N$_2$O and NO Emissions as Affected by the Continuous Combined Application of Organic and Mineral N Fertilizer to a Soil on the North China Plain

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A field experiment was conducted to evaluate the influence of the continuous application of organic and mineral N fertilizer on N$_2$O and NO emissions under maize and wheat rotation on the North China Plain. This study included eight treatments: no fertilizer (control); mineral N fertilizer (Nmin) at a rate of 200 kg N ha$^{-1}$ per season; 50% mineral fertilizer N plus 50% cattle manure N (50% CM), 50% chicken manure N (50% FC) or 50% pig manure N (50% FP); 75% mineral fertilizer N plus 25% cattle manure N (25% CM), 25% chicken manure N (25% FC) or 25% pig manure N (25% FP). The annual N$_2$O and NO emissions were 2.71 and 0.39 kg N ha$^{-1}$, respectively, under the Nmin treatment, with an emission factor of 0.50% for N$_2$O and 0.07% for NO. Compared with the Nmin treatment, N$_2$O emissions did not differ when 50% of the mineral N was replaced with manure N (50% CM, 50% FC and 50% FP), while annual NO emissions were significantly reduced by 49.0% and 27.8% under 50% FC and 50% FP, respectively. In contrast, annual N$_2$O emissions decreased by 21–38% compared to the Nmin treatment when 25% of the mineral N was replaced with manure N (50% CM, 50% FC and 50% FP), while annual NO emissions were significantly reduced by 49.0% and 27.8% under 50% FC and 50% FP, respectively. In contrast, annual N$_2$O emissions decreased by 21–38% compared to the Nmin treatment when 25% of the mineral N was replaced with manure N (25% CM, 25% FC and 25% FP). Most of the reduction occurred during the maize season. The 25% CM, 25% FC and 25% FP treatments had no effect on NO emissions compared to the Nmin treatment. There was no obvious difference in annual N$_2$O and NO emissions among the organic manures at the same application rate, probably due to their similar C/N ratio. Replacing a portion of the mineral fertilizer N with organic fertilizer N did not significantly affect crop grain yield, except for the 50% FC treatment in the wheat season. Overall, the results suggest that the combined application of 25% organic manure N plus 75% mineral fertilizer N had the most potential to mitigate N$_2$O emissions while not affecting crop yield in the maize and wheat rotation system in this area of China.

**Keywords:** nitrous oxide; nitric oxide; emission factor; organic manure

1. Introduction

Nitrous oxide (N$_2$O) is a trace and stable greenhouse gas and has a global warming potential (GWP) 298 times higher than CO$_2$ on a centennial scale [1]. As well, N$_2$O contributes to the depletion of stratospheric ozone [2]. The atmospheric N$_2$O concentration reached a new high value of 329.9 ± 0.1 ppb

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in 2017, representing a 122% increase on the pre-industrial (before 1750) levels [3], and it continues to increase at a rate of 0.73 ± 0.01 ppb yr\(^{-1}\) [4]. Nitric oxide (NO) is one of the main sources of air pollution. It is involved in the formation of stratospheric ozone and leads to the formation of photochemical smog and acid rain [1]. Globally, the \(\text{N}_2\text{O}\) and NO emissions from agricultural activities have been estimated at 4.1 and 1.6 Tg \(\text{N}\) yr\(^{-1}\), respectively [5–7], accounting for 60–70% and 10% of global anthropogenic \(\text{N}_2\text{O}\) and NO production, respectively [6]. Over the past 30 years, mineral N fertilizer has played an important role in food security for the growing human population [8]. Poor fertilizer practices have also caused a series of environmental problems, such as the chemical degradation of soil, the contamination of air and water, and large gaseous losses of \(\text{N}_2\text{O}\) and \(\text{NH}_3\) [9,10]. \(\text{N}_2\text{O}\) and NO emissions from agricultural soils are largely caused by N inputs including mineral N fertilizer [11,12]. Therefore, improving agricultural management practices has become a high priority to reduce the negative impacts of agriculture on climate while achieving food security [13].

Organic fertilizers are commonly applied to soil to supply essential plant nutrients and improve soil fertility [14–16]. However, reports of the effects of organic fertilizer application on \(\text{N}_2\text{O}\) and NO emissions have been inconsistent [17,18]. Qiao et al. [19] reported that the combined application of organic fertilizer (composted swine manure) and mineral fertilizer induced a 2.9-fold increase of \(\text{N}_2\text{O}\) emissions compared with mineral fertilizer due to the higher available N and labile organic C for nitrifiers and denitrifiers [20,21]. In contrast, Cai et al. [22] exhibited that, compared with mineral fertilizer, \(\text{N}_2\text{O}\) emissions declined by 22.8% and 41.7%, respectively, under organic fertilizer alone and in combination with mineral fertilizer. Yao et al. [13] also found that the application of organic fertilizer improved fertilizer nitrogen use efficiency (NUE) and decreased \(\text{N}_2\text{O}\) emissions compared to mineral fertilizer in their field study. Interestingly, Pu et al. [23] showed that, compared with mineral fertilizer, a single application of pig manure significantly enhanced \(\text{N}_2\text{O}\) emissions, while its application in combination with mineral fertilizer reduced \(\text{N}_2\text{O}\) emissions. The \(\text{N}_2\text{O}\) emissions of 50% organic fertilizer substitution were obviously lower than those of 25% organic fertilizer application for the same organic fertilizer type, and the \(\text{N}_2\text{O}\) emissions under pig manure amendment were significantly higher than under chicken manure application [24]. Das et al. [25] reported that the \(\text{N}_2\text{O}\) emissions of poultry manure treatment were 24% higher than those of compost treatment when 25% mineral fertilizer N was replaced by organic fertilizer. Differences in N form, chemical composition and application amounts of organic materials have been suggested to result in divergent effects on soil \(\text{N}_2\text{O}\) emissions [20,26–28].

