Swine Manure Reduces Nitrous Oxide Emissions from Acidic Red Soil Due to Mineral N Immobilization and Alleviated Acidification

Lu Zhang 1,2,3, Tusheng Ren 3 Ø, Jiwen Li 1, Kiya Adare 1 Ø, Nano Alemu Daba 1 Ø, Md Ashraful Alam 1, Shilin Wen 1,2 and Huimin Zhang 1,2, *

1 State Key Laboratory of Efficient Utilization of Arid and Semi-Arid Arable Land in Northern China, The Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China; zhanglu01@caas.cn (L.Z.); frankie716@163.com (J.L.); kiyaadare2006@gmail.com (K.A.); nanoalemu2001@gmail.com (N.A.D.); ashriful_bd22@yahoo.com (M.A.A.); wenshilin@caas.cn (S.W.)
2 Qiyang Farmland Ecosystem National Observation and Research Station, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Qiyang 426182, China
3 College of Land Science and Technology, China Agricultural University, Beijing 100193, China; tsren@cau.edu.cn
* Correspondence: zhanghuimin@caas.cn

Abstract: Swine manure is widely used for ameliorating red soil acidification, but little information is available about its effect on N2O emissions. To explore the effects, a 35-day incubation experiment was conducted with two soils under different fertilization history: chemical fertilizers only (F) and combination of chemical fertilizers with swine manure (M). The treatments included no fertilizer (control), 100% N from urea (M0), and urea plus swine manure, which supplied 20% (M20), 40% (M40), 60% (M60), and 100% (M100) of total N. Soil N2O emission rates, pH, exchangeable acidity, mineral N species, dissolved organic carbon and nitrogen, microbial biomass carbon, and their inner relationships were examined. The N2O emission rates markedly increased following the treatments, reached peaks before day 2, and thereafter decreased sharply to the level of the control by day 25, 25, 23, 15, and 9 in F soil and by day 25, 25, 23, 19, and 11 in M soil for M0, M20, M40, M60, and M100 treatments, respectively. As swine manure application rate increased, the cumulative N2O emissions of F soil decreased significantly, while, for M soil, there was no significant difference among M0, M20, M40, and M60 treatments, which were higher than the M100 treatment. At the end of incubation, soil pH in F and M soils followed the order M0 < M20 < M40 < M60 < control < M100 and vice versa for exchangeable Al3+ and acidity. F soil had relatively higher NH4+-N concentration in M0 treatment and higher NO3−-N concentrations in M0 and M20 treatments than M soil. Soil pH and NH4+-N had the greatest relative contribution to N2O emissions. Overall, this study indicates that partial chemical N replacement by swine manure could effectively mitigate N2O emissions from acidic red soil primarily because of mineral N immobilization and alleviated red soil acidification. Thus, swine manure has the potential to co-ameliorate red soil acidification and N2O emission. Further research is needed to determine the effect of swine manure on N2O emission reductions under field conditions and the overall benefit in effective N management.

Keywords: chemical fertilization; manure; soil pH; nitrous oxide; nitrification

1. Introduction

Nitrous oxide (N2O), as a potent greenhouse gas, has received great attention due to its atmospheric longevity (about 120 years) and global warming potential (273 times that of CO2 on a per-molecule basis over a 100-year period) [1]. The main source of anthropogenic N2O is agriculture, accounting for 60% of the global N2O emissions due to overuse of nitrogen fertilizers for food production [2]. Acidic soil comprises approximately one-third of the global ice-free land area, and it is the hotspot of global N2O emissions [3,4]. In China,
most acidic soil is distributed in the red soil region. Overuse of chemical nitrogen fertilizer has intensified red soil acidification, which seriously limits crop growth in the region [5]. Swine manure rich in alkalinity is widely used for preventing red soil acidification, in addition to introducing a large amount of carbon and other nutrients [6–8]. Swine manure’s effect on red soil \(\text{N}_2\text{O}\) emissions is not fully understand.

