Effects of salt stress on nitrification and denitrification rates and N_2O emissions in soil using a ^{15}N isotope tracing approach

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Abstract: Nitrous oxide (N_2O), a long-lived greenhouse gas, is primarily produced in agricultural soils through biological nitrification and denitrification processes. However, the effects of soil salinity on nitrogen transformation processes remain insufficiently understood, hindering the development of effective nitrogen management in salt-affected farmlands. In this study, laboratory incubation experiments combined with a ^{15}N stable isotope tracing technique were conducted to quantify the effects of salt stress on nitrification and denitrification rates and their contributions to N_2O emissions. The results showed that during the first week of incubation, slight and moderate salinity (NaCl contents of 0.04% and 0.10%; $EC_{1:5}$≤1.10 dS/m) enhanced both nitrification and denitrification rates, whereas strong salinity (0.20% NaCl; $EC_{1:5}$≥1.42 dS/m) inhibited these processes. In the first week of incubation, nitrification and denitrification contributed approximately 65%-70% and 30%-35% to the total N_2O emissions under 60% water-filled pore space, respectively. These results indicate that nitrification represents the predominant source of N_2O production in saline soils during the first week following fertigation. The findings suggest that nitrogen management practices for inhibiting the nitrification process (e.g., the addition of nitrification inhibitors in companion with fertigation) may mitigate N_2O emissions in saline fields. This provides a scientific basis for optimizing nitrogen management in saline soils and reducing greenhouse gas emissions.

Keywords: nitrogen transformation, soil salinity, soil N_2O emissions, stable isotope tracer
DOI: 10.25165/j.ijabe.20261903.10307

Citation: Du L J, Pan C Z, Wang C Y, Chen Z J, Xiong Y W, Huang G H. Effects of salt stress on nitrification and denitrification rates and N_2O emissions in soil using a ^{15}N isotope tracing approach. Int J Agric & Biol Eng, 2026; 19(3): 191–197.

1 Introduction

Global warming is one of the most critical environmental challenges facing humanity, primarily driven by the massive greenhouse gas (GHG) emissions. Among these greenhouse gases, nitrous oxide (N_2O) is a long-lived and potent GHG that not only contributes significantly to global warming but also catalyzes the depletion of the stratospheric ozone layer[1]. Existing studies have shown that N_2O remains in the atmosphere for approximately 116 years and has a global warming potential about 300 times greater than that of carbon dioxide over a 100-year period[2]. Therefore, the reduction of N_2O emissions is crucial for achieving climate change mitigation goals.

Agricultural soils represent a primary anthropogenic source of N_2O emissions, driven by microbially mediated nitrification and denitrification processes[1,3]. While synthetic nitrogen (N) fertilizers support the nutritional needs of nearly half the global population, their utilization is strikingly inefficient[4,5]. Currently, only about 47% of the applied nitrogen fertilizer is converted into harvested products[6], while the remainder is lost to the environment through leaching and gaseous emissions[7,8]. The environmental consequences of these N losses, however, are increasingly compounded by concurrent land degradation processes, most notably soil salinization.

Soil salinization is an escalating global crisis, closely intertwined with climate change and unsustainable irrigation[9]. Rising temperatures and shifting precipitation patterns accelerate salt accumulation, particularly in arid and semi-arid regions, by increasing surface evaporation and reducing leaching efficiency[10,11]. It is estimated that more than 900×10^6 hm^2 of land (approximately 20% of the cultivated lands and 33% of irrigated lands) worldwide is affected by various degrees of salinity stress, posing a serious threat to global food security[12]. Under these constrained conditions, achieving a synergy among crop productivity, environmental sustainability, and economic profitability becomes increasingly complex.