The North China Plain is an area for staple food production in China, accounting for 23% of the country’s total cultivated land [29]. The main cropping system in this region is summer maize and winter wheat rotation, contributing 35.6% and 20.9% of the national maize and wheat supply, respectively. Due to the low soil organic C content, N fertilizer is often overused to meet the demands of crop production [30], leading to low NUE and increased N losses via \(\text{N}_2\text{O}\) and NO emissions [31,32]. Previous work has shown that replacing a portion of the mineral fertilizer with organic fertilizer can reduce N losses and also improve soil fertility [12,19]. Here, a long-term field experiment established in 2010 was used to evaluate the effects of the combined application of different ratios of organic and mineral fertilizers on \(\text{N}_2\text{O}\) and NO emissions and crop yield. It was hypothesized that (i) the combined application of organic and mineral fertilizer would reduce soil \(\text{N}_2\text{O}\) and NO emissions, especially with a higher application ratio of organic fertilizer; (ii) different types of organic fertilizer would have distinct effects on \(\text{N}_2\text{O}\) and NO emissions.

2. Material and Methods

2.1. Study Site and Experimental Design

The field experiment was carried out at the Fengqiu National Station for Agroecological Observation and Research, Henan Province, China (35°00’ N, 114°24’ E). The area on the lower reaches of the Yellow River forms part of the North China Plain. It has a semi-arid, sub-humid monsoon climate with a mean annual temperature of 13.9 °C and precipitation of 615 mm. The soil of the
Experimental site is a fluvo-aquic soil. The traditional cropping system is maize (Zea mays L.) grown in summer rotated with wheat (Triticum aestivum L.) cultivated in winter.

A long-term field experiment had been established in September 2010 to examine the influence of organic fertilizer type and rate on soil organic carbon and greenhouse gas emissions. The study included eight treatments with three replicates: control without N fertilization (control); mineral fertilizer (Nmin); 50% mineral fertilizer N plus 50% cattle manure N (50% CM), 50% chicken manure N (50% FC) or 50% pig manure N (50% FP); 75% mineral fertilizer N plus 25% cattle manure N (25% CM), 25% chicken manure N (25% FC) or 25% pig manure N (25% FP). The plots measured 3.5 × 3.5 m and were arranged based on a randomized block design. For each crop, the application rates of N, P and K were 200 kg N ha⁻¹, 120 kg P₂O₅ ha⁻¹ and 120 kg K₂O ha⁻¹, respectively. N fertilizer was applied twice to each crop at a ratio of basal to supplemental fertilizer of 2:3 for maize and 3:2 for wheat. In the treatments where organic fertilizer replaced 50% of the mineral fertilizer N, all applied basal fertilizer N came from organic fertilizer, while in the treatments where 25% of the mineral fertilizer was replaced with organic fertilizer, half of the basal fertilizer N was derived from organic fertilizer. All organic fertilizer and P and K fertilizer were applied as basal fertilizer. The mineral N, P and K fertilizer used was urea, calcium superphosphate and potassium sulphate, respectively. The detailed application rates of mineral N and organic fertilizers are shown in Table 1. Basal fertilizers were evenly broadcast onto the soil surface by hand and immediately incorporated into the soil by ploughing to a depth of 20 cm. The supplemental fertilizer urea was spread and washed into the soil with flood irrigation (ca. 40 mm) in order to reduce ammonia volatilization. The organic fertilizers were purchased from Shanghai Sennong Environmental Protection Technology Co., Ltd. (Shanghai, China), and their properties are listed in Table 2.

The field measurements for the present study were conducted from June 2018 to May 2019. The basal fertilizers for the maize season were applied on 9 June 2018, and ploughing, sowing and irrigating were completed on the same day. Since the soil was too dry, irrigation (ca. 40 mm) was carried out 3 days before ploughing for the wheat season, and the basal fertilizer application, ploughing and sowing were carried out on 15 October 2018. The supplemental fertilizer urea was applied on

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**Table 1.** Fertilizer nitrogen forms and application rates (kg N ha⁻¹).

|              | **Maize Season** |              | **Wheat Season** |
|--------------|-----------------|--------------|-----------------|
|              | Basal Fertilizer |              | Total           | Basal Fertilizer |              | Total           |
|              | Manure Urea     |              | Manure Urea     |
| Control      | 0 0 0           | 0            | 0               | 0               | 0            | 0               |
| Nmin         | 80 0 120        | 200          | 0               | 120             | 80           | 200             |
| 50% CM       | 40 40 120       | 200          | 60              | 60              | 80           | 200             |
| 25% CM       | 80 0 120        | 200          | 60              | 60              | 80           | 200             |
| 25% FC       | 40 40 120       | 200          | 60              | 60              | 80           | 200             |
| 50% FP       | 80 0 120        | 200          | 60              | 60              | 80           | 200             |
| 25% FP       | 40 40 120       | 200          | 60              | 60              | 80           | 200             |

**Table 2.** Properties of organic fertilizers.

|                | Cattle Manure | Chicken Manure | Pig Manure |
|----------------|--------------|----------------|------------|
| TC (g C kg⁻¹) | 355.64 a     | 327.62 b       | 301.43 c   |
| TN (g N kg⁻¹) | 16.47 a      | 16.90 a        | 13.50 b    |
| C/N           | 21.58 a      | 19.38 a        | 22.32 a    |
| NH₄⁺-N (mg N kg⁻¹) | 15.11 b | 29.63 b       | 61.36 a   |
| NO₃⁻-N (g N kg⁻¹) | 2.68 b | 2.56 b        | 3.44 a    |
| DOC (g C kg⁻¹) | 3.08 a       | 2.33 b         | 3.26 a     |

TC, total organic C; TN, total N; C/N, ratio of total organic C to total N; DOC, dissolved organic C. Values followed by different letters within the same row denote significant differences between organic fertilizers at p < 0.05.
28 July 2018 for the maize and 6 March 2019 for the wheat. The maize and wheat were harvested on 23 September 2018 and 2 June 2019, respectively. Grains and straw were separated manually, dried at 60 °C and weighed to calculate the yield and aboveground biomass.