\(\text{N}_2\text{O}\) is produced primarily via microbial nitrification and denitrification processes. Swine manure is rich in easily degradable organic carbon, such as soluble organic carbon and volatile fatty acids, which could effectively alleviate the denitrification inhibition caused by the insufficient supply of carbon substrates, which is conducive to promoting \(\text{N}_2\text{O}\) production [9,10]. The input of unstable organic materials accompanied with manure could create a more anoxic soil environment and favor \(\text{N}_2\text{O}\) production from the denitrification process [11]. In addition, soil pH is recognized as an important factor influencing \(\text{N}_2\text{O}\) production by altering microbial-driven N-cycling processes [12–14]. For example, Cheng et al. [15] found that organic fertilizer increased acidic red soil \(\text{N}_2\text{O}\) emissions due to the soil pH elevation, which reduced the relative proportion of \(\text{N}_2\text{O}\) emissions caused by heterotrophic nitrification, but this might be masked by the increase in overall \(\text{N}_2\text{O}\) emissions stimulated by carbon input. The effect of pH stimulation on the rate of autotrophic nitrification and \(\text{NO}_3^-\) accumulation, as well as the increase in carbon availability, further facilitated the denitrification process [16].

However, opposite results have been obtained regarding the effect of organic fertilizer application on \(\text{N}_2\text{O}\) emissions. Li et al. [17] showed that, compared with chemical fertilizer, organic fertilizer amendment could significantly reduce \(\text{N}_2\text{O}\) emissions. The possible reasons are that the soil \(\text{N}_2\text{O}\) emissions were mainly from the chemical fertilizer nitrogen, rather than the mineralization and transformation of organic matter, while the available nitrogen in the soil after the addition of organic fertilizer was lower than that after inorganic fertilizer treatments [18,19]. Thus, the addition of organic N relative to chemical N could slow the mineralization of soil N for nitrification and denitrification; thus, it had the potential to depress the N losses of \(\text{N}_2\text{O}\). Considerable field investigations have shown that decreasing the amount of chemical fertilizer input and replacing it with organic fertilizer (e.g., manure) could substantially reduce soil \(\text{N}_2\text{O}\) emissions [17–19]. However, the measurements affected by partial chemical N replacement by manure were not consistent due to substitution rates and soil properties.

The contributions of various combinations of swine manure-N with urea-N and of soil improvement from manure amendment to \(\text{N}_2\text{O}\) emissions are not fully clear. The purpose of this study was to determine the potential effects of swine manure as amendment for alleviating red soil acidification on \(\text{N}_2\text{O}\) emissions and N transformation processes in comparison with chemical fertilizers only. Therefore, a 35-day incubation experiment was designed to investigate the effect of the proportion gradients of different swine manure to replace chemical nitrogen fertilizer on the acidity of red soil and \(\text{N}_2\text{O}\) emissions. Our hypothesis was that the increase in swine manure substitution ratio could effectively mitigate red soil acidification and reduce \(\text{N}_2\text{O}\) emissions.

2. Materials and Methods

2.1. Experimental Materials

Soils (0–20 cm) used in the study were collected in February 2022 from two different fertilization plots of a long-term (13 years) field experiment located at the Red Soil Experimental Station (26°45′12″ N, 111°52′32″ E, with an altitude of 120 m above sea level) of the Chinese Academy of Agricultural Sciences, Qiyang City, Hunan Province, China. The field experiment was conducted in September 2009, and the cropping system was single spring maize. The fertilization treatments included chemical N, P, and K fertilizers without manure (F), and chemical NPK plus swine manure at 60% of the total N supply (M). The total N application rate was 225 kg N ha \(^{-1}\) year \(^{-1}\). Urea (\(\text{CH}_4\text{N}_2\text{O}\)) (46.6% N) was used as the chemical N fertilizer source. Superphosphate (\(\text{Ca(H}_2\text{PO}_4\text{)}_2\)) (12.5% \(\text{P}_2\text{O}_5\)) and potassium chloride (KCl) (60.0% K\(_2\)O) were applied at 33 kg P ha \(^{-1}\) year \(^{-1}\) and 62 kg K.
ha$^{-1}$ year$^{-1}$ to both treatments. All fertilizers were applied to the soil together. Lime (CaO) (71.5% Ca) was applied at rate of 1500 kg ha$^{-1}$ for F treatment at the end of 2019 due to severe red soil acidification from chemical fertilizers. The study area is a subtropical humid monsoon climate, and the details can be found in Cai et al. [8]. The soil is Ferralic Cambisol according to the FAO or World Soil Classification and derived from quaternary red earth. The soil texture is clay, and the main clay mineral is kaolin. Surface soils (0–20 cm) were collected, air-dried, ground, and sieved to pass through a 2.0-mm sieve for incubation experiment.

The swine manure used for incubation experiment was collected from local farmyards and air-dried. For achieving uniform treatment, the swine manure was ground, sieved to pass through a 2.0-mm sieve, and thoroughly mixed before incubation experiment. Selected chemical properties of the soils and the swine manure are shown in Table 1.