To sustain productivity amidst growing food demand, farmers in salt-affected regions are often compelled to increase N inputs to compensate for the low N availability and limited microbial N-fixation capacity inherent in saline soils[13]. However, excessive nitrogen application further exacerbates soil-borne N_2O emissions, which are regulated by a complex interplay of soil texture, moisture, and salinity[14,15]. Despite the urgency of managing nitrogen in these sensitive ecosystems, the precise response of N_2O emissions to salinity remains a subject of intense scientific debate. For instance,

Received date: 2025-10-31 **Accepted date:** 2026-05-07
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Ghosh et al.[16] observed a positive correlation between soil electrical conductivity (ECe, 0.44-7.2 dS/m) and N_2O emissions in laboratory incubations, whereas Zhang et al.[17] reported a negative correlation between salinity and cumulative N_2O emissions over 15 d. Conversely, Jia et al.[18] found that cumulative N_2O emissions increased with salinity under nitrogen fertilization treatments. Similarly, Li et al.[19] reported that in field conditions, moderate salinity (1.0 dS/m) resulted in significantly higher N_2O emissions than both non-saline and high-salinity (5.0 dS/m) treatments, suggesting the existence of a threshold effect of salinity on soil N_2O emissions. A critical limitation of these existing studies is their reliance on total N_2O flux measurements, which fail to distinguish between the specific mechanistic contributions of nitrification and denitrification. Understanding these individual pathways is essential for developing targeted mitigation strategies in salt-affected croplands.

To address this knowledge gap, the present study aimed to quantify the effects of salt stress on nitrification and denitrification processes and their contributions to N_2O emissions using a ^{15}N stable isotope tracer technique. This approach enables direct partitioning of N_2O sources between nitrification and denitrification, providing novel insights into nitrogen transformation dynamics in saline agroecosystems. These findings are expected to offer a theoretical basis for optimizing nitrogen management and mitigating N_2O emissions in salt-affected agricultural regions.

2 Materials and methods

2.1 Soil sample collection

Soil samples were collected from a sunflower field in the Hetao Irrigation District (41°09′N, 107°39′E), a representative region of soil salinization dominated by sodium chloride (NaCl) due to shallow groundwater and intensive evaporation[20]. Soils at a depth of 0-15 cm were collected. The nitrogen application rate in the field was 250 kg/hm^2 of N in previous cultivation. The initial physicochemical properties of the soil are presented in Table 1.

Table 1 Physical and chemical properties of the tested soil

Soil property	Value
Sand/%	46.16
Silt/%	43.54
Clay/%	10.30
Texture class	Loam
pH	7.03
$EC_{1:5}$/dS·m^{-1}	0.32
NO_3-N/mg·kg^{-1}	46.21
NH_4^+-N/mg·kg^{-1}	8.02

2.2 Experimental setup

The incubation experiments were conducted in a controlled environment. The experiments were established in 500 mL Kirner jars with gas-tight caps and gas sampling ports. Sodium chloride (NaCl) was added to soil to generate three soil salinity levels, i.e., 0.04% (slight salinity), 0.10% (moderate salinity), and 0.20% (strong salinity) of NaCl content in soil (g/g). The soil without NaCl addition served as the control (non-saline). Each treatment had six replicates, three replicates used for gas sampling and three replicates for soil sampling. After thoroughly mixing different concentrations of NaCl with soil, 210 g of soil samples were packed into 500 mL incubation bottles with the bulk density of 1.4 g/cm^3. Each bottle was filled to a volume of 150 mL. After packing, the soil surface area was 58 cm^2. The bottles were then numbered, and deionized water was uniformly sprayed onto the soil surface using a 10 mL syringe to achieve a soil with water-filled pore space (WFPS) of 40%. The bottles were sealed with perforated sealing film and placed in a constant temperature and humidity incubator at (25±2)°C and 50% relative humidity in the dark for 7 d for pre-incubation to ensure uniform soil moisture distribution and activate soil microorganisms. Salinity treatments were established prior to the pre-incubation phase, during which soil moisture was kept at 40% WFPS.