2.2. Gas Flux Measurement

Soil N\textsubscript{2}O fluxes were measured using the static closed chamber method [33]. During the maize season, cylindrical polyvinyl chloride (PVC) plastic tubes (10 cm long, 10 cm inner diameter) were installed approximately 5 cm into the soil, and one plant was subsequently established in the center of each PVC tube. A stainless steel rectangular chamber base (70 × 30 × 10 cm) with a 5 cm groove around the upper edge was fitted 10 cm into the soil around the above mentioned PVC tube. In order to collect gas samples, a separate PVC pipe (35 cm in height, 10 cm outer diameter) with an airtight rubber seal was placed in the existing PVC tube to exclude the maize plants and avoid the need to raise the chamber height. A stainless steel rectangular chamber (70 × 30 × 30 cm) with a 10 cm diameter center opening (for the PVC pipe) was fitted to the base by inserting the flange of the chamber into the groove of the chamber base, with water in the groove for airtightness. The chamber consisted of two separate parts joined by two hinges and an airtight rubber seal, and it was covered with white plastic foam to minimize the effect of solar heating during the sampling process. The chamber was equipped with a small, silicon-sealed aperture for sampling and another port for measuring chamber temperature. During the wheat season, gas samples were obtained by covering the soil including the wheat plants with a similar chamber (50 × 50 × 50 cm) after inserting a stainless steel base (50 × 50 × 10 cm) with a 5 cm groove around the upper edge. The height of the chamber was increased to 100 cm if the wheat height exceeded 50 cm [34].

Gas fluxes were collected twice per week during the maize season and for part of the wheat season, reducing to weekly or twice monthly in winter. Each sampling was conducted at the same time of day between 08:00 and 11:00 to minimize diurnal variation. Four gas samples were obtained from the chamber using 50 mL syringes at 0, 10, 20, and 30 min after chamber closure, then injected into pre-evacuated 20 mL glass vials fitted with butyl rubber stoppers. The air temperature inside the chamber was simultaneously measured with a thermometer (Glass rod thermometer, Thermometer factory of Wuqiang County, Hebei, China). Gas samples were analyzed using a gas chromatograph (Agilent 7890, Agilent Technologies, Santa Clara, CA, USA) equipped with an electron capture detector. The N\textsubscript{2}O fluxes were calculated from the slope of the linear increase in concentration during the chamber closure period [33].

NO fluxes were also measured using the static chamber method [35]. Approximately 2 L of chamber gas was extracted using a syringe at the beginning and end of chamber closure and stored in Teflon gas bags. The gas was then immediately analyzed using a chemiluminescent NOx analyzer (Model 42i, Thermo Fisher Scientific Inc., Boston, MA, USA).

2.3. Measurement of Environmental, Soil and Organic Fertilizer Variables

Air temperature and precipitation were monitored at a meteorological station in the vicinity of the study field. Soil temperatures at depths of 5, 10 and 15 cm were measured with thermometers (Glass rod thermometer, Thermometer factory of Wuqiang County, Hebei, China). Soil moisture was measured at a depth of 5 cm using a time domain reflectometry (TDR) probe and expressed as water-filled pore space (WFPS, %) using the following equation:

\[
WFPS = \frac{\text{volumetric water content}}{\text{total soil porosity}} \times 100
\]  

(1)

where total soil porosity = 1 − (soil bulk density/2.65), with 2.65 (g cm\textsuperscript{-3}) being the assumed particle density of the soil. Soil bulk density was determined using the intact core method.

After the flux measurements, soil surface (0–20 cm) samples were randomly collected from five different locations in each plot using a 5 cm diameter stainless steel sampler, and then thoroughly
mixed to form a composite sample. Exchangeable NH$_4^+$ and NO$_3^-$ were extracted from the soil and organic fertilizer samples using 2 M KCl solution at a soil and organic fertilizer to solution ratio of 1:10, and measured colorimetrically using a continuous-flow autoanalyzer (San++ System, Skalar Analytical BV, Breda, The Netherlands). The dissolved organic C (DOC) content of the soil and organic fertilizer samples was determined using a TOC analyzer (Vario TOC Cube, Elementar, Hanau, Germany) after mixing 50 mL of deionized water with fresh soil, equivalent to 10 g on an oven-dried basis, shaking for 30 min, centrifuging for 15 min, and filtering through a 0.45-µm polyethersulfone membrane filter.

The soil pH was determined using soil–water suspensions (1:2.5 v/v). The total C content of the soil and organic fertilizer samples was measured by wet digestion with H$_2$SO$_4$-K$_2$Cr$_2$O$_7$ and titration, and total N (TN) in the soil, organic fertilizers and plant material was measured using the Kjeldahl method [36].

2.4. Data Analysis and Statistics

The fluxes of N$_2$O and NO were calculated as follows:

$$ F = \rho \times (V/S) \times (dC/dt) \times (273/(273 + T)) $$

where $F$ is the gas flux ($\mu$g N m$^{-2}$ h$^{-1}$); $\rho$ is the N$_2$O–N density at the standard temperature and pressure (1.25 kg N m$^{-3}$); $V$ is the volume of the chamber (m$^3$); $S$ is the area of the chamber (m$^2$); $dC/dt$ is the change in gas concentration with time ($10^{-9}$ mol mol$^{-1}$ h$^{-1}$); $T$ is the mean temperature inside the chamber during sampling (K).

