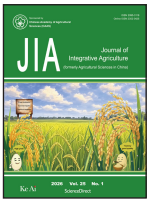
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RESEARCH ARTICLE

Long-term manure amendment enhances N_2O emissions from acidic soil by alleviating acidification and increasing nitrogen mineralization

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Highlights

- Manure amendment, especially combined with inorganic fertilizer, increased soil N_2O emissions.
- Increased N_2O emissions resulted from alleviated acidification and enhanced gross N mineralization.
- Manure amendment favored N_2O-producing over N_2O-consuming denitrifiers.
- Higher soil aggregate stability closely correlated with increased N_2O emissions.

Abstract

Long-term manure application has the potential to alleviate soil acidification, and increase carbon sequestration and nutrient availability, thus improving cropland fertility. However, the mechanisms behind greenhouse gas N_2O emissions from acidic soil mediated by long-term manure application remain poorly understood. Herein, we investigated N_2O emission and its linkage with gross N mineralization and nitrification rates, as well as nitrifying and denitrifying microbes in an acidic upland soil subjected to 36-year fertilization treatments, including an unfertilized control (CK), inorganic fertilizer (F), 2× rate of inorganic fertilizer (2F), manure (M), and the combination of inorganic fertilizer and manure (FM) treatments. Compared to the CK treatment (1.34 μg N kg^{-1} d^{-1}), fertilization strongly increased N_2O emissions by 34-fold on average, with more pronounced increases in the manure-amendment (10.6–169 μg N kg^{-1} d^{-1}) than those in the inorganic fertilizer treatments (3.26–5.51 μg N kg^{-1} d^{-1}). The manure amendment-stimulated N_2O emissions were highly associated with increased soil pH, mean weight diameter of soil aggregates, substrate availability (e.g., particulate organic carbon, NO_3^- and available phosphorus), gross N mineralization rates, denitrifier abundances and the (*nirK*+*nirS*)/*nosZ* ratio. These findings suggest that the increased N_2O emissions primarily resulted from alleviated acidification, increased substrate availability and improved soil structure, thus enhancing microbial N mineralization and favoring N_2O-producing denitrifiers over N_2O consumers. Moreover, ammonia-oxidizing bacteria (AOB) rather than ammonia-oxidizing archaea (AOA) positively correlated with soil NO_3^- concentration and N_2O emissions, indicating that nitrification indirectly contributed to N_2O production by supplying NO_3^- for denitrification. Collectively, manure amendment potentially stimulates N_2O emissions, primarily resulting from alleviated soil acidification and increased substrate availability, thus enhancing N mineralization and denitrifier-mediated N_2O production. Our findings suggest that consideration should be given to the greenhouse gas budgets of agricultural ecosystems when applying manure for managing the pH and fertility of acidic soils.

Keywords: long-term manure amendment, N_2O emission, nitrogen mineralization, denitrification, (*nirK*+*nirS*)/*nosZ*

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1. Introduction

Nitrous oxide (N_2O) is a long-lived greenhouse gas (GHG) that depletes the stratospheric ozone layer (Ravishankara *et al.* 2009), and has a global warming potential 273-fold higher than that of carbon dioxide (CO_2) over a 100-year timescale (Tian *et al.* 2020; IPCC 2021). Agricultural soils are dominant sources of the increased atmospheric N_2O concentration, and account for around 52% of global anthropogenic N_2O emissions, primarily driven by intensive nitrogen (N) fertilization (Tian *et al.* 2020; Cheng *et al.* 2021; Maaz *et al.* 2021). Understanding the mechanisms of N fertilization regulating N_2O emissions from agricultural soils is crucial for optimizing management strategies, so as to curtail GHG emissions arising from N fertilization for soil fertility and productivity improvement (Butterbach-Bahl *et al.* 2013; Xu *et al.* 2020).

N_2O emissions are closely related to microbial N transformation processes in diverse soil N pools, typically including the organic N pool *via* N mineralization, NH_4^+ pool *via* nitrification, and NO_3^- pool *via* denitrification (Butterbach-Bahl *et al.* 2013; Xu *et al.* 2023). These processes occur simultaneously in soils, albeit in different micro-habitats (Wu *et al.* 2017, 2023). Gross N mineralization, converting organic N into NH_4^+ and NO_3^-, reflects the capacity of available N supplies for nitrification- and denitrification-derived N_2O production (Wang *et al.* 2023; Xu *et al.* 2023). Nitrification is the process that converts NH_4^+ into NO_2^- by ammonia oxidation, followed by further oxidation to NO_3^- which serves as a substrate for denitrification (Cheng *et al.* 2021). The oxidation of hydroxylamine (NH_2OH) to nitrite (NO_2^-), the rate-limiting step of nitrification, is regulated by ammonia-oxidizing archaea (AOA) and bacteria (AOB), and produces N_2O as a byproduct (Hu *et al.* 2015). Microbial denitrification involves the sequential anaerobic reduction of NO_3^- to NO_2^-, N_2O and N_2, encoded by *narG*, *nirK*/*nirS* and *nosZ* genes, respectively (Butterbach-Bahl *et al.* 2013). The ratio between N_2O-producing denitrifiers (*nirK* and *nirS*) and N_2O-consuming denitrifiers (*nosZ*) controls characterizes the soil $N_2O/(N_2O+N_2)$ ratio, with a larger ratio indicating higher N_2O emission potential (Qu *et al.* 2014; Wu *et al.* 2022). Soil N_2O emissions are primarily regulated by the processes of N mineralization, nitrification and denitrification, along with the related microbial functional genes encoding the enzymes for N_2O production and consumption (Wan *et al.* 2023; Xu *et al.* 2023; Liao *et al.* 2024). However, the interactions among these N processes and their collective regulation of N_2O emissions remain elusive (Cheng *et al.* 2021; Wan *et al.* 2023). Disentangling the responses of N_2O emissions and their linkage with N transformations and associated microbial functional genes in relation to management practices, such as fertilization, is crucial for developing climate-smart sustainable agricultural management.

The increased N_2O emissions from agricultural ecosystems are closely linked to the fertilization-induced changes in soil N availability for microbial nitrification and denitrification (Tian *et al.* 2020). Numerous studies regarding the fertilization effects on N_2O emissions have mainly focused on the short period following nutrient addition (Wu *et al.* 2017; Guenet *et al.* 2021; Xu *et al.* 2023). However, the responses of N_2O emission to fertilization in the long term may differ from its response in the short term (Wu *et al.* 2020), because of changes in soil biotic and abiotic properties caused by fertilization. Intensive long-term application of N fertilizers inevitably causes soil acidification, high N surplus and nutrient imbalances, consequently reducing productivity while enhancing N_2O emissions (Qu *et al.* 2014; Cheng *et al.* 2021). Manure amendment has been widely adopted as an effective management practice to alleviate acidification, and enhance nutrient availability and carbon sequestration, thus optimizing microbial communities and improving soil fertility (Wan *et al.* 2023; Liang *et al.* 2024). Long-term manure amendment largely mediates microbial N transformation processes and consequent N_2O emissions (Cheng *et al.* 2021; Wan *et al.* 2023). However, the manure-amendment effects on N_2O emissions from arable soils are highly variable (Hayakawa *et al.* 2009; Guenet *et al.* 2021; Kong *et al.* 2024). There are two contrasting perspectives regarding the role of manure amendment in regulating soil N_2O emissions. One perspective suggests that manure application decreases N_2O emissions, due to increased N immobilization resulting in decreased N availability and increased pH stimulating complete denitrification (Zhang *et al.* 2020; Wan *et al.* 2023; Tang *et al.* 2024). Conversely, the other perspective suggests that manure amendment has the potential to increase soil C and nutrient availability, and alleviate acidification, thus stimulating N_2O emissions (Cheng *et al.* 2021; Liao *et al.* 2024; Wu *et al.* 2024). The manure amendment-induced enhanced N_2O emissions may offset the benefits of increased soil carbon sequestration on mitigating global warming (Zhou *et al.* 2017; Guenet *et al.* 2021). These divergent results suggest that the above-mentioned mechanisms could both individually and interactively regulate N_2O emissions in response to manure amendment (Zhou *et al.* 2017; Liao *et al.* 2024). The interplay between N transformation processes with soil abiotic properties should be considered when addressing the N_2O emissions in response to manure amendment (Wan *et al.* 2023; Xu *et al.* 2023). Therefore, it is urgent to comprehensively evaluate the long-term application of inorganic fertilizer versus manure effects on N_2O emissions and their linkage with N transformation processes and N cycling functional genes, so as to develop efficient GHG mitigation strategies and improve N use efficiency in agricultural ecosystems.