2.2. Experimental Design and Incubation Procedure

Two soils (F and M) were respectively set up with 6 treatments: (1) no fertilizer (Control), (2) 100% chemical fertilizer nitrogen (M0), (3) 20% N from manure and 80% N from chemical N (M20), (4) 40% N from manure and 60% N from chemical N (M40), (5) 60% N from manure and 40% N from chemical N (M60), and (6) 100% N from manure (M100). An amount of air-dried soil equivalent to 1.5 kg of oven-dried soil was weighed in a sterilized glass bottle (2500 mL volume capacity) for 36 duplicates. These soil samples were moistened to 60% water holding capacity by DI water. The bottles were pre-incubated in a constant-temperature and -humidity incubator (HSP-350B Kuntian, Shanghai, China) at 25 ± 1 °C for 21 days to recover microbial activity. The urea and manure were added to reach the final treatment rates, respectively. The soil, urea, and manure were thoroughly mixed, and the soil water content was adjusted to 20% ($w/w$), equivalent to 80% of water holding capacity (25%, $w/w$). Then, the bottles continued to incubate for 35 days. Each bottle was sealed with PM996 sealing film and was equipped with 30 small pinholes to reduce water loss during gas exchange. During the incubation, the weight of each bottle was recorded every 2 or 3 days to calculate the water lost through evaporation, and DI water was added to soil.

2.3. Gas Sampling and Analysis

Gas samples were collected on days 0, 0.25, 1, 2, 3, 4, 5, 6, 7, 9, 11, 15, 17, 19, 21, 23, 25, 28, 30, and 35 after adding fertilizers. Prior to gas sampling, the sealing film was removed so that it was in equilibrium with the surrounding air for 30 min, and then the bottle was sealed with a rubber cork with a glass tube (diameter 4.6 mm) at a 120° angle at the top, which connected to a rubber tube and a three-way valve for gas sampling. The gas samples were collected in 12.5 mL vacuum glass bottles with a 30 mL syringe at 0, 1, and 2 h after sealing. The concentrations of N$_2$O were determined using a meteorological chromatograph (Agilent Technologies 7890A, Inc., Santa Clara, CA, USA). The N$_2$O emission rate was calculated by the following formula [20]:

$$ Fl = \rho \times V/W \times \Delta c/\Delta t \times 273/(T + 273) $$

(1)

$Fl$ is the N$_2$O emission rate ($\mu g$ kg$^{-1}$ h$^{-1}$), $\rho$ is the N$_2$O density under the standard condition (kg m$^{-3}$), $V$ is the volume of gas in the incubation bottle (m$^3$), $W$ is the mass of drying soil in the incubation bottle (kg), $\Delta c$ is the change in N$_2$O concentration during the sealing time (ppm), $\Delta t$ is the sealing time (h), and $T$ is the incubation temperature (25 °C).

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

(2)

$E$ denotes the cumulative N$_2$O emissions ($\mu g$ kg$^{-1}$), and $F_{t}$ and $F_{t+1}$ are the N$_2$O emission rates at time $t_{t}$ and $t_{t+1}$, respectively.
Table 1. Selected chemical properties of the soils and the swine manure.

| Materials | pH | NH$_4^+$-N (mg kg$^{-1}$) | NO$_3^-$-N (mg kg$^{-1}$) | AN (mg kg$^{-1}$) | DON (mg kg$^{-1}$) | TN (g kg$^{-1}$) | DOC (mg kg$^{-1}$) | TOC (g kg$^{-1}$) | C/N | MBC (mg kg$^{-1}$) | AP (mg kg$^{-1}$) | TP (g kg$^{-1}$) | AK (mg kg$^{-1}$) | TK (g kg$^{-1}$) | BD (g cm$^{-3}$) |
|-----------|----|---------------------------|--------------------------|------------------|------------------|----------------|----------------|----------------|-----|----------------|----------------|-------------|----------------|---------------|---------------|--------------|
| F Soil    | 5.60 | 2.22 | 9.27 | 68.48 | 26.80 | 0.94 | 109.94 | 8.20 | 8.72 | 116.43 | 14.26 | 0.91 | 121.76 | 13.30 | 1.26 |
| M Soil    | 5.67 | 2.88 | 9.78 | 74.91 | 23.70 | 0.96 | 97.06 | 9.41 | 9.80 | 81.47 | 117.35 | 2.10 | 225.69 | 13.40 | 1.40 |