The cumulative gas emissions ($E$, kg N ha$^{-1}$) were computed according to the following equation:

$$ E = \sum_{i=1}^{n-1} \frac{F_i + F_{i+1}}{2} \times (t_{i+1} - t_i) \times 24 $$

where $F_i$ and $F_{i+1}$ are the N$_2$O or NO fluxes at the $i^{th}$ and $(i+1)^{th}$ measurement time ($\mu$g N m$^{-2}$ h$^{-1}$), respectively; $(t_{i+1} - t_i)$ is the interval between the $i^{th}$ and $(i+1)^{th}$ measurement time (d); $n$ is the total number of measurements.

The N$_2$O or NO emission factor ($EF$, %) of the applied N fertilizer was calculated as follows:

$$ EF = \frac{E_{\text{fertilizer}} - E_{\text{control}}}{N_{\text{applied}}} \times 100 $$

where $E_{\text{fertilizer}}$ and $E_{\text{control}}$ are the cumulative N$_2$O or NO emissions (kg N ha$^{-1}$) from the fertilized and control treatments, respectively; $N_{\text{applied}}$ is the N application rate as urea or manure (200 kg N ha$^{-1}$ for each treatment).

Yield-scaled N$_2$O or NO emission (g N kg$^{-1}$ grain) was calculated as follows:

$$ \text{Yield-scaled emission} = \frac{\text{Cumulative emission}}{\text{crop yield}} $$

where cumulative emission is the cumulative N$_2$O or NO emission (kg N ha$^{-1}$) from each treatment and crop yield is the amount of grain harvested from each treatment (kg ha$^{-1}$).

All data were analyzed using the SPSS software package for Windows (Version 18.0, SPSS Inc., Chicago, IL, USA). Differences in soil properties, cumulative N$_2$O and NO emissions, grain yield and N uptake among treatments were evaluated using one-way ANOVA, followed by least significant difference (LSD) tests at $p < 0.05$. Correlations between the N$_2$O or NO fluxes and environmental factors or soil properties were analyzed using Pearson correlation coefficients at a 0.05 or 0.01 probability level.
3. Results

3.1. Environmental Variables

Annual precipitation was 416.4 mm (Figure 1a), which was lower than the 30-year average of 615 mm. Daily air temperature ranged from −6.2 °C to 32.4 °C with a mean of 14.9 °C, which was slightly higher than the 30-year mean of 13.9 °C. During the maize season, the average air temperature was 26.1 °C with a range of 13.8 °C to 31.7 °C, and average soil temperature at 5 cm depth was 26.8 °C (Figure 1b). Cumulative rainfall was 344.4 mm during the maize season, accounting for 83% of total annual rainfall. The soil moisture varied greatly, from 6.2% to 68.3% WFPS (Figure 1c). During the wheat season, the average air temperature was 9.3 °C, with a range of −6.2 °C to 27.1 °C, and the total precipitation was only 72.0 mm. In December of 2018, the air temperature decreased to below 0 °C, and remained there until early February 2019. Soil WFPS ranged from 5.4% to 70.3%, with an average of 36.4%. Over the experimental period, there were no significant differences in soil temperature or moisture between treatments.

3.2. Soil Variables

The soil organic carbon (SOC) content in the Nmin treatment was 6.84 g C kg\(^{-1}\), and was significantly increased by 37.3%, 16.7% and 27.2% under 25% CM, 25% FC and 25% FP, respectively, in comparison to the Nmin treatment (supplemental material Table S1). These represent an increase rate of 64.3%, 49.9% and 57.6% for 50% CM, 50% FC and 50% FP, respectively.

During the maize season, soil exchangeable NH\(_4^+\) concentrations in all fertilized treatments reached the highest value following supplemental fertilization, and then rapidly decreased to a relatively stable level (<8 mg N kg\(^{-1}\)) (Figure 2a). The soil exchangeable NO\(_3^-\) concentrations in the Nmin treatment reached the highest value 3 days after basal fertilization (Figure 2b), while those
under the treatments with organic fertilizers peaked following supplemental fertilizer application, and fluctuated greatly over the next 20 days, varying from 13.27 to 91.72 mg N kg⁻¹.

![Figure 2](image-url)

**Figure 2.** Temporal dynamics of soil exchangeable NH₄⁺ (a), NO₃⁻ (b), and DOC (c) concentrations in all treatments during the maize and wheat seasons. Vertical bars denote the standard errors of the means (n = 3). The solid and dash arrows indicate the application time of basal and supplemental fertilizer, respectively. The shaded area represents the wheat season.

During the wheat season, the soil exchangeable NH₄⁺ concentrations under the Nmin and 25% organic fertilizer N treatments reached their maximum values of 29.0–76.7 mg N kg⁻¹ within 1 week of basal fertilizer application. In the 50% organic fertilizer N treatments, the peak values of 25.9–39.7 mg N kg⁻¹ appeared after supplemental fertilization. Soil exchangeable NO₃⁻ concentrations fluctuated greatly after basal fertilizer application, except for the control (Figure 2b).

The mean soil DOC concentrations varied from 15.9–46.0 mg C kg⁻¹ during the maize season (Figure 2c). Compared with Nmin, the average DOC concentration under the 50% CM, 50% FC and 50% FP treatments increased by 86.0%, 53.3% and 64.6%, respectively, and by 18.4%, 10.9% and 17.0%, respectively, under the 25% CM, 25% FC and 25% FP treatments. During the wheat season, soil DOC concentrations among all treatments showed similar variation patterns as those in the maize season. The annual average soil DOC concentrations varied from 30.4 to 57.8 mg C kg⁻¹ under the organic fertilizer treatments, which was significantly higher than that under the Nmin treatment (22.2 mg C kg⁻¹).