We hypothesized that long-term manure amendment would stimulate N_2O emission in acidic soils, mainly due to alleviated acidification and enhanced N mineralization increasing substrate availability for nitrification and denitrification. The objectives of the present study were to: (1) evaluate the responses of N_2O emissions and associated N transformation processes to long-term application of inorganic fertilizers and manure in an acidic upland soil, and (2) clarify the underlying mechanisms of long-term manure amendment regulating N_2O emissions.

2. Materials and methods

2.1. Site description and soil sampling

The soil samples for the present study were collected from

a long-term fertilization experiment (over 36 years) located at the station of the Institute of Red Soil and Germplasm Resources in Jiangxi Province, China (28°35′N, 116°17′E). This region has a subtropical monsoon climate, with mean annual temperature of 18.1°C and average precipitation of 1,537 mm yr^{-1} over the past 50 years. The soil, classified as a *Ferralic Cambisol* (WRB 2014), is developed from Quaternary red clay dominated by kaolinites. The long-term fertilization experiment began in 1986 and was continuously cropped with maize (*Zea mays* L.), with five treatments selected for this study: (1) unfertilized control (CK), (2) recommended application rates of inorganic N, phosphorus (P) and potassium (K) fertilizer (F), (3) 2× the recommended application rates of inorganic fertilizer (2F), (4) pig manure alone (M), and (5) recommended inorganic fertilizer rate combined with pig manure (FM). Details regarding the fertilizer types and application rates can be found in Wu *et al.* (2019). The experimental treatments were arranged in a randomized complete block design with three replicate plots of 4 m×6 m for each treatment.

After the maize harvest in September 2022, five soil cores (2 cm in diameter) were randomly collected from the top 20 cm layer and combined to form a composite soil sample in each field plot. The fresh soil samples were transported to the laboratory to remove the visible stones and crop residues, and the samples were homogenized and passed through a 2-mm mesh sieve and then stored at 4°C on the day of sampling for further use within one week. Subsamples were stored at −80°C for DNA extraction and air-dried for basic property analysis.

2.2. Experimental design and N_2O measurement

N_2O effluxes were determined for field-moist soil samples using a microcosm incubation method. Prior to incubation, the soil water content of each soil sample was measured gravimetrically, and the water holding capacity (WHC) was determined. The fresh soil samples were weighed (equivalent to 40 g dry soil) into airtight 500-mL Mason jars each fitted with a lid containing a rubber gas sampling septum. The samples were adjusted to 65% WHC by adding deionized water, and pre-incubated at 25°C for 3 days to activate microbial activity and minimize disturbance effects. After pre-incubation, the soil samples were continuously incubated at 25°C in the dark for 7 days. The soil moisture content of samples was regularly checked and maintained at 65% WHC over the incubation period.

For gas sampling, the jars were left open for 10 min to ventilate with ambient air, then lids with rubber gas sampling septa were used to seal the jars. A gas-tight syringe was filled and then expelled back into the headspace of each jar three times to mix the headspace gases after lid closure, and 20 mL gas samples were collected from the headspace of each jar at 0, 30 and 60 min. The collected gas samples were immediately injected into 12-mL vacuum vials (Labco Limited, UK) for N_2O and CO_2 analysis using a gas chromatograph (7890A, Agilent Technologies, USA) (Wu *et al.* 2017). The effluxes of N_2O and CO_2 were calculated from the linear regression slope of gas concentrations over time after jar closure (correlation coefficient R^2>0.85):

$$N_2O(CO_2)\ \text{efflux}=\frac{\Delta C}{\Delta t}\times\frac{V_h}{V_m}\times\frac{M}{m_s} \quad (1)$$

where $\frac{\Delta C}{\Delta t}$ is the rate of gas accumulation in the headspace (μL L^{-1} d^{-1}), V_h is the headspace volume (L), V_m is the molar gas volume (L mol^{-1}), M is the molar mass of the gas (g mol^{-1}) and m_s is the soil dry weight (g).

The N_2O and CO_2 effluxes were measured daily for 7 days. Cumulative emissions of N_2O and CO_2 were calculated by multiplying the average daily gas effluxes from two consecutive measurements by the time interval between sampling events.

2.3. Measurements of N transformation processes

Gross rates of soil N mineralization and nitrification were determined using the ^{15}N isotope pool dilution technique (Murphy *et al.* 2003). The $(^{15}NH_4)_2SO_4$ (98 atom% ^{15}N) and $K^{15}NO_3$ (98 atom% ^{15}N) used in the present study were obtained from Shanghai Chem-Industry Institute, China. The pre-incubated soil samples were weighed (equivalent to 20 g dry soil) into 120 mL serum vials and either 1.2 mL $(^{15}NH_4)_2SO_4$ (98 atom% ^{15}N, 0.5 mol L^{-1}) or $K^{15}NO_3$ (98 atom% ^{15}N, 1.0 mol L^{-1}) solution was added. The samples were then incubated for 4 h (t_1) or 24 h (t_2) at 65% WHC and 25°C. The soil samples were destructively extracted with 2 mol L^{-1} KCl solution (soil:KCl ratio of 1:5) after the incubation period. The NH_4^+ and NO_3^- concentrations were determined using a continuous flow analyzer (Skalar, Breda, The Netherlands). For ^{15}N enrichment analysis, the NH_4^+ and NO_3^- in extracts were separated using micro-diffusion methods with MgO and Devarda's alloy, absorbed by oxalic acid, and measured using an isotope ratio mass spectrometer coupled to an elemental analyzer (MAT253, Bremen, Germany). Soil gross N mineralization and nitrification rates (mg N kg^{-1} d^{-1}) were calculated according to eqs. (2) and (3), respectively (Hart *et al.* 1994):

$$\text{Gross N mineralization rate}=\frac{[NH_4^+]t_1-[NH_4^+]t_2}{t_2-t_1}\times\frac{\text{Ln}(A_1t_1/A_2t_2)}{\text{Ln}([NH_4^+]t_1/[NH_4^+]t_2} \quad (2)$$

$$\text{Gross nitrification rate}=\frac{[NO_3^-]t_1-[NO_3^-]t_2}{t_2-t_1}\times\frac{\text{Ln}(B_1t_1/B_2t_2)}{\text{Ln}([NO_3^-]t_1/[NO_3^-]t_2} \quad (3)$$

where $[NH_4^+]_{t_1}$ and $[NH_4^+]_{t_2}$ are the NH_4^+ concentrations (mg N kg^{-1}) at t_1 and t_2, respectively. A_1t_1 and A_2t_2 are the ^{15}N atom% in the $^{15}NH_4^+$-labeled extracts at t_1 and t_2, respectively. $[NO_3^-]_{t_1}$ and $[NO_3^-]_{t_2}$ are the NO_3^- concentrations (mg N kg^{-1}) at t_1 and t_2, respectively. B_1t_1 and B_2t_2 are the ^{15}N atom% in the $^{15}NO_3^-$-labeled extracts at t_1 and t_2, respectively.

Microbial nitrogen use efficiency (NUE) was determined using the ^{18}O-H_2O tracer incubation method as decreased by Wang *et al.* (2023).

2.4. Measurements of soil physicochemical and biological properties

The soil samples were taken for determination of soil

aggregate fraction, pH, SOC, total nitrogen (TN), total phosphorus (TP), particle organic carbon (POC), dissolved organic nitrogen (DON), mineral N (NH_4^+ and NO_3^-), available phosphorus (AP), and microbial biomass-C (MBC) and -N (MBN), prior to incubation assays. The soil aggregate fractions were separated using a wet-sieving method according to Elliott (1986). Soil pH was measured in a soil:water suspension (1:2.5, w/v) using a digital pH meter (Radiometer, Copenhagen). Soil sub-samples were air-dried and ground using a ball-mill, and SOC and TN concentrations were measured by dry combustion with an elemental analyzer (EA3000, Milan, Italy). Soil TP was digested with H_2SO_4-$HClO_4$ and measured using the molybdenum blue colorimetric method (Lu 1999). The POC was measured after sodium hexametaphosphate dispersion and size fractionation (>53 μm) as described by Yu *et al.* (2022). Soil NH_4^+ and NO_3^- were extracted with 2 mol L^{-1} KCl solution at a soil: water ratio of 1:5, and determined colorimetrically with an automated flow-injection analyzer (Tecator FIA Star 5000 Analyzer, Foss Tecator, Sweden) (Wu *et al.* 2017). The dissolved organic N (DON) concentration was calculated as the difference between the total dissolved N and mineral N concentration. Soil available P (AP) was extracted with 0.5 mol L^{-1} $NaHCO_3$ solution and determined using the molybdenum blue colorimetric method (Murphy and Riley 1962). The concentrations of MBC and MBN were determined by the differences in extractable organic C and total N between the fumigated and corresponding non-fumigated soil samples using the chloroform-fumigation K_2SO_4 extraction method, and divided by the correction factors of 0.45 and 0.54, respectively (Brookes *et al.* 1985; Wu *et al.* 1990).