Subsequent to pre-incubation, $NH_4{}^{15}NO_3$ (10 atom% ^{15}N enrichment in NO_3^--N) was dissolved in deionized water and applied uniformly to the soil surface using a syringe at a rate equivalent to 250 kg N/hm^2. Subsequently, deionized water was used to adjust the soil water-filled pore space to 60%. The bottles were then hermetically sealed and incubated in the dark at (25±2)°C with 50% relative humidity. To minimize priming effects due to the addition of water, soil moisture adjustment was performed after gas sampling, and soil water-filled pore space was maintained at 60% throughout the formal incubation period[21].

2.3 Gas sample collection and analysis

The day of $NH_4{}^{15}NO_3$ application was considered as day 0 of incubation. Gas samples for analyzing atom% ^{15}N of N_2O-N were collected at 9:00-10:00 a.m. on days 1, 3, 5, and 7 of incubation. Each time 20 mL of gas sample was taken into a pre-vacuumed gas bag. Before gas sampling, a 100 mL syringe was pumped several times to ensure thorough gas mixing within the bottle headspace. After gas sampling, the bottle was opened to ventilate for 2-3 min before sealing and incubating in preparation for the collection of greenhouse gases. Gas samples for analyzing the atom% ^{15}N of N_2O-N were analyzed within 24 h using an isotope ratio mass spectrometer with a concentrator system (IRMS; Delta V Plus-Precon, Thermo Fisher Scientific, Bremen, Germany). The precision of atom% ^{15}N measurement was 0.5‰. The calculation formula for $\delta^{15}N$ is shown in Equation (1):

$$\delta^{15}\mathrm{N} = {}^{15}R^{i}_{sample} / {}^{15}R_{standard} \quad (1)$$

where, $\delta^{15}N$ denotes nitrogen isotope ratio of N_2O; ${}^{15}R^{i}_{sample}$ denotes the value of $^{15}N/^{14}N$ of N_2O samples; ${}^{15}R_{standard}$ denotes the value of $^{15}N/^{14}N$ of standard atmospheric N_2.

When the ^{15}N atomic percentage of N_2O was measured, international standards for ^{15}N atomic percentage provided by atmospheric N_2 was compared. The calibration was performed using a standard reference gas (Air Liquide America, Specialty gas LLC).

Gas samples for measuring greenhouse gas emissions were collected at 10:00 a.m.-12:00 p.m. on days 0, 1, 3, 5, 7, 10, and 14 during the incubation. Each sampling was completed within 2 hours, with 20 mL of gas sample taken and stored in pre-evacuated gas bags. After sampling, 20 mL of air was injected into the incubation bottle to ensure pressure balance. After each gas sampling operation, the bottle cap was opened, and the bottle was ventilated for 2-3 min before being sealed again for incubation. During the incubation period, the headspace air in the culture bottles was refreshed every 2 d. The gas samples were analyzed using a Shimadzu GC-2014 gas chromatograph (SHIMADZU, Japan) within 48 h. The greenhouse gas emission flux was calculated using Equation (2)[22]:

$$F = \frac{p_0}{p} \times \frac{273}{273+T} \times \frac{M}{V_m} \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \quad (2)$$

where, F is the N_2O emission flux, mg/m^2·h; p_0 is the atmospheric pressure at the experimental site, hPa; p is the local standard

atmospheric pressure (1013 hPa); and T is the average temperature during the incubation process, °C; M is the molar mass of N_2O (44 g/mol); V_m is the molar volume under standardized conditions (22.4 L/mol); V is the headspace volume of the incubation bottle, m^3; A is the cross-sectional area of the soil in the incubation bottle, m^2; and $\Delta c/\Delta t$ is the rate of change of N_2O concentration in the incubation bottle, ppm/h; Δt is 0.5 h. The experiment showed that Δc and Δt follow a linear relationship, with n=4 and $R^2 \in [0.85, 0.99]$. Therefore, this study considers it feasible to calculate N_2O flux values using linear regression.