### 3.3. N₂O Emissions

During the maize season, N₂O flux peaks occurred after basal and supplemental fertilizer application under the Nmin and 25% organic fertilizer N treatments; however, the N₂O flux peaks appeared only after supplemental fertilization in the 50% organic fertilizer N treatments (Figure 3a). The peaks in the 50% organic fertilizer N treatments were significantly higher than those in the other treatments. The highest N₂O flux was 1748 μg N m⁻² h⁻¹ in the 50% FC treatment. N₂O fluxes were significantly correlated with soil WFPS and with soil exchangeable NH₄⁺ in all treatments except the control, but not with soil temperature in the maize season (Table 3).
Figure 3. Dynamic variation of N$_2$O (a) and NO (b) fluxes and ratios of NO/N$_2$O fluxes (c) in all treatments during the maize and wheat seasons. The solid and dash arrows indicate the application time of basal and supplemental fertilizer, respectively. The shaded area represents the wheat season.

Table 3. Correlations between N$_2$O or NO flux and soil temperature at 5 (T$_5$), 10 (T$_{10}$) and 15 (T$_{15}$) cm depth, soil WFPS, exchangeable inorganic N (NH$_4^+$, NO$_3^-$) and dissolved organic C (DOC) during the maize and wheat season.
Table 3. Cont.

| Gas  | Crop | Treatment | T5 | T10 | T15 | WFPS | NH4⁺ | NO₃⁻ | DOC |
|------|------|----------|----|-----|-----|------|------|------|-----|
| NO   | Wheat| Control  | 0.01 | 0.03 | 0.04 | 0.30 * | 0.09 | 0.14 | 0.14 |
|      |      | Nmin     | 0.18 | 0.25 | 0.27 | 0.06 | 0.67 ** | 0.55 ** | 0.32 |
|      |      | 50% CM   | 0.17 | 0.19 | 0.19 | 0.14 | 0.11 | 0.01 | −0.26 |
|      |      | 25% CM   | 0.21 | 0.25 | 0.32 * | 0.09 | 0.63 ** | 0.39 * | −0.01 |
|      |      | 50% FC   | 0.08 | 0.10 | 0.09 | 0.21 | 0.17 | 0.09 | 0.18 |
|      |      | 25% FC   | 0.25 | 0.29 * | 0.34 * | 0.13 | 0.50 ** | 0.57 ** | 0.51 |
|      |      | 50% FP   | 0.20 | 0.26 | 0.27 | 0.12 | 0.11 | 0.22 | −0.24 |
|      |      | 25% FP   | 0.24 | 0.29 * | 0.33 * | 0.10 | 0.66 ** | 0.64 ** | 0.19 |

Asterisks indicate significant correlation at * \( p < 0.05 \) or ** \( p < 0.01 \).

The \( \text{N}_2\text{O} \) fluxes were very low in all treatments during the wheat season (Figure 3a). The highest \( \text{N}_2\text{O} \) flux was 10.3 \( \mu \text{g N m}^{-2} \text{ h}^{-1} \) under the control treatment, and it reached 32.6–41.0 \( \mu \text{g N m}^{-2} \text{ h}^{-1} \) under the 50% organic fertilizer N treatments, significantly higher than those in the 25% organic fertilizer N treatments. \( \text{N}_2\text{O} \) flux was correlated with soil temperature in all treatments during the wheat season, but not with soil WFPS, inorganic N or DOC concentrations (Table 3).

The cumulative \( \text{N}_2\text{O} \) emission during the maize season was 2.28 kg N ha\(^{-1}\) under the Nmin treatment, which was 4.54-fold that of the control (Table 4). Compared with Nmin, the 50% organic fertilizer N treatments did not significantly affect \( \text{N}_2\text{O} \) emissions, but the 25% organic fertilizer N treatments reduced \( \text{N}_2\text{O} \) emissions by 21.8–42.9%. \( \text{N}_2\text{O} \) emissions during the maize season accounted for 71.8–85.1% of the annual emissions. During the wheat season, the cumulative \( \text{N}_2\text{O} \) emission was 0.43 kg N ha\(^{-1}\) under the Nmin treatment, which was similar to those under the 50% organic fertilizer N treatments except for 50% FP. The cumulative \( \text{N}_2\text{O} \) emissions were 0.35–0.37 kg N ha\(^{-1}\) under the 25% organic fertilizer N treatments and were significantly lower than under the Nmin treatment. The annual \( \text{N}_2\text{O} \) emission factor (EF) for the applied N was 0.50% under the Nmin treatment, a value that was close to those under the 50% organic fertilizer N treatments, but which was significantly decreased to 0.24–0.36% under the 25% organic fertilizer N treatment.

3.4. NO Emissions

During the maize season, NO flux peaks were observed after basal and supplemental fertilization under the Nmin and 25% organic fertilizer N treatments, while the peaks occurred after supplemental fertilization under the 50% organic fertilizer N treatments (Figure 3b). The NO fluxes were significantly correlated with soil WFPS and exchangeable NH\(_4^+\) concentrations in the fertilization treatments (Table 3). During the wheat season, the NO flux reached the highest value 1 day after basal fertilization under the 50% organic fertilizer N treatments, and about 1 week after the basal fertilization under the Nmin and 25% organic fertilizer N treatments. No significant differences were observed in NO fluxes between the Nmin and 25% organic fertilizer N treatments, but there were significantly lower values under the 50% organic fertilizer N treatments.