The activities of β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) were measured using the fluorogenic methylumbelliferone-based substrates method (Marx *et al.* 2001). Specifically, 1.5 g of fresh soil was weighed and mixed with 125 mL of 50 mmol L^{-1} sodium acetate buffer for 1 min. Then, the soil suspension (200 μL) and 50 μL of 200 μmol L^{-1} fluorometric substrates were pipetted into 96-well microplates. The standard fluorometric substrates were 4-methylumvelliferyl for BG, CBH and NAG, and 7-amino-4-methylcoumarin for LAP. The microplates were incubated at 25°C in the dark for 4 h, and the fluorescence was measured using a fluorometric spectrometer (Synergy H1M, BioTek, USA) with excitation at 365 nm and emission at 450 nm. The enzyme activities were expressed in units of nmol g^{-1} dry soil h^{-1}.

The copy numbers of functional genes related to N mineralization (*sub*, encoding protease; *chiA* and *exo-chi*, encoding chitinase), nitrification (AOA and AOB) and denitrification (*nirK*, *nirS* and *nosZ*) were quantified using the fluorescent dye SYBR-Green with a CFX384 real-time qPCR detection system (BioRad, USA). Briefly, total DNA was extracted from 0.25 g of freeze-dried soil sample in triplicate using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted DNA was checked on a 1% agarose gel, and the concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). For each DNA sample, qPCR was performed in triplicate. The gene-specific primers, reaction systems, and procedures for qPCR are shown in Appendix A. The PCR reaction mixtures contained 12.5 μL of 2×SYBR Premix *Ex Taq* (SinoGene, China), 0.5 μL of each forward and reverse primer, 10.5 μL of DNA-free water, and 1 μL of target DNA template. All qPCR assays were performed in duplicate for each sample and standard. The abundances of functional genes were analyzed by a qPCR detection system (StrataGene Mx3005P, CA, USA). Standard curves for all functional genes targeted were established by using a 10-fold series dilution of a plasmid, with R^2>0.99.

2.5. Statistical analyses

All data were checked for normality and homogeneity using the Shapiro–Wilk and Bartllet tests, respectively, before performing an analysis of variance (ANOVA). Natural log-transformation was conducted to normalize non-normally distributed data. A one-way ANOVA with the post-hoc Tukey HSD test was used to examine the effects of fertilization on the cumulative emissions of N_2O and CO_2, and soil biophysicochemical properties. Correlation analysis was performed to evaluate relationships between N_2O emissions and the measured variables. Structural equation modeling (SEM) was performed to elucidate the direct and indirect effects of key soil physicochemical and microbial properties on N_2O emissions. Prior to using the SEM procedure, principal component analysis was conducted to remove redundant variables. The best-fitting model was selected by step-wise removal of non-significant path. Chi-square (χ^2, P>0.05), root mean square error of approximation (RMSEA<0.05), and comparative fit index (CFI>0.9) were used to assess model fit. Statistically significant differences were tested at P<0.05. All statistical analyses were conducted using SPSS software 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. N_2O and CO_2 emissions

Long-term fertilization largely affected the effluxes and cumulative emissions of N_2O (P<0.05; Fig. 1). The N_2O effluxes were consistently higher in the FM treatment compared to the other treatments (P<0.05; Fig. 1-A). Compared to the CK treatment (9.35 μg N kg^{-1}), inorganic fertilization (22.8–38.6 μg N kg^{-1}) and manure amendment (74.0–1,187 μg N kg^{-1}) strongly increased the 7-day cumulative N_2O emissions, with more pronounced effects observed in the manure amendment treatments (P<0.05; Fig. 1-B). The N_2O emissions were significantly higher in the FM treatment than in the M treatment (Fig. 1-B).

The CO_2 effluxes were the highest on the first day of incubation, and rapidly decreased over time across all fertilization treatments (Appendix B). Cumulative CO_2 emissions were significantly higher (P<0.05) in manure amended treatments ((48.24±0.81) and (48.06±0.46) mg C kg^{-1} soil for M and FM treatments, respectively), than in treatments with inorganic fertilization ((20.53±0.44)

and (24.23±0.24) mg C kg^{-1} soil for F and 2F treatments, respectively) and the CK treatment ((16.26±1.77) mg C kg^{-1} soil) (P<0.05; Appendix B).

3.2. Soil nitrogen transformation processes and related functional genes

Long-term fertilization markedly affected microbial NUE and gross rates of N mineralization and nitrification (Fig. 2; Appendix C). Manure application decreased NUE by 20–46% and increased gross N mineralization rates by 4.63–8.31 folds, as compared to the CK, F and 2F treatments among which no significant difference was observed (Fig. 2-A; Appendix C). The gross nitrification rates in the M and FM treatments were 27–55% lower than those in the F and 2F treatments, but 77–130% higher than in the CK treatment (Fig. 2-B).

The abundances of N mineralization, nitrifying and denitrifying functional genes significantly differed among

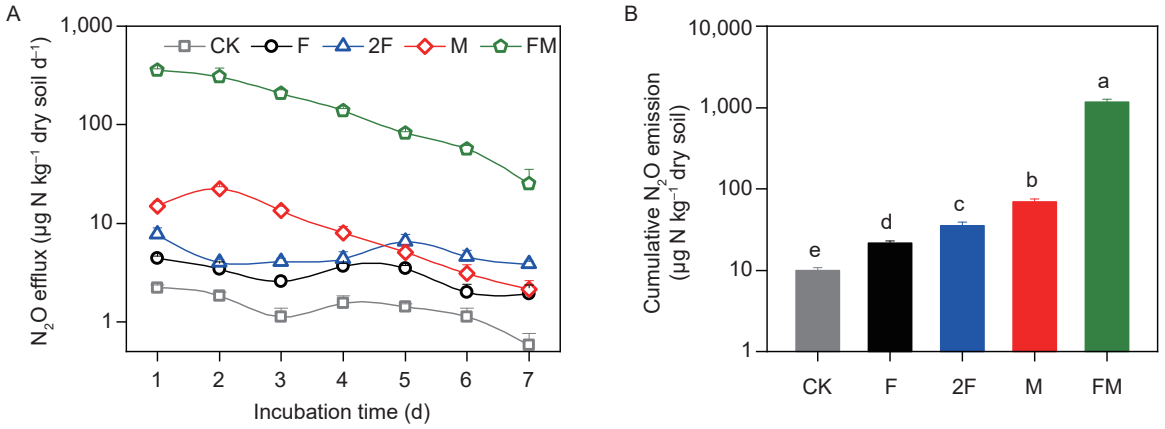


Fig. 1 Temporal effluxes (A) and cumulative emissions (B) of N_2O over the 7-day incubation period from soil subjected to five long-term fertilization treatments. CK, unfertilized control; F, inorganic fertilizer application; 2F, 2× rate of inorganic fertilizer application; M, manure application; FM, inorganic fertilizer (single rate) plus M application. Error bars represent standard errors of the mean values (n=3). Different letters above the bars indicate significant differences among the fertilization treatments at P<0.05.