2.4 Soil sample collection and analysis

Soils were destructively sampled on tdays 1, 3, 5, 7, 10, and 14 of incubation in soil sampling bottles. Sampling was done in a fan-shaped pattern to minimize variability among soil samples, and the samples were stored in labeled sterile self-sealing bags at 4°C. Soil pH and electrical conductivity values were measured using a soil-water ratio of 1:5. The atom% ^{15}N of NH_4^+-N and NO_3^--N in the soil were determined using an IRMS (Delta V Plus-Precon, Thermo Fisher Scientific, Bremen, Germany). The atom% ^{15}N of NH_4^+-N ($\delta^{15}N$-NH_4^+) measurement was performed using the diffusion method to collect NH_4^+, followed by analysis with an isotope ratio mass spectrometer and an elemental analyzer (EA-IRMS, isoprime100-vario PYRO cube, Elementar, Berlin, Germany) to measure $\delta^{15}N$ of NH_4^+-N. The denitrifier method (*Pseudomonas chlororaphis subsp. aureofaciens ATCC 13985*) was used to convert NO_3^- to N_2O for determining $\delta^{15}N$ of NO_3^--N ($\delta^{15}N$-NO_3^-). The calibration standards for NH_4^+ were IAEA-N-1 and IAEA-N-2, and for NO_3^- the calibration standards were USGS32, USGS34, and USGS35[23].

2.5 Determination of the contribution of nitrification and denitrification to N_2O emissions

To quantify the amount of N_2O produced from the two distinct functional nitrogen pools, i.e., nitrification and denitrification pathways, a two-source isotope mixing model was employed (Equation 3). The f_d (denitrification pool, assumed to be equivalent to the NO_3^--N nitrogen pool) and f_n (nitrification pool, assumed to be equivalent to the NH_4^+-N nitrogen pool) represent the atom% ^{15}N in the denitrification pool and nitrification pool, respectively. It is assumed that the N_2O in the air at the headspace air of the incubation bottle prior to accumulation is negligible. f_m represents the atom% ^{15}N of the mixed N_2O-N produced from the two different nitrogen pools. The contributions of nitrification and denitrification to N_2O emissions were calculated using Equation (3)[24]:

$$f_m = d \times f_d + (1-d) \times f_n \tag{3}$$

where, f_d represents the ^{15}N atoms percentage in NO_3^--N ($\delta^{15}N$-NO_3^-); f_n represents the ^{15}N atoms percentage in NH_4^+-N ($\delta^{15}N$-NH_4^+); f_m represents the ^{15}N atoms percentage in N_2O ($\delta^{15}N$-N_2O) produced from the two different nitrogen pools; d represents the contribution of denitrification to N_2O emissions; and 1–d represents the contribution of nitrification to N_2O emissions.

2.6 Calculation of nitrogen transformation rates

Based on the principle of isotope dilution and the conceptual model of nitrogen transformation, gross nitrification and denitrification rates in soils with different salinity levels were calculated. The detailed mathematical principles of this conceptual model can be found in Di et al.[25]. Since this experiment was conducted as a laboratory incubation experiment, atmospheric nitrogen deposition was neglected. Throughout the incubation period, no additional nitrogen fertilizer was added except for the initial addition of $NH_4{}^{15}NO_3$ at the start of the formal incubation. Therefore, F_1 will represent primarily the nitrification rate. Since there is no leaching of NO_3^--N and no nitrogen loss due to plant uptake, F_0 represents the sum of denitrification and microbial nitrogen immobilization rates. The calculation formula was:

$$F_1 = \left[(Q_1 - Q_n) \times \ln\left(\frac{A_1}{A_n}\right)\right] / [(t_n - t_1) \times \ln(Q_1/Q_n)] \tag{4}$$

$$F_0 = F_1 - [(Q_n - Q_1)/(t_n - t_1)] \tag{5}$$

where, F_1 represents the nitrification rate; F_0 represents the sum of denitrification rate and microbial immobilization (in this experiment, F_0 is approximately denoted as denitrification rate); Q_1 and Q_n represent the NO_3^--N content on day 1 and day n of incubation, respectively, mg/kg; A_1 and A_n represent the ^{15}N atomic percent excess of NO_3^--N on day 1 and day n of incubation, respectively; t_1 and t_n represent the first day and the n-th day of incubation.

