$), and negligibly correlated with the soil moisture and temperature ($P > 0.05$).

4 Discussion

4.1 Effects of different fertilizer management practices on N₂O emissions

Soil N₂O is primarily generated during the transformation of N via nitrification and denitrification processes, which are dominated by microorganisms (Smith, 1997; Ding et al., 2007; Akiyama, et al., 2010). N₂O emissions from wheat fields are controlled by fertilization management practices. In the present study, N₂O emissions under different N management practices exhibited distinct variations, and prominent N₂O peaks were observed at the seedling-jointing-filling stages (Figure 2A), which corresponded

to the findings of Liu et al. (2015) and Lei et al. (2021). During the three key wheat growth stages due to the application of N fertilizer, the soil available N content was increased, which provided rich substrates for nitrification and denitrification, particularly for nitrification, during which ammonia oxidizing bacteria and archaea oxidize ammonia gas that stimulate soil N₂O emissions (Liu et al., 2015; Dong et al., 2018; Zhang et al., 2023). Further, during the seeding to germination stages, wheat grows slowly with a low demand for N, which results in limited N uptake and creates a favorable soil environment for N₂O production (Liu et al., 2015).

In addition, N₂O emissions from wheat fields were intimately related to the application rates and types of N fertilizer (Sainju et al., 2020). Earlier studies revealed that the application rates of N fertilizer had a significant impact on N₂O emissions (Zou et al., 2005; Sun et al., 2017; Hou et al., 2018), with emissions increasing along with N fertilizer application rates from 41% to 229% (Xia et al., 2020). In this study, the NH₄⁺-N and NO₃⁻-N contents in the soil increased by 59% and 70%, respectively, (Table 2), when the N fertilizer application rates were from 90 to 270 kg N ha⁻¹. This increased the reaction substrates for nitrification and denitrification, which promoted N₂O emissions. The N₂O emissions under N270 were significantly higher (by 30%) than those under N90, which also confirmed the conclusions above (Figure 2B). However, compared with the single urea application, the addition of organic fertilizer to N fertilizer significantly reduced the soil N₂O emissions (Miao et al., 2020). In this study, N₂O emissions under 80%N90 + 20%OF90 and 80%N270 + 20%OF270 were significantly lower than N90 and N270 by 20% and 38%, respectively, which aligned with the report of Miao et al. (2020). Under equivalent N fertilization when

organic fertilizers partially replaced N fertilizers, the slower nutrient release rate from organic fertilizers translated to a decrease in the total readily available soil N content. This reduced the substrates for nitrification and denitrification to subsequently decrease the production and release of N₂O (Azam et al., 2002). The application of organic fertilizer increased the exogenous carbon (C) and organic matter contents of the soil, which sequestered the readily available N and promoted the conversion of N₂O to N₂ under denitrification, thereby reducing N₂O emissions (Azam et al., 2002; Miao et al., 2020). Furthermore, increased C sources not only enhanced the effectiveness of soil nutrients and improved the physical soil properties, but also greatly stimulated the activities of soil microbial communities; accelerating their metabolism and increasing the soil microbial C and N contents (Debosz et al., 1999; Jackson et al., 2003; Lu and Han, 2011). However, soil N₂O emissions were negatively correlated with the soil microbial C and N contents (Xie et al., 2015). Consequently, mixing organic fertilizer with N fertilizer can significantly decrease N₂O emissions in wheat fields. However, we found that the organic fertilizer application rate had no significant effect on soil N₂O emissions (Figure 2C), which may have been related to the lower N content of the organic fertilizer.

4.2 Effects of different fertilizer management practices on grain yield and quality

Recently, increased wheat grain production and quality have become one of the primary agronomic goals pursued by many countries, due to rapid population growth and continuous improvements in human living standards (Zhang et al., 2023; De Vita et al., 2007). However, grain yields and the quality of wheat are controlled by genetic traits, environmental conditions, and cultivation practices, particularly the application of N fertilizer (e.g., application rate, method, and timing) (Pierre et al., 2008; Liu et al., 2015; Marinaccio et al., 2016; Tang et al., 2021; Zhang et al., 2023). The suitable application of N fertilizers can effectively improve wheat yields and quality, while excessive N application may have the opposite effect (Zhang et al., 2023).

In this study, the additional application of N fertilizers not only significantly increased the wheat spikelet number by 20.1%, thousand grain weight by 6.8%, and yield by 33.4%, but also substantially increased the protein content by 5.8% and starch content by 2.4%. Compared with N fertilizer alone, a 20% reduction in N combined with organic fertilizer significantly increased the wheat spikelet number, thousand grain weight, and yield. Numerous preceding studies demonstrated increasing trends in grain yields and quality with higher N and organic fertilizer application rates (Liu et al., 2015; Tang et al., 2021; Xiao et al., 2023; Zhang et al., 2023).