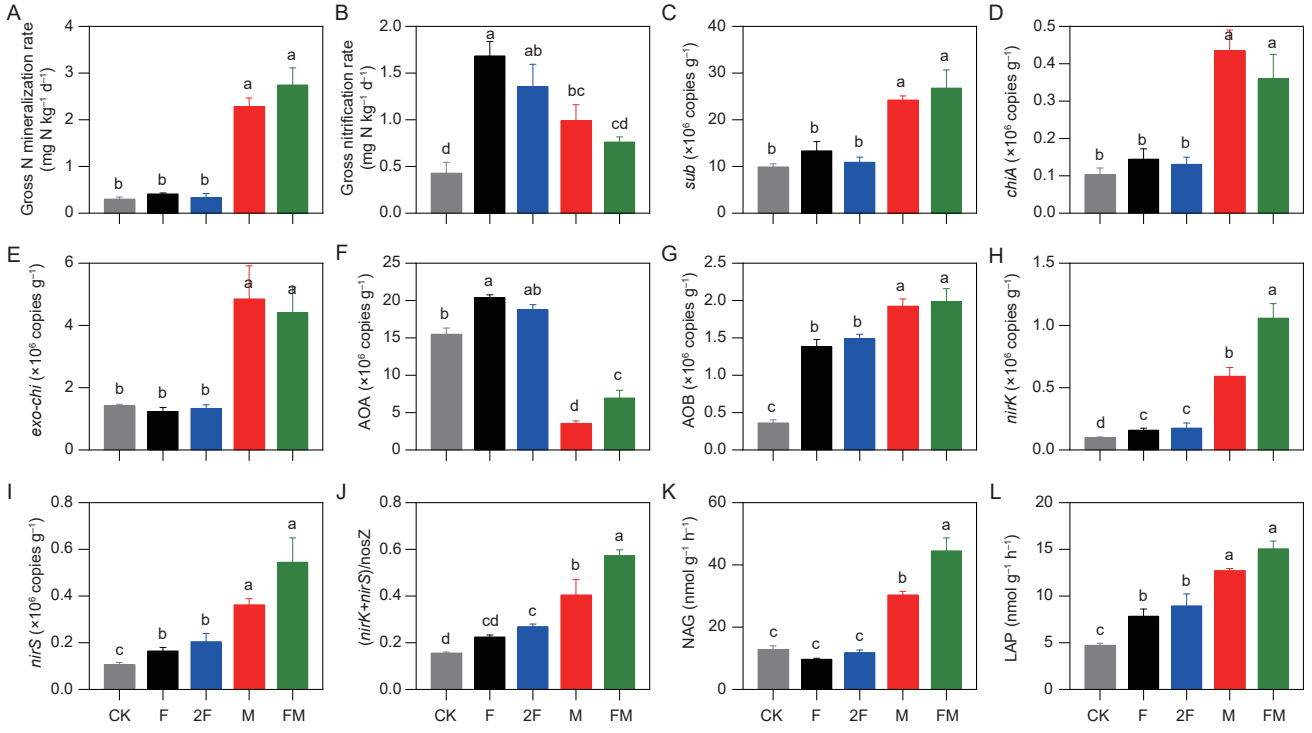


Fig. 2 Gross rates of N mineralization (A) and nitrification (B), N cycling gene abundances (C–I), (*nirK*+*nirS*)/*nosZ* ratio (J) and the enzymatic activities of NAG (K) and LAP (L) for the soil subjected to five long-term fertilization treatments. CK, unfertilized control; F, inorganic fertilizer application; 2F, 2× rate of inorganic fertilizer application; M, manure application; FM, inorganic fertilizer (single rate) plus M application. AOB, ammonia oxidation bacteria; AOA, ammonia oxidation archaea; NAG, β–1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase. Error bars represent standard errors of the mean values (n=3). Different letters above the bars indicate significant differences among the fertilization treatments at P<0.05.

fertilization treatments (Fig. 2-C–J; Appendix C). Manure amendment strongly increased the abundances of *sub*, *chiA* and *exo-chi* genes by 81–170%, 150–325% and 211–295%, respectively, compared to the CK, F and 2F treatments among which there was no significant difference (Fig. 2-C–E). Compared to the CK and inorganic fertilization (F and 2F) treatments, manure application significantly increased the gene abundances of AOB, *nirK*, *nirS* and *nosZ*, and the (*nirK*+*nirS*)/*nosZ* ratio, but decreased AOA abundance and the (AOA+AOB)/(*nirK*+*nirS*+*nosZ*) ratio (Fig. 2-F–J; Appendix C). The abundance of AOA was ten folds higher than that of AOB across fertilization treatments (Fig. 2-F and G).

3.3. Soil physicochemical and biological properties

The soil physicochemical and biological properties significantly varied among fertilization treatments (P<0.05; Table 1; Fig. 2-K–L; Appendix C). Manure application largely increased the mass proportion of macroaggregates, and decreased that of microaggregates, thereby increasing MWD by 7–29%, as compared to the CK, F and 2F treatments (P<0.05; Table 1; Appendix C). The soil pH was significantly lower in the inorganic fertilization treatments, and higher in the manure treatment, than in the CK treatment. The concentrations of SOC, TN and TP were significantly higher in the manure amendment treatments than in the inorganic fertilization and CK treatments (P<0.05; Table 1). The POC concentration was significantly lower in the CK and F treatments than in the 2F, M and FM treatments (Table 1). Manure amendment also strongly increased soil DON concentration as compared to the CK, F and 2F treatments (P<0.05; Table 1). Soil NH_4^+ was significantly increased by inorganic fertilization, but was little changed by manure amendment (Table 1). Fertilization, especially by the M and FM treatments, greatly increased soil NO_3^- concentration compared to the CK treatment (Table 1). Fertilization greatly increased soil AP concentration, with the most pronounced effects observed in the M and FM treatments (Table 1). Manure application strongly increased MBC, MBN, and the activities of NAG, LAP, BG and CBH, as compared to the CK, F and 2F treatments (Table 1; Fig. 2-K and L; Appendix C).

Table 1 Properties of the acidic upland soil subjected to five long-term fertilization treatments[1)]

Soil property[2)]	CK	F	2F	M	FM
MWD (mm)	0.70 d	0.76 c	0.79 c	0.84 b	0.92 a
pH	4.90 b	4.63 c	4.58 c	6.12 a	5.93 a
SOC (g kg^{-1})	8.63 b	9.82 b	9.11 b	12.22 a	12.45 a
TN (g kg^{-1})	0.85 c	1.01 b	1.03 b	1.23 a	1.27 a
TP (g kg^{-1})	0.75 d	0.84 cd	1.08 c	1.85 b	2.34 a
POC (g kg^{-1})	1.49 c	1.48 c	2.48 b	2.37 b	3.45 a
DON (mg kg^{-1})	7.37 b	7.40 b	5.57 b	18.52 a	18.15 a
NH_4^+-N (mg kg^{-1})	9.89 c	23.67 b	38.80 a	7.80 c	8.74 c
NO_3^--N (mg kg^{-1})	6.82 c	15.56 b	18.05 b	27.73 a	26.56 a
AP (mg kg^{-1})	13.86 c	24.76 c	51.32 b	254.47 a	258.29 a
MBC (mg kg^{-1})	111.85 c	175.48 b	184.27 b	245.28 a	278.15 a
MBN (mg kg^{-1})	10.08 c	13.99 c	14.86 c	26.51 b	32.87 a

[1)] CK, unfertilized control; F, inorganic fertilizer application; 2F, 2× rate of inorganic fertilizer application; M, manure application; FM, inorganic fertilizer (single rate) plus M application.
[2)] MWD, mean weight diameter; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; POC, particle organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.
Values followed by different lowercase letters in the same horizontal row indicate significant difference at P<0.05.

3.4. Factors affecting N_2O emission and nitrogen transformation processes

Variance partitioning analysis revealed that soil biophysicochemical properties and microbial N cycling functional genes together explained 85% of the variation in N_2O emissions among the fertilization treatments (Fig. 3-A). Pearson correlation analysis between N_2O emission and soil properties further showed that N_2O emissions exhibited strong positive correlations with the main soil physicochemical properties, including MWD, pH, SOC, POC, TN, DON, NO_3^-, TP and AP (Fig. 3-B). Moreover, the increase in N_2O emission by manure amendment was significantly positively associated with gross N mineralization, CO_2 emission, MBC, MBN, and gene abundances of *sub*, *chiA*, *exo-chi*, AOB, *nirK* and *nirS*, and (*nirK*+*nirS*)/*nosZ* ratio, and negatively correlated with the NUE (Fig. 3-B). No quantitative relationship was found between N_2O emission and NH_4^+ concentration, gross nitrification or AOA gene abundances (Fig. 3-B).