The cumulative NO emission during the maize season was 0.25 kg N ha\(^{-1}\) under the Nmin treatment, which was similar to those under the 25% organic fertilizer N treatments, but was reduced by 46.4% and 21.8% under the 50% FC and 50% FP treatments (Table 4). During the wheat season, the cumulative NO emission under the Nmin treatment was 0.14 kg N ha\(^{-1}\), which was significantly higher than those under the 50% organic fertilizer N treatments. Annual NO emissions under the 50% FC and 50% FP treatments were significantly reduced, by 49.0% and 27.8%, respectively, compared with Nmin, but those under the 25% organic fertilizer N treatments were not affected.
Table 4. Effects of the combined application of organic and mineral N fertilizer on cumulative N$_2$O and NO emissions and the emission factor of applied N as N$_2$O or NO.

| Treatment | Cumulative Emission (kg N ha$^{-1}$) | Emission Factor (%) |
|-----------|-------------------------------------|---------------------|
|           | Maize | Wheat | Annual | Maize | Wheat | Average |
| N$_2$O    |       |       |        |       |       |         |
| Control   | 0.50 ± 0.05 c | 0.20 ± 0.02 d | 0.70 ± 0.04 c | - | - | - |
| Nmin      | 2.28 ± 0.21 a | 0.43 ± 0.01 a | 2.71 ± 0.21 a | 0.89 ± 0.10 a | 0.12 ± 0.00 a | 0.50 ± 0.05a |
| 50% CM    | 2.35 ± 0.30 a | 0.41 ± 0.01 a | 2.76 ± 0.30 a | 0.92 ± 0.15 a | 0.11 ± 0.00 b | 0.51 ± 0.08 a |
| 25% CM    | 1.56 ± 0.19 b | 0.35 ± 0.01 bc | 1.91 ± 0.20 b | 0.53 ± 0.10 b | 0.08 ± 0.00 d | 0.30 ± 0.05 b |
| 50% FC    | 2.31 ± 0.38 a | 0.41 ± 0.01 a | 2.72 ± 0.37 a | 0.91 ± 0.19 a | 0.11 ± 0.00 b | 0.51 ± 0.09 a |
| 25% FC    | 1.30 ± 0.07 b | 0.37 ± 0.01 bc | 1.67 ± 0.07 b | 0.40 ± 0.04 b | 0.08 ± 0.00 cd | 0.24 ± 0.02 b |
| 50% FP    | 2.03 ± 0.32 ab | 0.38 ± 0.00 b | 2.40 ± 0.32 ab | 0.76 ± 0.16 ab | 0.09 ± 0.00 c | 0.43 ± 0.08 ab |
| 25% FP    | 1.78 ± 0.28 b | 0.35 ± 0.00 c | 2.13 ± 0.28 ab | 0.64 ± 0.14 b | 0.08 ± 0.00 d | 0.36 ± 0.07 b |
| NO        |       |       |        |       |       |         |
| Control   | 0.06 ± 0.01 e | 0.04 ± 0.00 d | 0.10 ± 0.01 e | - | - | - |
| Nmin      | 0.25 ± 0.04 bc | 0.14 ± 0.01 a | 0.39 ± 0.06 bc | 0.09 ± 0.02 bc | 0.05 ± 0.00 a | 0.07 ± 0.01 b |
| 50% CM    | 0.41 ± 0.02 a | 0.11 ± 0.01 b | 0.52 ± 0.03 a | 0.17 ± 0.01 a | 0.03 ± 0.01 b | 0.10 ± 0.01 a |
| 25% CM    | 0.29 ± 0.03 b | 0.16 ± 0.01 a | 0.45 ± 0.03 abc | 0.12 ± 0.02 b | 0.06 ± 0.00 a | 0.09 ± 0.01 ab |
| 50% FC    | 0.13 ± 0.02 d | 0.06 ± 0.01 c | 0.19 ± 0.03 d | 0.04 ± 0.01 d | 0.01 ± 0.01 c | 0.02 ± 0.01 c |
| 25% FC    | 0.25 ± 0.02 bc | 0.14 ± 0.01 a | 0.39 ± 0.02 bc | 0.10 ± 0.01 bc | 0.05 ± 0.01 a | 0.07 ± 0.01 b |
| 50% FP    | 0.19 ± 0.01 cd | 0.08 ± 0.00 bc | 0.28 ± 0.01 d | 0.07 ± 0.00 cd | 0.02 ± 0.00 bc | 0.05 ± 0.00 c |
| 25% FP    | 0.32 ± 0.02 b | 0.15 ± 0.01 a | 0.47 ± 0.02 ab | 0.13 ± 0.01 b | 0.06 ± 0.00 a | 0.09 ± 0.00 ab |

Means ± SE (n = 3). Different letters indicate statistically significant differences between treatments within the same column at $p < 0.05$. 
3.5. Ratio of NO/N$_2$O Fluxes

The average NO/N$_2$O flux ratios ranged from 0.18 under Nmin to 0.31 under 25% CM during the maize season. During the wheat season, the average NO/N$_2$O flux ratios ranged from 0.28–0.80; however, they were slightly greater than 1 during the 3 days after basal fertilization under the 50% organic fertilizer N treatments, and during the 2 weeks after basal fertilization under the Nmin and 25% organic fertilizer N treatments (Figure 3c).

3.6. Crop Yield and Yield-Scaled Nitrogenous Gas Emission

Maize grain yield under the Nmin treatment was 10,337 kg ha$^{-1}$, which was similar to those under the organic fertilizer treatments (Figure 4a). Replacing a portion of the mineral fertilizer N with organic fertilizer also did not significantly affect wheat grain yield, except for the 50% FC treatment (Figure 4c).

![Figure 4](image_url)

Figure 4. Crop biomass and amount of N uptake as affected by organic and mineral fertilization during the maize (a,b) and wheat (c,d) seasons. Vertical bars denote the standard errors of the means (n = 3). Different capital and lowercase letters indicate significant differences in the yield and N uptake amount of crop grain between treatments and in the weight and N uptake amount of crop straw between treatments at $p < 0.05$, respectively.