NH$_4^+$-N, ammonium nitrogen; NO$_3^-$-N, nitrate nitrogen; AN, alkali-hydrolyzable nitrogen; DON, dissolved organic nitrogen; TN, total nitrogen; DOC, dissolved organic carbon; TOC, total organic carbon; MBC, microbial biomass carbon; AP, available phosphorus; TP, total phosphorus; AK, available potassium; TK, total potassium; BD, bulk density.
2.4. Soil Sampling and Analysis

Soil samples were collected on days 0, 0.25, 1, 3, 5, 7, 11, 15, 21, 28, and 35 after fertilizer addition. The soil in the incubation bottle was thoroughly mixed with a long-handled stainless-steel weighing spoon before sampling, and then 50 g of soil was taken for soil property analysis. Soil ammonium nitrogen (NH$_4^+$-N) and nitrate nitrogen (NO$_3^-$-N) were extracted with 2 mol L$^{-1}$ KCl and analyzed by a flow injection analyzer (SEAL Auto Analyzer AA3, Norderstedt, Germany) [21]. Soil microbial biomass carbon (MBC) was estimated using the chloroform–fumigation–extraction method [22]. Soil dissolved organic carbon (DOC) and nitrogen (DON) were extracted with 0.5 mol L$^{-1}$ K$_2$SO$_4$ solution [23]. MBC, DOC, and DON were analyzed by a total organic carbon analyzer (multi N/C 3100 Analitick Jena, Germany). Soil pH was determined by a pH meter (FE28, Mettler, Toledo, OH, USA) with a mixture of soil and water at a ratio of 1:2.5 (soil-to-water ratio) [24]. For total exchangeable acidity (Al$^{3+}$ and H$^+$), soils were extracted with 1 mol L$^{-1}$ KCl, and then titrated with 0.02 mol L$^{-1}$ NaOH to phenolphthalein endpoint [25].

2.5. Data Analysis

One-way analysis of variance (ANOVA) and the independent-sample Duncan test were conducted to compare differences in mean N$_2$O emissions and soil properties among different treatments at a significant level of 0.05 by SPSS v. 19.0 (IBM, Armonk, NY, USA). Pearson correlation analysis was used to calculate correlation coefficients between N$_2$O emissions and soil properties by Origin 2023 (OriginLab, Northampton, MA, USA). All figures and tables were created using SigmaPlot 10.0 and Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). All the data are presented as the mean plus or minus standard error.

3. Results

3.1. N$_2$O Emission

There were no significant changes in N$_2$O emissions from no fertilizer (control) of the F and M soils throughout the whole incubation period. However, the N$_2$O emission rates of F soil were markedly increased following fertilization and reached the peaks by day 0.25 for M40, M60, and M100 treatments, and by day 1 and day 2 for M20 and M0 treatment, respectively, indicating that the peaks significantly increased with the manure application rate ($p < 0.05$). For M100 treatment, the N$_2$O emission rates decreased sharply thereafter ($p < 0.05$), in spite of some fluctuation between 0.58 and 0.77 µg kg$^{-1}$ h$^{-1}$ during days 1 to 5, and further decreased to 0.03–0.06 µg kg$^{-1}$ h$^{-1}$ after day 9, becoming similar to the control. For M40 and M60 treatments, a further decrease in N$_2$O emission rates was observed by day 15 and 11, respectively ($p < 0.05$). The second peaks were found by day 7 and 15 for M0 and M20 treatments, respectively, and a further decrease was observed after day 19 ($p < 0.05$). For M soil, the peaks of N$_2$O emission rates were observed by day 0.25 in all fertilization treatments and decreased sharply ($p < 0.05$) thereafter to the level of the control by 25, 25, 23, 19, and 11 in M0, M20, M40, M60, and M100 treatments, respectively (Figure 1).

The cumulative N$_2$O emissions showed a significant difference among treatments and soils. As compared with the control, all fertilization treatments significantly increased the cumulative N$_2$O emissions for both soils. For F soil, the cumulative N$_2$O emissions decreased with the increase in manure application rates. At the end of incubation, the highest (282.17 µg kg$^{-1}$) cumulative N$_2$O emissions were observed in M0 treatment, and the lowest (146.81 µg kg$^{-1}$) were found in M100 treatment. For M soil, there was no significant difference among M0, M20, M40, and M60 treatments, which were higher than the M100 treatment (Figure 2).
N2O emission rate (μg kg\(^{-1}\) h\(^{-1}\) )

0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Incubation days (day)

(a) F soil

(b) M soil

Figure 1. Change in nitrous oxide emission rates of the red soils after different fertilizations. The initial soils were from two fertilization treatments of a long-term field experiment. (a) F soil N2O emission rate, (b) M soil N2O emission rate. Error bars are the standard error of the mean.