2.7 Statistical analysis

A one-way analysis of variance (ANOVA) and the Tukey's post hoc test were used to analyze the effects of soil salinity on cumulative N_2O emissions and nitrogen transformation rates, with significance level set at p<0.05. Pearson correlation analysis was used to assess the correlation between salinity levels and the relative contributions of nitrification and denitrification to N_2O emissions. Statistical analysis was performed using SPSS 26.0 software (IBM SPSS Statistics, Chicago, USA).

3 Results

3.1 N_2O emission fluxes

Figure 1 presents the temporal variations in N_2O fluxes under different salinity levels. During the initial stage of incubation (days 1–3), N_2O fluxes in the moderate-salinity treatment (0.10% NaCl) were slightly higher (0.001–0.005 mg/m²·h) than those in the slight salinity treatment (0.04% NaCl). Similarly, the strong salinity treatment (0.20% NaCl) exhibited fluxes of 0.002–0.003 mg/m²·h, which also exceeded those in the slight salinity treatment. These initial fluctuations likely reflect a short microbial adaptation phase following salinity adjustment. Starting from day 5, N_2O fluxes across all treatments exhibited a general decline with increasing salinity levels. On day 14, however, N_2O fluxes were significantly higher than those recorded between days 1 and 10, suggesting that peak emissions likely occurred around this time. Nevertheless, the precise timing of the maximum flux could not be determined within the current observation window.

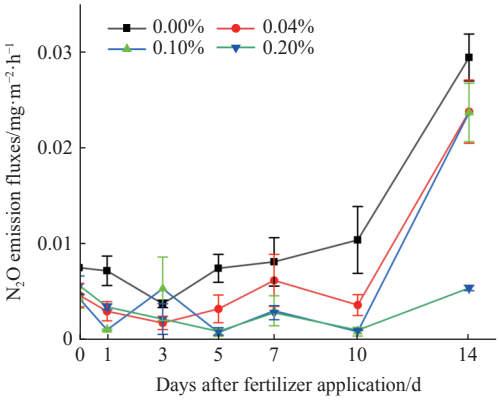
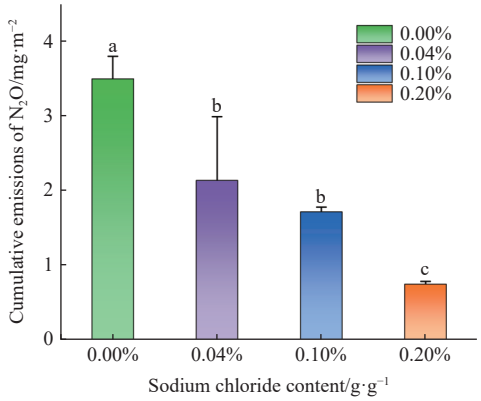


Figure 1 Temporal dynamics of N_2O fluxes under different salinity levels

Figure 2 illustrates the cumulative N_2O emissions over the 14 d incubation period. Overall, cumulative N_2O emissions decreased

with increasing salinity levels. The slight- and moderate-salinity treatments yielded cumulative emissions of 2.13 and 1.71 mg/m^2, representing 61.21% and 49.14% of the non-saline control (3.48 mg/m^2), respectively. The strong-salinity treatment showed the lowest cumulative emissions (0.74 mg/m^2), which were significantly lower than those of all other treatments (p<0.05).



Note: Different lowercase letters denote significant differences at p<0.05.

Figure 2 Cumulative N_2O emissions under different salinity treatments

3.2 Effects of salinity on nitrification and denitrification rates

Nitrification and denitrification rates exhibit a non-linear response to salinity (Figure 3). Under slight and moderate salinity (NaCl=0.04% and 0.10%; $EC_{1:5}$≤1.101 dS/m), both nitrification and denitrification rates were higher than those of the non-saline control. In contrast, strong salinity (NaCl=0.20%; $EC_{1:5}$≥ 1.421 dS/m) markedly inhibited both processes. This pattern indicates that mild salt stress may stimulate microbial activity and substrate availability, while excessive salinity suppresses microbial metabolism and oxygen diffusion.