The SEM analysis showed that soil physicochemical properties were the primary drivers of N_2O emission, and indirectly regulated N_2O emission *via* changing microbial N mineralization, nitrification and denitrification processes (Fig. 4). Microbial N mineralization determined N_2O emission through direct effects on nitrification and denitrification processes. Nitrification genes were indirectly related to N_2O emission *via* the production of NO_3^- for denitrification. Denitrification genes had strong direct and positive effects on N_2O emission (path coefficient=0.93). The results of the pathway collectively explained 86% of the variation in N_2O emissions induced by long-term fertilization (Fig. 4).

Correlation analysis revealed that gross N mineralization and related functional gene abundances of *sub*, *chiA* and *exo-chi* were positively associated with the mass proportion of macroaggregates, MWD, pH, SOC, POC, TN, DON, AP, MBC, MBN, and LAP and NAG enzyme activities (Appendix D). Manure amendment significantly increased denitrification functional gene abundances (*nirK*, *nirS* and *nosZ*) and the (*nirK*+*nirS*)/*nosZ* ratio by increasing soil MWD, pH, concentrations of POC, TN, NO_3^- and AP, and soil respiration (Appendix D).

4. Discussion

Our study showed that compared to inorganic fertilizer alone, manure amendment strongly increased N_2O emissions (by 20-fold on average), with the positive effects being even more pronounced when manure was combined with inorganic fertilizer, in an acidic upland soil (P<0.05; Figs. 1 and 5). These findings aligned with some previous studies that manure amendment of acidic soils greatly increases N_2O emission (Cheng *et al.* 2021; Wu *et al.* 2024). The increases in N_2O emissions by manure amendment were primarily attributed to shifts in soil substrate availability (including available C, N and P) and pH, both of which are critical to the

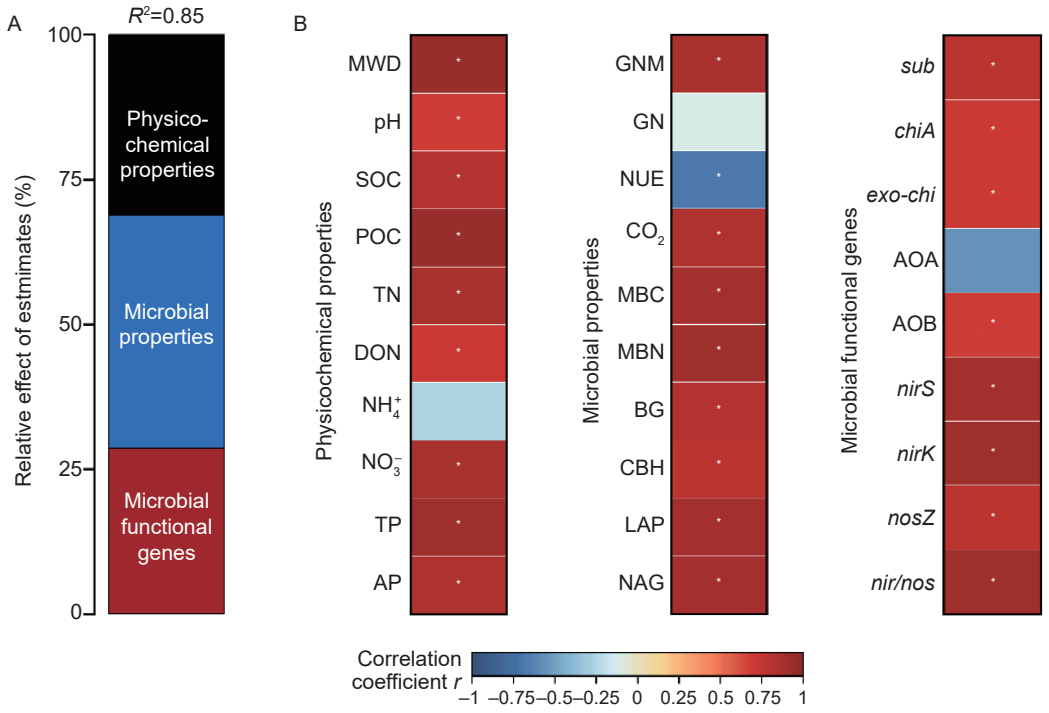


Fig. 3 Effects of soil biophysicochemical properties and microbial N cycling functional genes on N_2O emissions in soil subjected to long-term fertilization treatments. A, variance partitioning analyses for soil biophysicochemical properties and microbial N cycling functional gene effects on N_2O emissions. B, Pearson correlation matrix heatmap between N_2O emissions and soil biophysicochemical properties and microbial N cycling functional genes, with the color indicating the strength and direction of the relationship. MWD, mean weight diameter; SOC, soil organic carbon; POC, particle organic carbon; TN, total nitrogen; DON, dissolved organic nitrogen; TP, total phosphorus; AP, available phosphorus; GNM, gross nitrogen mineralization rate; GN, gross nitrification rate; NUE, nitrogen use efficiency; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; BG, β-1,4-glucosidase; CBH, cellobiohydrolase; LAP, leucine aminopeptidase; NAG, β-1,4-N-acetylglucosaminidase; AOA, gene copies of ammonia oxidation archaea; AOB, gene copies of ammonia oxidation bacteria. * indicates relationships are significant at P<0.05.

regulation of soil N mineralization and subsequent nitrification and denitrification, ultimately affecting N_2O emission (Figs. 4 and 5).

Compared to the inorganic fertilization treatments, manure amendment significantly increased soil C and N availability (Table 1; Fig. 5), thus enhancing microbial N mineralization and denitrification-derived N_2O emission (Wu *et al.* 2020; Wu *et al.* 2024). This was evidenced by the fact that the N_2O emissions were strongly and positively correlated with TN, NO_3^- and POC concentrations, but unrelated to soil NH_4^+ concentration (Figs. 3 and 4). This phenomenon suggests that NO_3^- served as the primary substrate for N_2O production *via* heterotrophic denitrification-mediated processes under manure amendment (Wu *et al.* 2020, 2022). High soil NO_3^- concentration could also inhibit N_2O reduction to N_2, due to the preference for NO_3^- as a terminal electron acceptor during denitrification (Pan *et al.* 2013; Senbayram *et al.* 2019, 2022). Besides C and N availability, our results demonstrated that the N_2O emissions were positively linked to available P concentration (Fig. 3), suggesting the important role of soil P availability in regulating N_2O production (Wei *et al.* 2017). P availability controls microbial activity due to the P requirement for the synthesis of genetic and cellular components, metabolism and energy transfer (Duan *et al.* 2024). High P availability promotes N transformation processes, leading to increased N_2O emission (Mehnaz and Dijkstra 2016; Mehnaz *et al.* 2018). Positive relationships between available P and gross N mineralization, and the functional genes related to N mineralization and denitrification processes were found in our study (Appendix D), providing mechanistic insights into the impacts of P availability on microbial N cycling and N_2O emission (Mehnaz and Dijkstra 2016; Wei *et al.* 2017). Our study highlights the need for in-depth studies on the linkage between soil N and P transformation, in order to improve our understanding of N_2O emissions from agroecosystems. Overall, these results demonstrate that the increased N_2O emissions following manure amendment largely resulted from increased substrate availability favoring microbial N mineralization and denitrification processes (Figs. 4 and 5).