Compared with Nmin, the 50% organic fertilizer N treatments had no significant effect on the yield-scaled N$_2$O emissions (Table 5). In contrast, the 25% organic fertilizer N treatments reduced yield-scaled N$_2$O emissions by 19.9–35.1% compared with Nmin. Unlike the yield-scaled N$_2$O emissions, the 25% organic fertilizer N treatments had no significant effect on yield-scaled NO emissions compared with Nmin. In the 50% FC and 50% FP treatments, yield-scaled NO emissions were decreased by 42.3% and 30.4%, respectively.
Table 5. Effects of the combined application of organic and mineral N fertilizer on yield-scaled N$_2$O and NO emissions (g N kg$^{-1}$ grain) during the maize and wheat seasons.

| Treatment | Maize $N_2$O | Maize NO | Wheat $N_2$O | Wheat NO | Total $N_2$O | Total NO |
|-----------|--------------|----------|---------------|----------|--------------|----------|
| Control   | 0.125 ± 0.012 b | 0.015 ± 0.003 e | 0.146 ± 0.013 a | 0.026 ± 0.003 a | 0.130 ± 0.007 ab | 0.018 ± 0.003 cd |
| Nmin      | 0.221 ± 0.020 a | 0.024 ± 0.004 cd | 0.056 ± 0.001 bc | 0.018 ± 0.001 bc | 0.151 ± 0.012 a | 0.021 ± 0.003 b |
| 50% CM    | 0.226 ± 0.028 a | 0.039 ± 0.002 a | 0.058 ± 0.001 bc | 0.015 ± 0.002 c | 0.159 ± 0.017 a | 0.030 ± 0.002 a |
| 25% CM    | 0.149 ± 0.018 b | 0.028 ± 0.003 bc | 0.048 ± 0.001 c | 0.021 ± 0.001 b | 0.107 ± 0.011 b | 0.025 ± 0.002 ab |
| 50% FC    | 0.236 ± 0.039 a | 0.014 ± 0.003 e | 0.066 ± 0.001 b | 0.010 ± 0.002 d | 0.170 ± 0.023 a | 0.012 ± 0.002 e |
| 25% FC    | 0.134 ± 0.007 b | 0.026 ± 0.002 bc | 0.050 ± 0.001 c | 0.019 ± 0.002 bc | 0.098 ± 0.004 b | 0.023 ± 0.001 bc |
| 50% FP    | 0.184 ± 0.029 ab | 0.018 ± 0.001 de | 0.050 ± 0.000 c | 0.011 ± 0.000 cd | 0.129 ± 0.002 ab | 0.015 ± 0.001 de |
| 25% FP    | 0.177 ± 0.028 ab | 0.032 ± 0.002 b | 0.046 ± 0.000 c | 0.020 ± 0.001 b | 0.121 ± 0.016 b | 0.026 ± 0.001 ab |

Means ± SE (n = 3). Different letters indicate statistically significant differences between treatments within the same column at $p < 0.05$. 
4. Discussion

4.1. Effect of Proportion of Organic Manures on \( \text{N}_2\text{O} \) and NO Emissions

There are contradictory findings about the effect of organic fertilizers on \( \text{N}_2\text{O} \) emissions: both stimulation [37,38] and suppression [39,40] have been reported. For example, the application of soybean cake fertilizer in a subtropical tea plantation significantly increased \( \text{N}_2\text{O} \) emissions, and in contrast, livestock manures exhibited a suppressive effect compared with mineral fertilizer [41]. It has been suggested that soybean cake N could remain in the soil longer than urea N, potentially due to lower losses via NH\(_3\) volatilization, runoff, and leaching through the sustained release of inorganic N during mineralization, thereby increasing soil exchangeable NH\(_4^+ \) and NO\(_3^- \) availability for \( \text{N}_2\text{O} \) production [42,43]. Unexpectedly, in the current study, replacing 50% of the mineral fertilizer with organic fertilizer did not affect annual \( \text{N}_2\text{O} \) emissions significantly compared with Nmin. Under the 50% organic fertilizer N treatments, \( \text{N}_2\text{O} \) flux peaks after amendment with organic manures were lower compared with Nmin. He et al. [41] pointed out that the ratios between the soil \( \text{N}_2\text{O} \) emissions from organic fertilizer application and those from mineral fertilizer N application were correlated with the C/N ratios of the organic fertilizers, and the threshold value for the organic fertilizer C/N ratio, above which responses of soil \( \text{N}_2\text{O} \) emissions change from positive to negative, was approximately 8.6. The C/N ratios in the organic manures in the current study were higher than this threshold value, resulting in a negative response of soil \( \text{N}_2\text{O} \) emissions to organic fertilizer. It has been reported that a C/N ratio of 41 in organic fertilizers was the break-even point between net N immobilization and the mineralization of organic materials after incorporation into soils [44]. In our study, the organic manures showed net N release; however, this was less than from urea, therefore reducing the overall availability of N for nitrifiers and denitrifiers and \( \text{N}_2\text{O} \) production after basal fertilization compared to the Nmin treatment.

Interestingly, \( \text{N}_2\text{O} \) flux peaks after the application of supplemental fertilizer urea were significantly higher under the 50% organic fertilizer N treatments than under Nmin. This indicated that supplemental urea N was more efficiently converted into \( \text{N}_2\text{O} \) in the 50% organic fertilizer plots than in the Nmin plots, although the rate was identical. Thus, the fact that there was no apparent response of total soil \( \text{N}_2\text{O} \) emissions is probably because the reduced \( \text{N}_2\text{O} \) emissions after basal organic fertilizer application were exactly offset by increased \( \text{N}_2\text{O} \) emissions after the application of supplemental fertilizer urea in the 50% organic fertilizer N treatments.