Cumulative N2O emission (μg kg\(^{-1}\))

(a) F soil

(b) M soil

Figure 2. Change in cumulative N2O emissions of the red soils after different fertilizations. (a) F soil cumulative N2O emission, (b) M soil cumulative N2O emission. Error bars are the standard error of the mean. Different letters indicate a significant (\(p < 0.05\)) difference between the treatments and soils.

3.2. Soil pH Change

The changes in soil pH during the study period are shown in Figure 3. Soil pH was significantly affected by application rates of swine manure in both the F and the M soils. Without fertilizer addition (control), the soil pH showed no change throughout the incubation period. As compared with the control, soil pH significantly increased for all fertilization treatments of the two soils at the beginning of the incubation experiment (\(p < 0.05\)). Thereafter, F soil pH significantly decreased (\(p < 0.05\)) and then stabilized by days 28, 21, 15, 11, and 7, and the stable pH was 4.58, 4.79, 4.99, 5.27, and 5.78 for M0, M20, M40, M60, and M100 treatments, respectively. In M soil, it took 28, 21, 15, 11, and 5 days to reach the stable pH of 4.63, 4.74, 4.97, 5.25, and 5.81 for M0, M20, M40, M60, and M100 treatments, respectively. For both soils, the final pH significantly increased with swine manure application rates (\(p < 0.05\)), and the M100 treatment had much higher soil pH than the control treatment (\(p < 0.05\)). At the end of incubation, the pH of soils followed the order M0 < M20 < M40 < M60 < control < M100, and there was no significant difference between the F and the M soil at the same fertilization application rates in the incubation study.
significant difference among M0, M20, M40, and M60 treatments, which were higher than
the control treatment of F soil. As compared with the control, the M0 and M20 treatments of F soil increased exchangeable Al
p< 0.05). There were no significant differences in exchangeable Al³⁺ or acidity between the control and M40 for F soil, and between the control and M60 for M soil. The M60 and M100 treatments had much lower concentrations of exchangeable Al³⁺ and acidity than the control treatment of F soil. For M soil, the lowest exchangeable Al³⁺ and acidity were observed in the M100 treatment.

3.4. Soil Mineral Nitrogen Change

The changes in soil mineral N species (NH₄⁺-N and NO₃⁻-N) during the 35-day incubation experiment are shown in Figure 5. Soil N species were significantly affected by the application rates of swine manure in both the F and the M soils. Without fertilizer addition (control), NH₄⁺-N and NO₃⁻-N of the two soils showed no change throughout the incubation period. As compared to the control, soil NH₄⁺-N concentration of both F and M soils decreased as the swine manure application rate increased. Thereafter, the NH₄⁺-N of F soil significantly decreased to the level of the control by days 28, 21, 15, 11, and 7 for M0, M20, M40, M60, and M100 treatments, respectively. In M soil, it took 28, 21, 15, 11, and 5 days to decrease to the level of the control for M0, M20, M40, M60, and M100 treatments, respectively. The concentration of NH₄⁺-N in the M0 treatment of F soil was higher than that from M soil until day 11 (Figure 5a,b).

Figure 3. Change in pH of the red soils after different fertilizations. (a) F soil pH, (b) M soil pH. The initial soils were from two long-term field fertilization treatments. Error bars are the standard error of the mean.

3.3. Soil Exchangeable Acidity

The exchangeable H⁺ and Al³⁺ and the acidity differed between the two soils and were also significantly affected by swine manure application rates (Figure 4). After 35-day incubation, the exchangeable H⁺ and Al³⁺ and the acidity of the two soils significantly decreased as the swine manure application rate increased. The highest exchangeable H⁺ was found in the M0 treatment of M soil. As compared to the control, exchangeable H⁺ increased by 33.33% in the M0 treatment of F soil (p < 0.05), and increased by 51.67% and 26.67% for the M0 and M20 treatments of M soil, respectively (p < 0.05) (Figure 4a,b). The exchangeable H⁺ of the M0 and M20 treatments of M soil was much higher compared to F soil (p < 0.05).