Long-term fertilization affects microbial gross N mineralization and NUE, thus regulating N availability for nitrification and denitrification-derived N_2O production (Wang *et al.* 2023; Xu *et al.* 2023). The low gross N mineralization rates and high NUE limited N availability and lowered N_2O emissions in the unfertilized and inorganic fertilization treatments (Table 1; Figs. 2 and 3; Appendix C). The large N_2O emissions under manure amendment partly resulted from the decreased microbial NUE and enhanced gross mineralization rates increasing N availability (Xu *et al.* 2023). Soil pH was positively related to gross N mineralization, suggesting the importance of acidification alleviation in enhancing N mineralization (Appendix D), thus increasing soil

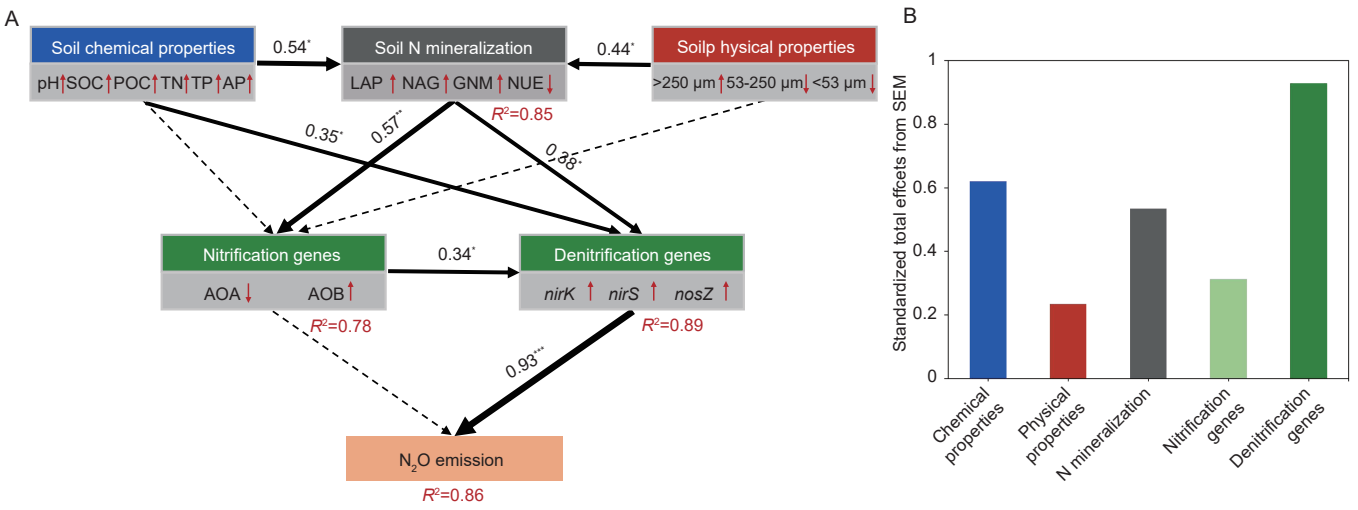


Fig. 4 Structural equation model (SEM) describing the mechanism pathways of N_2O emissions driven by long-term fertilization (A), and the standardized effects on N_2O emissions derived from SEM (B). SOC, soil organic carbon; POC, particle organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus; LAP, leucine aminopeptidase; NAG, β-1,4-N-acetylglucosaminidase; GNM, gross nitrogen mineralization rate; NUE, nitrogen use efficiency; AOA, gene copies of ammonia oxidation archaea; AOB, gene copies of ammonia oxidation bacteria; *nirK*, genes encoding nitrite reductases; *nirS*, genes encoding nitrite reductases; *nosZ*, genes encoding nitrous oxide reductases. The width of arrows is proportional to the strength of path coefficients (standardized correlation coefficients). Continuous and dashed arrows represent significant and insignificant relationships, respectively. Values adjacent to arrows represent standardized path coefficients. R^2 denotes the proportion of variance explained. The factors listed in the rectangles with "↑" or "↓" indicate a positive or negative correlation between the factors and the first component from the PCA. *, ** and *** indicate significance at $P<0.05$, $P<0.01$, and $P<0.001$, respectively. The goodness-of-fit was used to assess the model.

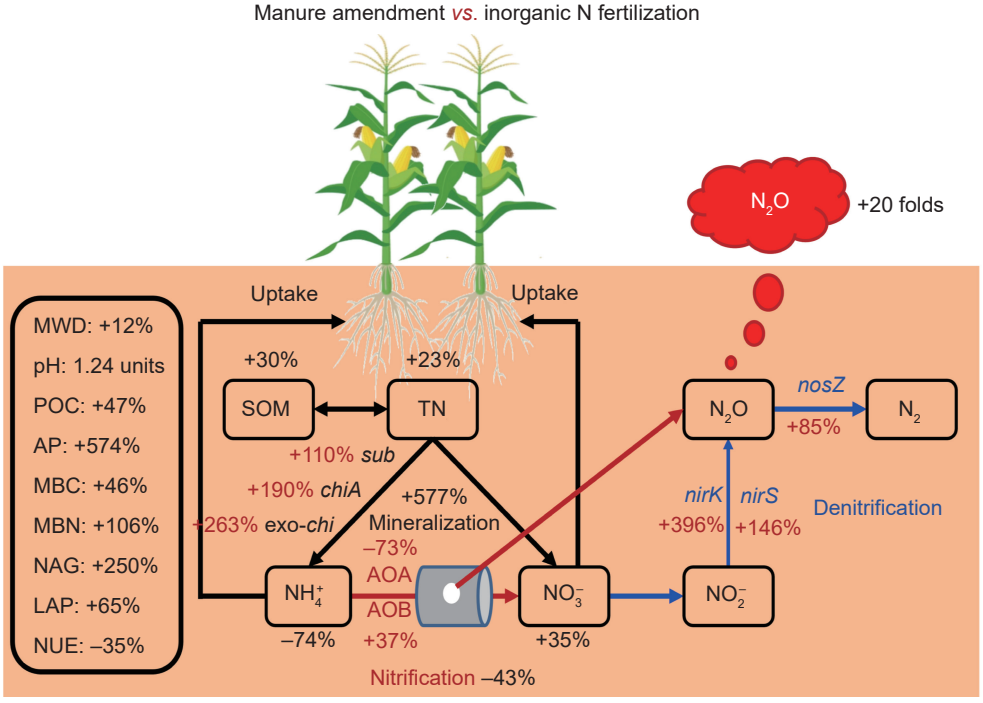


Fig. 5 Conceptual diagram illustrating the pathways of N_2O emissions associated with soil N transformation processes, N-cycling genes and biophysicochemical properties for manure amendment relative to inorganic N fertilization treatments for a maize cropping system in acidic soil. MWD, mean weight diameter; SOM, soil organic matter; TN, total nitrogen; POC, particle organic carbon; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; NAG, β-1,4-N-acetylglucosaminidase; LAP, leucine aminopeptidase; NUE, nitrogen use efficiency. The black arrows refer to the nitrogen mineralization process, red arrows refer to the nitrification process, and blue arrows refer to the denitrification process. The numbers adjacent to the parameters represent changes for the manure-amendment relative to inorganic N fertilization treatments.

N supply capacity (Wang *et al.* 2023). Manure amendment alleviated the inhibition effect of acidification on microbial metabolism, creating favorable conditions for N mineralization and supply, thereby increasing N_2O emissions in acidic soils

(Qu *et al.* 2014; Zhou *et al.* 2017; Cheng *et al.* 2021). Our study highlights the strong linkage between soil N_2O emission and N mineralization and NUE, with manure amendment largely increasing N availability for microbial N_2O production due to acidification alleviation (Figs. 4 and 5).

The differences in N_2O emission among fertilization treatments could also partly result from the niche differentiation of nitrifiers (AOA and AOB), due to fertilization-induced soil pH changes (Wu *et al.* 2020; Zhang *et al.* 2021). Soil pH correlated negatively with the abundance of AOA and positively with AOB, thus regulating the community composition of nitrifiers, with AOA abundance outnumbering AOB in acidic soils (He *et al.* 2012). However, the AOA abundance and gross nitrification rates were not correlated with N_2O emission (Fig. 3-B), implying that nitrification might have little direct contribution to N_2O emission as evidenced by the SEM result (Fig. 4). Furthermore, the AOB rather than AOA abundances were strongly and positively correlated with NO_3^- and N_2O emission (Fig. 3; Appendix D). These results indicate that nitrification acted as a key process for N_2O production indirectly through the supply of NO_3^- for denitrification under manure amendment (Zhou *et al.* 2017; Senbayram *et al.* 2019; Wu *et al.* 2023).