Increased N application rates have been shown to significantly impact the soil N content, especially the NO₃⁻-N content (Table 2). The increased availability of soil NO₃⁻-N was beneficial for the development of branches and roots of crops and contributed to the improved crop production (Li et al., 2013). However, a poor soil NO₃⁻-N content can result in inadequate N supplies for the grain filling stage and reduced crop grain yields (Li et al., 2018).

Furthermore, increased N fertilizer application rates significantly enhance the N content of wheat plant organs, improve crop N use efficiencies, and increase crop yields (Ru et al., 2022; Zhang et al., 2023). This may explain why the wheat grain yield of N270 was 33.4% higher than that of N90. The rapid release of N nutrients in applied inorganic N fertilizers can result in insufficient N supplies during the later stages of wheat growth. However, when organic fertilizers are combined with inorganic N fertilizers, the slow-release characteristics of the former help to replenish the soil N content, improve soil fertility, and ensure continuous N supplies during the wheat growing period. This is conducive to wheat grain formation and filling, and promotes high wheat yields (Huang et al., 2021; Yin et al., 2020; Zhang et al., 2023). Additionally, the application of organic fertilizers significantly improves the soil organic matter content, which stimulates the decomposition of nutrients by soil microorganisms and enzymes, leading to a sustained increase in soil available nutrients. This ensures an adequate nutrient supply during the wheat growing period (Liu et al., 2020; Zhang et al., 2023). Consequently, under equivalent N applications, wheat yields under organic fertilizers combined with inorganic fertilizers were significantly higher than that of single N fertilizers.

The N fertilizer application rate had a strong impact on the wheat grain quality (e.g., protein content, wet gluten content and settlement value) (Pierre et al., 2008; Marinaccio et al., 2016; Zhang et al., 2023). The protein contents of crops were mediated by the application of N, where insufficient N supplies prolonged the N transit time, thereby reducing the grain protein content (Gao et al., 2019). Previous studies indicated that the protein, wet gluten, and starch contents, and settlement values of wheat grains increased significantly under the increased application of N (Dai et al., 2019; Tang et al., 2021; Zhang et al., 2023), which was consistent with the results of this study. Further, the organic fertilizer combined with inorganic N fertilizer not only altered the soil N cycling process but also enhanced soil C storage and micronutrients, which ensured the nutrient supply and absorption during wheat grain formation and filling and improved the quality of wheat grains (Ling et al., 2021; Zhang et al., 2023). In the present study, 20%OF90 + 80%N90 significantly increased the wheat grain quality, while 20%OF270 + 80%N270 improved only the wheat starch content, with a tendency to increase the wet gluten and protein contents, albeit not significantly.

4.3 The future study focus of this experiment

Different N management practices were significantly altered temperature and moisture in soil and the content of C and N in wheat fields, which affected N₂O emission, grain yield and quality. Soil N₂O is produced mainly by the microbial processes of nitrification and denitrification (Ding et al., 2007). However, we did not measure the variations of functional genes of N₂O producing microorganisms under different N management practices in this study. In addition, we only emphasized N₂O emissions in wheat fields, but did not consider the N use efficiency of wheat plants under different N management practices. Therefore, we should emphasize the above two aspects to clarify the microbial driving mechanism of N₂O production in wheat fields during N transformation in future studies.

5 Conclusion

The specific objective of this study was to investigate the effects of mixed organic and N fertilizers at different N application rates on the N₂O emissions, grain yields, and quality of a wheat field in China. N₂O emissions under different N management practices showed significant differences. The N fertilizer application rate drastically increased the N₂O emissions in wheat fields. However, the co-application of organic and N fertilizers resulted in a notable 18.6%–27.2% reduction in N₂O emissions. The application rate of organic fertilizer had a negligible effect on the N₂O emissions.

Furthermore, we found that 20% OF90 + 80% N90 treatment significantly increased the wheat grain yield and quality. The starch content also increased significantly, while the settlement value substantially decreased under the N270 treatment application rates, while here was no significant difference in the wet gluten and protein contents. Thus, to mitigate climate change and ensure food security, we suggest adjusting the N fertilizer application rates and adopting the combined application of organic and inorganic fertilizers for wheat production.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

YJ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing–original draft, Writing–review and editing. HC: Data curation, Software,

Writing–original draft. XT: Conceptualization, Writing–original draft. LZ: Conceptualization, Writing–original draft, Data curation. JY: Conceptualization, Software, Writing–original draft. SL: Writing–original draft, Investigation. YC: Investigation, Writing–original draft. XL: Writing–original draft, Writing–review and editing. HW: Writing–original draft, Writing–review and editing. XX: Writing–review and editing, Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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