There was the same change trend in exchangeable Al³⁺ and acidity of the two soils (Figure 4c–f). The highest exchangeable Al³⁺ and acidity were both observed in M0 of F soil. As compared with the control, the M0 and M20 treatments of F soil increased exchangeable Al³⁺ and acidity by 46.99–105.60% and 42.01–83.80% (p < 0.05); the M0, M20, and M40 treatments of M soil increased exchangeable Al³⁺ and acidity by 59.74–126.59% and 29.34–92.29% (p < 0.05). There were no significant differences in exchangeable Al³⁺ or acidity between the control and M40 for F soil, and between the control and M60 for M soil. The M60 and M100 treatments had much lower concentrations of exchangeable Al³⁺ and acidity than the control treatment of F soil. For M soil, the lowest exchangeable Al³⁺ and acidity were observed in the M100 treatment.
Figure 4. Change in exchangeable acidity of the red soils after different fertilizations. (a) F soil exchangeable H⁺, (b) M soil exchangeable H⁺, (c) F soil exchangeable Al³⁺, (d) M soil exchangeable Al³⁺, (e) F soil exchangeable acidity, (f) M soil exchangeable acidity. Error bars are the standard error of the mean. Different letters indicate a significant (p < 0.05) difference between the treatments and soils.

A decrease in soil NH₄⁺-N concentration was accompanied by an increase in NO₃⁻-N concentration indicating nitrification. Soil NO₃⁻-N concentration significantly increased for all fertilization treatments of the two soils following addition and stabilized by days 15, 15, 15, 15, and 3 for M0, M20, M40, M60, and M100 treatments, respectively (p < 0.05). The stable NO₃⁻-N concentrations of F soil were 146.3, 120.3, 106.7, 76.8, and 35.0 mg kg⁻¹ for M0, M20, M40, M60, and M100 treatments, respectively. In M soil, the stable NO₃⁻-N concentrations were 111.3, 108.1, 90.0, 64.7, and 33.3 mg kg⁻¹ for M0, M20, M40, M60, and M100 treatments, respectively. The stable NO₃⁻-N concentrations of M0 and M20 in F soil were higher than those from M soil at the same fertilization (Figure 5c,d).
A decrease in soil NH$_4^+$−N concentration was accompanied by an increase in NO$_3$−N concentration indicating nitrification. Soil NO$_3$−N and NH$_4^+$−N concentrations increased firstly and then stabilized as the incubation time increase. The increase rates of DOC in F soil were 2.30–3.63 mg kg$^{-1}$ d$^{-1}$ in the first 15 days, and slightly decreased in days 15–21. Soil DOC of M40, M60, and M100 treatments were stable at 133.08–152.97 mg kg$^{-1}$ after day 21, and the concentration increased with the swine manure application rate ($p < 0.05$). Soil DOC in the control treatment increased by 15.32% in the control treatment for both F and M soils. DON concentrations in all fertilization treatments showed a similar trend in soil dissolved organic nitrogen (DON) concentrations as compared with the initial values ($p < 0.05$), and the highest was found in the M100 treatment. The DOC of the control and M0 treatments in the M soil continued to increase throughout the incubation period and increased by 18.14% and 44.85% as compared to initial value. At the end of the incubation experiment, the final concentrations of DOC increased with swine manure application rates ($p < 0.05$), and the highest was found in the M100 treatment. The soil DOC of F soil was higher than that from M soil for the same fertilization treatments (Figure 5).

3.5. Soil Dissolved Organic Carbon and Nitrogen, and Microbial Biomass Carbon

Soil dissolved organic carbon (DOC) concentrations increased firstly and then stabilized as the incubation time increase. The increase rates of DOC in F soil were 2.30–3.63 mg kg$^{-1}$ d$^{-1}$ in the first 15 days, and slightly decreased in days 15–21. Soil DOC of M40, M60, and M100 treatments were stable at 133.08–152.97 mg kg$^{-1}$ after day 21, and the concentration increased with the swine manure application rate ($p < 0.05$). Soil DOC in the control treatment increased by 15.32% in the first 7 days and then stabilized around 124.10 mg kg$^{-1}$. For M soil, DOC of M20, M40, M60, and M100 increased by 24.40–31.21% as compared with the initial values ($p < 0.05$), and then decreased slightly after 21 days. The DOC of the control and M0 treatments in the M soil continued to increase throughout the incubation period and increased by 18.14% and 44.85% as compared to initial value. At the end of the incubation experiment, the final concentrations of DOC increased with swine manure application rates ($p < 0.05$), and the highest was found in the M100 treatment. The soil DOC of F soil was higher than that from M soil for the same fertilization treatments (Figure 6a,b).