Ding et al. [45] and Niu et al. [34] reported that \( \text{N}_2\text{O} \) was mainly produced by nitrification, and denitrification was limited by carbon availability in a nearby maize field soil with a light texture [46]. In contrast, a NO/\( \text{N}_2\text{O} \) flux ratio of less than one, especially during the maize season, suggested that \( \text{N}_2\text{O} \) was predominantly sourced from denitrification in the test soil with a heavy texture [47]. In the current study, soil exchangeable NO\(_3^- \) concentrations after supplemental fertilization across all fertilization treatments were higher than the threshold for denitrification of 5 mg N kg\(^{-1} \) [48], indicating that NO\(_3^- \) was not a key factor limiting \( \text{N}_2\text{O} \) production. It has been suggested that organic fertilizers increase \( \text{N}_2\text{O} \) emissions by providing more labile C for denitrification in the presence of high levels of N fertilizer [49–51]. In the current study, it was found that the 50% organic fertilizer N treatments increased soil DOC by 53-86% compared with Nmin in the maize season. It is likely that the increased DOC concentrations stimulated soil respiration, which in turn increased the occurrence of anaerobic microenvironments that favored the production of \( \text{N}_2\text{O} \) via denitrification [52].

In contrast, it was found that the 25% organic fertilizer N treatments did reduce \( \text{N}_2\text{O} \) emissions by 21–38% compared with Nmin. Most of the reduction occurred during the maize season and \( \text{N}_2\text{O} \) emissions accounted for 71.8-85.1% of the annual emissions, while the low \( \text{N}_2\text{O} \) emissions in the wheat season was primarily due to low temperature [33]. This suggests that, as well as C/N ratios, the application rate also affected the responses of soil \( \text{N}_2\text{O} \) emissions to organic fertilizers in the test soil. In contrast to the 50% organic fertilizer N treatments, the 25% organic fertilizer N treatments increased soil DOC concentration in the maize season by only 11–18% compared with Nmin. Chen et al. [54]...
reported that N$_2$O fluxes were not only correlated with NO$_3^-$ and DOC concentrations but also with the NO$_3^-$/DOC ratios. In this study, the highest N$_2$O emissions during the maize season occurred at a NO$_3^-$/DOC ratio of 0.48 mg N mg$^{-1}$ C, while the NO$_3^-$/DOC ratio was 0.59–0.63 under the 25% organic fertilizer N treatments. Thus, it was very likely that a higher ratio of NO$_3^-$ to available C, i.e., fewer electron donors, results in a less anaerobic environment for denitrification [22,38].

In this study, annual NO emissions were 0.19-0.52 kg N ha$^{-1}$ in the fertilization treatments, and the annual NO emission factor of the applied N was 0.07% under the Nmin treatment, which was at the low end of the range of 0.04-0.67% found in cropland in China [55]. According to a conceptual model proposed by Davidson [56], both nitrification and denitrification could occur. However, as mentioned above, denitrification was the dominant process here, thus NO emissions from nitrification might be quite low [57]. In addition, the high clay content in the test soil might prevent NO release into the atmosphere. Compared with Nmin, the annual NO emissions under 50% FC and 50% FP were reduced by 49.0% and 27.8%, respectively, mainly due to a lower net N release from organic manures [24,58]. It is also likely that the dominant denitrification process in the 50% organic fertilizer treatments reduced NO to N$_2$O and N$_2$, thereby decreasing NO emissions [37,38,59]. Unexpectedly, the 50% CM treatment stimulated NO emissions compared with Nmin. It was found that the 50% CM treatment had the highest SOC and lowest soil bulk density (Table 2). Previous studies have shown that increased SOC can synchronically stimulate the development of both micropores (<4 µm pores) [60] and macropores [61,62], and increased macropores might favor the release of NO into the atmosphere.

4.2. Effects of Organic Fertilizer Type on N$_2$O and NO Emissions

Previous studies have suggested that differences in N form and content in organic fertilizers can affect the responses of soil N$_2$O emissions [20,28]. Although the total nitrogen content in the pelleted manures was the same as in the other treatments, Hayakawa et al. [38] observed higher N$_2$O emissions in a pelleted poultry manure treatment and they attributed this to increased denitrification inside the pelleted manure. In the present study, no significant difference was found in annual N$_2$O emissions among the three organic manures, which were applied at the same N rate although the inorganic N content in the pig manure (FP) was significantly higher. Chen et al. [24] also reported that pig manure application more effectively stimulated N$_2$O emissions compared with chicken manure, despite the fact that the latter had a relatively larger inorganic N content. These findings indicated that the N form and content in organic fertilizers were not the primary controlling factors for N$_2$O and NO emissions.

5. Conclusions

This study provided important insights into the effects of the types and application rates of organic fertilizers on N$_2$O and NO emissions in a clay loam soil. The mean flux ratios of NO/N$_2$O were lower than 1, suggesting that denitrification was the dominant process for N$_2$O production. Compared with Nmin, the 50% organic fertilizer N treatments did not affect annual N$_2$O emissions, but the 50% FC and 50% FP treatments significantly reduced annual NO emissions by 49.0% and 27.8%, respectively. In contrast, annual N$_2$O emissions, but not NO emissions, were reduced by 21–38% in the 25% organic fertilizer N treatments compared with Nmin. There was no obvious difference in N$_2$O and NO emissions among the three organic manures applied at the same rate, probably because they had similar C/N ratios. These findings suggest that the combined application of 25% organic manure N plus 75% mineral fertilizer N could effectively mitigate N$_2$O emissions while not affecting crop yield in maize–wheat rotation systems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1965/s1, Table S1: Soil properties as affected by 8 years of continuous combined application of organic and mineral N fertilizer in June 2018.
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