Denitrification was the predominant pathway responsible for the increased N_2O emission by manure amendment in the present study (Figs. 4 and 5), primarily attributed to the following lines of evidence. First, manure amendment greatly increased the abundances of denitrifying genes (*nirK* and *nirS*) and the (*nirK*+*nirS*)/*nosZ* ratio and consequent N_2O emissions, as evidenced by the direct and positive relationships between N_2O emissions and *nirK*, *nirS* and (*nirK*+*nirS*)/*nosZ* ratio (Figs. 2-H–J, 3-B and 4). These results indicated that manure amendment was conducive to the growth and metabolism of denitrifiers, and altered the composition of denitrifying community (Fig. 5), thus favoring N_2O-producing over N_2O-consuming denitrifiers (Zhou *et al.* 2017; Cheng *et al.* 2021). Second, changes in soil pH explained the variation of denitrification-related genes and consequent N_2O emissions among fertilization treatments (Fig. 3-B; Appendix D). The decreased soil pH by inorganic fertilization inhibited the *nosZ* genes, likely decreasing N_2O consumption and thereby enhancing N_2O emissions (Raut *et al.* 2012; Wu *et al.* 2022). Elevated soil pH by manure amendment has the potential to reduce N_2O emissions, due to the increased *nosZ* gene abundances resulting in N_2O consumption (Qu *et al.* 2014; Tang *et al.* 2024). In contrast, as revealed by our results, raising the pH of acidic soils by manure amendment significantly increased N_2O emissions, due to the increased *nirK* and *nirS* abundances and a higher (*nirK*+*nirS*)/*nosZ* ratio inducing increased N_2O production, outweighing N_2O consumption (Senbayram *et al.* 2019). Third, manure amendment potentially increases labile C and N supply, provides sufficient electrons and creates favorable conditions for denitrifiers to support N_2O production (Zhou *et al.* 2017). Our results showed a significant and positive correlation between POC and the abundances of *nirK* and *nirS* genes and the (*nirK*+*nirS*)/*nosZ* ratio (Appendix D), suggesting that POC was the main energy source and electron donor triggering the denitrifying community structure and activities for N_2O production. The increased POC by manure amendment facilitates increased N_2O emissions indirectly by stimulating heterotrophic respiration inducing O_2 depletion, thus creating local anaerobic microsites conducive to denitrification–derived N_2O production (Hayakawa *et al.* 2009; Jahangir *et al.* 2012; Li *et al.* 2016; Wu *et al.* 2022). This interpretation is supported by the increased CO_2 emissions and their positive association with N_2O emissions (Fig. 3-B; Appendix B). Manure amendment also increased the mass proportion of macroaggregates, thus facilitating N_2O production from denitrification by promoting aggregation formation when excess N substrate was available (Wang *et al.* 2019; Jiang *et al.* 2021). The interactions of high NO_3^- availability along with sufficient available C and optimal soil aggregation stimulated denitrification-derived N_2O emissions under manure amendment (Weier *et al.* 1993; Zhou *et al.* 2017).

In summary, compared to the unfertilized and inorganic fertilization treatments, long-term manure amendment significantly increased substrate availability, alleviated soil acidification, and enhanced the abundances of functional genes related to N mineralization and denitrification, consequently resulting in higher N_2O emissions from acidic upland soils (Fig. 5). These findings suggest that the potential benefits of manure amendment for soil carbon sequestration should consider the offsetting effects of increased N_2O emission when evaluating overall GHG balance (Zhou *et al.* 2017; Liao *et al.* 2024). Our study provides a mechanistic understanding of manure amendment in mediating N transformation processes that lead to cascading effects on N_2O emissions from an acidic upland soil. These findings are helpful in developing effective N fertilizer management strategies, so as to curtail reactive N emissions while maintaining soil fertility and ensuring crop production within agricultural ecosystems. However, some limitations still exist and need to be discussed. First, it is worth noting that we inferred the changes in denitrification from the variation of functional genes, but did not directly detect the denitrification rates and their linkage with N_2O emissions. Second, the results reported here were derived from one site for acidic upland soil. Future research involving multiple field conditions with simultaneous and repeated measurements of N_2O emissions and N transformation processes over time is warranted to verify the robustness and applicability of results. Third, further field investigations should consider the integration of multiple measurement techniques, including isotopic tracer techniques coupled with metagenomic tools and inhibitor methods, to thoroughly understand the complexity of microbial interactions regulating N cycling processes and consequent N_2O emissions in response to manure amendment.

5. Conclusion

Long-term manure amendment strongly increased N_2O emissions in an acidic upland soil, largely resulting from increased substrate availability, alleviated acidification and enhanced aggregate stability, thus enhancing microbial N mineralization and favoring N_2O-producing denitrifiers

over N_2O consumers. Denitrification was the predominant pathway responsible for the increased N_2O emissions upon manure amendment. This was supported by the significant increases in abundances of denitrifying genes (*nirK* and *nirS*) and the (*nirK* + *nirS*)/*nosZ* ratio, along with sufficient C and NO_3^- availability from manure amendment, all of which positively correlated with N_2O emission. Collectively, these results suggest that long-term manure amendment potentially enhances N_2O emission from acidic soils, primarily due to increased substrate availability and alleviated soil acidification, both of which are conducive to the stimulation of gross N mineralization and denitrifier-mediated N_2O production.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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References

Brookes P, Landman A, Pruden G, Jenkinson D. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Butterbach-Bahl K, Baggs E M, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. 2013. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society*, **368**, 20130122.

Cheng Y, Zhang H, Chen Z, Wang J, Cai Z, Sun N, Wang S, Zhang J, Chang S X, Xu M, Cai Z, Müller C. 2021. Contrasting effects of different pH-raising materials on N_2O emissions in acidic upland soils. *European Journal of Soil Science*, **72**, 432–445.

Duan S, Feng G, Limpens E, Bonfante P, Xie X, Zhang L. 2024. Cross-kingdom nutrient exchange in the plant–arbuscular mycorrhizal fungus–bacterium continuum. *Nature Reviews Microbiology*, **22**, 773–790.

Elliott E T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil Science Society of America Journal*, **50**, 627–633.

Guenet B, Gabrielle B, Chenu C, Arrouays D, Balesdent J, Bernoux M, Bruni E, Caliman J P, Cardinael R, Chen S, Ciais P, Desbois D, Fouche J, Frank S, Henault C, Lugato E, Naipal V, Nesme T, Obersteiner M, Pellerin S, *et al.* 2021. Can N_2O emissions offset the benefits from soil organic carbon storage? *Global Change Biology*, **27**, 237–256.

Hart S C, Nason G E, Myrold D D, Perry D A. 1994. Dynamics of gross nitrogen transformations in an old-growth forest: The carbon connection. *Ecology*, **75**, 880–891.

Hayakawa A, Akiyama H, Sudo S, Yagi K. 2009. N_2O and NO emissions from an Andisol field as influenced by pelleted poultry manure. *Soil Biology and Biochemistry*, **41**, 521–529.

He J Z, Hu H W, Zhang L M. 2012. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. *Soil Biology and Biochemistry*, **55**, 146–154.

Hu H W, Chen D, He J Z. 2015. Microbial regulation of terrestrial nitrous oxide formation: Understanding the biological pathways for prediction of emission rates. *FEMS Microbiology Reviews*, **39**, 729–749.

IPCC (Intergovernmental Panel on Climate Change). 2021. Climate Change 2021: The Physical Science Basis. In: Masson-Delmotte V, Zhai P, Pirani A, Connors S L, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis M I, Huang M, Leitzell K, Lonnoy E, Matthews J B R, Maycock T K, Yelekçi W T O, Yu R, Zhou B, eds., *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Jahangir M M R, Khalil M I, Johnston P, Cardenas L M, Hatch D J, Butler M, Barrett M, O'Flaherty V, Richards K G. 2012. Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. *Agriculture*, *Ecosystems & Environment*, **147**, 13–23.

Jiang M, Yang N, Zhao J, Shaaban M, Hu R. 2021. Crop straw incorporation mediates the impacts of soil aggregate size on greenhouse gas emissions. *Geoderma*, **401**, 115342.

Kong D, Zhang X, Yu Q, Jin Y, Jiang P, Wu S, Liu S, Zou J. 2024. Mitigation of N_2O emissions in water-saving paddy fields: Evaluating organic fertilizer substitution and microbial mechanisms. *Journal of Integrative Agriculture*, **23**, 3159–3173.

Li X, Sorensen P, Olesen J E, Petersen S O. 2016. Evidence for denitrification as main source of N_2O emission from residue-amended soil. *Soil Biology and Biochemistry*, **92**, 153–160.

Liang F, Guo Y, Liu A, Wang Y, Cao W, Song H, Li B, Chen J, Guo J. 2024. Is partial substitution of animal manure for synthetic fertilizer a viable N_2O mitigation option? An integrative global meta-analysis. *Field Crops Research*, **318**, 109574.