There was a similar trend in soil dissolved organic nitrogen (DON) concentrations to DOC concentrations. There were no significant changes in DON concentrations of the control treatment for both F and M soils. DON concentrations in all fertilization treatments for both F and M soils increased first ($p < 0.05$) and then stabilized by days 21, 21, 21, 21, and 7 for M0, M20, M40, M60, and M100 treatments, respectively. The highest DON concentrations of F soil were 226.1, 205.1, 183.0, 140.4, and 80.5 mg kg$^{-1}$ for M0, M20, M40, M60, and M100 treatments, respectively. In M soil, the stable DON concentrations were 203.8, 188.7, 174.8, 140.8, and 83.7 mg kg$^{-1}$ for M0, M20, M40, M60, and M100 treatments, respectively. The stable DON concentrations of M0 and M20 in F soil were higher than those from M soil at the same fertilization treatments (Figure 6c,d).
soil DOC of F soil was higher than that from M soil for the same fertilization treatments (Figure 6a,b). The soil microbial biomass carbon (MBC) content fluctuated greatly and increased with the swine manure application rate. After 35-day incubation, the MBC of M100 was significantly higher than that from other treatments. The soil MBC in M soil was higher than that from F soil for the same fertilization treatments (Figure 6e,f).

3.6. Relationship between N₂O Emission and Soil Properties

The N₂O emission rate was positively correlated with soil pH, NH₄⁺-N, and MBC, and the correlation coefficients were 0.59, 0.54, and 0.27, respectively (p < 0.001). The N₂O emission rate was significantly negatively correlated with soil NO₃⁻-N, DON, and DOC, and the correlation coefficients were −0.46, −0.44 (p < 0.001), and −0.18 (p < 0.05), respectively. There was no significant correlation between N₂O emission rate and MN (manure nitrogen) (Figure 7a). The results of random forest statistics showed that soil pH
had the greatest relative contribution to $N_2O$ emissions followed by $NH_4^+$-$N$, while MBC had the lowest impact (Figure 7b).

![Figure 7. Relationship and relative contribution of soil properties to $N_2O$ emission. (a) Relationship between soil properties and $N_2O$ emission, (b) relative importance of soil properties to $N_2O$ emission.](image)

4. Discussion

4.1. Difference in $N_2O$ Emission between the Two Soils

The current study showed that the cumulative $N_2O$ emissions of M0 and M20 treatments in F soil significantly increased by 23.24–68.25% ($p < 0.05$) compared with that from M soil (Figure 2). A possible reason was the reduction in soil $NH_4^+$-$N$ and $NO_3^-$-$N$ due to microbial assimilation from long-term carbon input in M soil [18,19]. This study showed, more than 90% $N_2O$ of fertilization treatments were released within the first 21 days of incubation period, implying that a substantial amount of $N_2O$ was produced through nitrification process. Swine manure as an organic carbon substrate for microbial growth could promote the assimilation of microbial nitrogen, which led to an intense competition for $NH_4^+$-$N$ between heterotrophic microorganisms and autotrophic nitrifiers, resulting in a decrease in $N_2O$ production [26]. Studies have shown that a combination with organic fertilizers could enhance the microbial immobilization [27,28] and reduce the soil nitrogen availability for $N_2O$ production [29]. Bhattacharyya et al. [30] also reported that a combined application of chemical and organic N reduced the conversion of fertilizer N to soil ammonium and nitrate by 20% as compared to chemical fertilizers only. Yao et al. [31] pointed out through meta-analysis that organic fertilizers decreased $N_2O$ emissions because of the limitation of the readily available nitrogen substrates.

The N transformation data in the current study also confirmed microbial assimilation as the concentration of $NH_4^+$-$N$ in M0 treatment of M soil was lower than that from F soil (Figure 5a,b), and the $NO_3^-$-$N$ concentrations of M0 and M20 in M soil were also lower than those from F soil (Figure 5c,d). The M soil with long-term swine manure application history had a relatively higher TOC and C/N ratio than F soil (Table 1), which might stimulate microbial immobilization, and it had reduced $N_2O$ production from nitrification compared to F soil. A high proportion of chemical nitrogen fertilizer (M0 and M20) was conducive to promoting the immobilization of nitrogen by microorganisms, thus reducing available nitrogen for nitrification and denitrification and, therefore, $N_2O$ emissions [32]. Huang et al. [33] also showed that soil $N_2O$ emission had a negative relationship with C/N.

4.2. Partial Chemical N Replacement by Swine Manure Mitigating Red Soil $N_2O$ Emission

This study showed that the cumulative $N_2O$ emission decreased with the increase in manure application rates, and a significant difference between F and M soils disappeared as the swine manure application rate increased to 40% (M40), 60% (M60), and 100% (M100) of total N (Figure 2). A possible reason is that the soil $N_2O$ emissions mainly came from hydrolysis and nitrification of urea-N rather than mineralization and transformation of swine
manure-N [18]. In the current study, the relative importance of soil NH$_4^+$-N concentration to N$_2$O production was only lower than that of pH (Figure 7). The ammonification of organic N within swine manure was much harder than that of hydrolysis of urea, and partial chemical N replacement by swine manure markedly decreased soil NH$_4^+$-N concentration, which substantially reduced the nitrification process, supported by a much lower NO$_3^-$-N concentration in swine manure amendment treatments. A decrease in soil NH$_4^+$-N showed a lack of nitrification substrate, which resulted in a significant reduction in N$_2$O emissions [19]. The lower soil NH$_4^+$-N concentration resulting from an increased swine manure substitution ratio decreased soil N$_2$O emissions at the initial stage. This is consistent with the results reported by Duan et al. [34], where N$_2$O emissions from nitrification in chemical nitrogen treatments were significantly higher than manure treatments.

In addition, the increased swine manure substitution ratio resulting in prevailing anoxic conditions might facilitate dissimilatory nitrate reduction to ammonium [35], resulting in a decrease in soil NO$_3^-$ concentrations (Figure 5). The N transformation data also suggested that NO$_3^-$ production through nitrification could have been impeded by O$_2$ limitations in the present study. Under the prevailing reductive soil conditions, denitrifiers might easily assimilate the highly reactive N$_2$O as an electron-acceptor or nitrogen source [36], thus lowering N$_2$O emissions with manure substitution. Xia et al. [37] showed that the combined application of synthetic and organic nitrogen fertilizers promoted the reduction of N$_2$O to N$_2$ during denitrification due to the supply of dissolved organic carbon by organic fertilizer addition.

In addition to a reduction in mineral N, soil pH was one of the most important factors for N$_2$O production, influencing nitrification, nutrient conversion, and microbial community structure in the soil [38,39]. Soil pH increased by 0.13–1.21 units after swine manure amendment (Figure 3). The increase in soil pH could enhance the activity of N$_2$O reductase encoded by nosZ gene, as well as promote the reduction of N$_2$O to N$_2$ [40]. Therefore, N$_2$O production decreased as the swine manure application rate increased (Figure 1). The abundance of N$_2$O-related bacteria might have also increased with the elevation of soil pH [41]; however, more N$_2$O-consuming bacteria could have dominated over the N$_2$O-producing bacteria and then eventually resulted in a net decrease in bacterial N$_2$O emission with more manure addition in this study. Wang et al. [3] also pointed out that, under the same N input, the N$_2$O emissions of acidic soil were higher than those of alkaline soil. Acidic conditions might favor the growth of fungal populations lacking N$_2$O reductase, terminating at N$_2$O. Therefore, the inhibition of N$_2$O reductase activity during bacterial denitrification under low-pH conditions might have been another reason for the high N$_2$O emissions [42]. In this study, the manure rich in alkalinity addition increased the soil pH; therefore, the increased N$_2$O reductase activity decreased the N$_2$O emissions [43]. In addition, higher soil pH might suppress the nitrate reductase activity that converted NO$_3^-$ into NO$_2^-$ and then decreased N$_2$O emissions [20].

In the future, it is still necessary to further explore the effects of adding different amounts of swine manure on soil carbon and nitrogen conversion processes and greenhouse gas emissions through isotope tracer technology, and study the community composition, abundance of key nitrogen-related microorganisms, and related enzymes in the process of nitrification and denitrification of red soil by applying organic materials combined with molecular study. This can provide technical support for the efficient use of nitrogen fertilizer in red soil production systems, reduce nitrogen gas loss, leaching loss, and environmental risks, and provide a scientific basis for nutrient management of red soil and improvement of soil cultivated land quality.

5. Conclusions

Our data indicated that partial chemical N replacement by swine manure could effectively mitigate N$_2$O emissions from acidic red soil primarily because of mineral N immobilization and alleviated red soil acidification. Thus, swine manure has the potential to co-ameliorate red soil acidification and N$_2$O emission. Further research is needed to
determine the effect of swine manure on N₂O emission reductions and related enzymes under field conditions and the overall benefits in effective N management.

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