Liao X, Müller C, Sun H, Yuan J, Liu D, Chen Z, He T, Jansen-Willems A, Luo J, Ding W. 2024. Increases of N_2O emissions due to enhanced nitrification in a sandy loam soil under long-term manure application. *Biology and Fertility of Soils*, **60**, 1127–1141.

Lu R. 1999. *Chemical Analysis of Agricultural Soil*. China Agricultural Science and Technology Press, Beijing. (in Chinese)

Maaz T M, Sapkota T B, Eagle A J, Kantar M B, Bruulsema T W, Majumdar K. 2021. Meta-analysis of yield and nitrous oxide outcomes for nitrogen management in agriculture. *Global Change Biology*, **27**, 2343–2360.

Marx M C, Wood M, Jarvis S C. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry*, **33**, 1633–1640.

Mehnaz K R, Dijkstra F A. 2016. Denitrification and associated N_2O emissions are limited by phosphorus availability in a grassland soil. *Geoderma* , **284**, 34–41.

Mehnaz K R, Keitel C, Dijkstra F A, 2018. Effects of carbon and phosphorus addition on microbial respiration, N_2O emission, and gross nitrogen mineralization in a phosphorus-limited grassland soil. *Biology and Fertility of Soils*, **54**, 481–493.

Murphy D V, Recous S, Stockdale E A, Fillery I R P, Jensen L S, Hatch D J, Goulding K W T. 2003. Gross nitrogen fluxes in soil: Theory, measurement and application of ^{15}N pool dilution techniques. *Advances in Agronomy*, **79**, 69–118.

Murphy J, Riley J P. 1962. Modified single solution method for the determination of phosphate in natural water. *Analytica Chimica Acta*, **27**, 31–36.

Pan Y, Ni B, Bond P L, Ye L, Yuan Z. 2013. Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water Research*, **47**, 3273–3281.

Qu Z, Wang J G, Almøy T, Bakken L R. 2014. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. *Global Change Biology*, **20**, 1685–1698.

Raut N, Dörsch P, Sitaula B K, Bakken L R. 2012. Soil acidification by intensified crop production in South Asia results in higher $N_2O/(N_2+N_2O)$ product ratios of denitrification. *Soil Biology and Biochemistry*, **55**, 104–112.

Ravishankara A, Daniel J S, Portmann R W. 2009. Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science*, **326**, 123–125.

Senbayram M, Budai A, Bol R, Chadwick D, Marton L, Gündogan R,

Wu D. 2019. Soil NO_3^- level and O_2 availability are key factors in controlling N_2O reduction to N_2 following long-term liming of an acidic sandy soil. *Soil Biology and Biochemistry*, **132**, 165–173.

Senbayram M, Wei Z, Wu D, Shan J, Yan X, Well R. 2022. Inhibitory effect of high nitrate on N_2O reduction is offset by long moist spells in heavily N loaded arable soils. *Biology and Fertility of Soils*, **58**, 77–90.

Tang Q, Moeskjær S, Cotton A, Dai W, Wang X, Yan X, Daniell T J. 2024. Organic fertilization reduces nitrous oxide emission by altering nitrogen cycling microbial guilds favouring complete denitrification at soil aggregate scale. *Science of the Total Environment*, **946**, 174178.

Tian H, Xu R, Canadell J G, Thompson R L, Winiwarter W, Suntharalingam P, Davidson E A, Ciais P, Jackson R B, Janssens-Maenhout G, Prather M J, Regnier P, Pan N, Pan S, Peters G P, Shi H, Tubiello F N, Zaehle S, Zhou F, Arneth A, *et al.* 2020. A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, **586**, 248–256.

Wan S, Lin Y, Fan J, Hu H W, Zhang J, Jin S S, Deng M, Müller C, He J Z. 2023. Different responses of nitrous oxide emissions to liming and manure amendment of an acidic ultisol are controlled by autotrophic and heterotrophic nitrification. *Soil Biology and Biochemistry*, **178**, 108960.

Wang B, Brewer P E, Shugart H H, Lerdau M T, Allison S D. 2019. Soil aggregates as biogeochemical reactors and implications for soil ― atmosphere exchange of greenhouse gases ― A concept. *Global Change Biology*, **25**, 373–385.

Wang J, Wu L, Xiao Q, Huang Y, Liu K, Wu Y, Li D, Duan Y, Zhang W. 2023. Long-term manuring enhances soil gross nitrogen mineralization and ammonium immobilization in subtropical area. *Agriculture, Ecosystems & Environment*, **348**, 108439.

Wei X, Hu Y, Peng P, Zhu Z, Atere C T, O'Donnell A G, Wu J, Ge T. 2017. Effect of P stoichiometry on the abundance of nitrogen-cycle genes in phosphorus-limited paddy soil. *Biology and Fertility of Soils*, **53**, 767–776.

Weier K L, Doran J W, Power J F, Walters D T. 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal*, **57**, 66–72.

WRB (World Reference Base for Soil Resources). 2014. *World Soil Resources Reports 106*. Food and Agriculture Organization of the United Nations, Rome.

Wu G, Liang F, Wu Q, Feng X G, Shang W D, Li H W, Li X X, Che Z, Dong Z R, Song H. 2024. Soil pH differently affects N_2O emissions from soils amended with chemical fertilizer and manure by modifying nitrification and denitrification in wheat–maize rotation system. *Biology and Fertility of Soils*, **60**, 101–113.

Wu J, Joergensen R G, Pommerening B, Chaussod R, Brookes P C. 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biology and Biochemistry*, **22**, 1167–1169.

Wu L, Hu R G, Tang S R, Shaaban M, Zhang W J, Shen H P, Xu M G. 2020. Nitrous oxide emissions in response to straw incorporation is regulated by historical fertilization. *Environmental Pollution*, **266**, 115292.

Wu L, Tang S R, He D D, Wu X, Shaaban M, Wang M L, Zhao J S, Khan I, Zheng X H, Hu R G. 2017. Conversion from rice to vegetable production increases N_2O emission via increased soil organic matter mineralization. *Science of the Total Environment*, **583**, 190–201.

Wu L, Tang S R, Hu R G, Wang J, Duan P P, Xu C, Zhang W J, Xu M G. 2023. Increased N_2O emission due to paddy soil drainage is regulated by carbon and nitrogen availability. *Geoderma*, **432**, 116422.

Wu L, Xiao Q, Wang J, Huang Y P, Wu D, Liu J, Wang B R, Zhang H M, Xu M G, Zhang W J. 2022. Liming decreases the emission and temperature sensitivity of N_2O following labile carbon addition. *Geoderma*, **425**, 116032.

Wu L, Zhang W J, Wei W J, He Z L, Kuzyakov Y, Bol R, Hu R G. 2019. Soil organic matter priming and carbon balance after straw addition is regulated by long-term fertilization. *Soil Biology and Biochemistry*, **135**, 383–391.

Xu C, Zhu H, Wang J, Ji C, Liu Y, Chen D, Zhang H, Wang J, Zhang Y. 2023. Fertilizer N triggers native soil N-derived N_2O emissions by priming gross N mineralization. *Soil Biology and Biochemistry*, **178**, 108961.

Xu R, Tian H, Pan S, Prior S A, Feng Y, Dangal S R S. 2020. Global N_2O emissions from cropland driven by nitrogen addition and environmental factors: Comparison and uncertainty analysis. *Global Biogeochemical Cycles*, **34**, e2020GB006698.

Yu W, Huang W, Weintraub-Leff S R, Hall S J. 2022. Where and why do particulate organic matter (POM) and mineral-associated organic matter (MAOM) differ among diverse soils? *Soil Biology and Biochemistry*, **172**, 108756.

Zhang X, Fang Q, Zhang T, Ma W, Velthof G L, Hou Y, Oenema O, Zhang F. 2020. Benefits and trade-offs of replacing synthetic fertilizers by animal manures in crop production in China: A meta-analysis. *Global Change Biology*, **26**, 888–900.

Zhang Y, Zhao J, Huang X, Cheng Y, Cai Z, Zhang J, Müller C. 2021. Microbial pathways account for the pH effect on soil N_2O production. *European Journal of Soil Biology*, **106**, 103337.

Zhou M, Zhu B, Wang S, Zhu X, Vereecken H, Brüggemann N. 2017. Stimulation of N_2O emission by manure application to agricultural soils may largely offset carbon benefits: A global meta-analysis. *Global Change Biology*, **23**, 4068–4083.

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