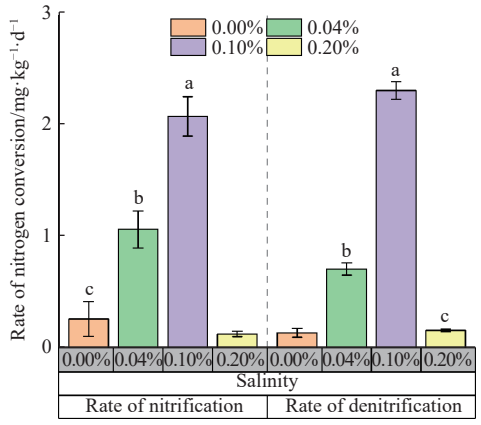
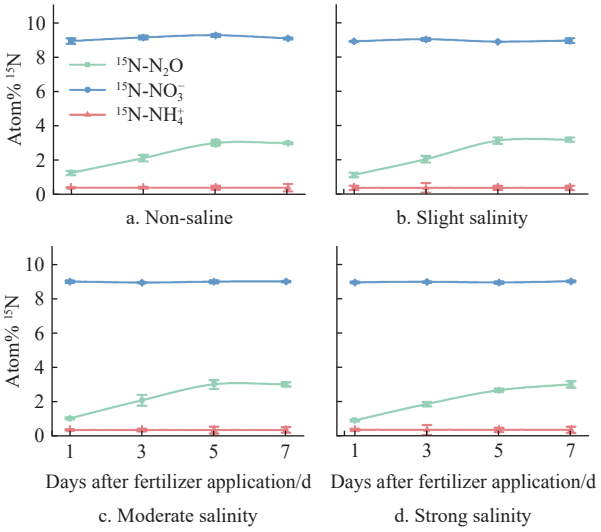


Figure 3 Effects of salinity on nitrification and denitrification rates (calculated from day 1 and day 7 data)

3.3 Contributions of nitrification and denitrification to N_2O emissions

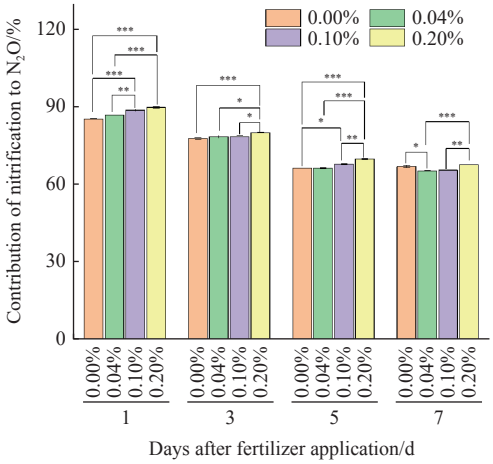
According to the isotope-dilution analysis, the ^{15}N abundance (atom%) of N_2O in all treatments was consistently bracketed by the values of the NO_3^- and NH_4^+ pools (Figure 4). Salinity had no significant influence on the ^{15}N abundance of N_2O, NO_3^-, or NH_4^+. During the incubation, the ^{15}N abundance of the NO_3^- pool stabilized at approximately 9 atom%. Meanwhile, the measured ^{15}N abundance of the NH_4^+ pool remained relatively stable, ranging from 0.3671 to 0.3701 atom% across all treatments, which was closely aligned with the natural abundance (0.3663 atom%). This indicates that N_2O was simultaneously derived from nitrification (the NH_4^+ pool) and denitrification (the NO_3^- pool).



Note: Vertical bars represent ±SE (n=3).

Figure 4 Temporal changes in ^{15}N enrichment (atom%) in NH_4^+, NO_3^-, and N_2O pools

The proportional contribution of nitrification to N_2O production decreased gradually with incubation time (Figure 5). During days 1-3, the relative contribution of nitrification followed an increasing trend with salinity, suggesting a transient stimulation by moderate salt levels. However, from day 5 onwards, both slight- and moderate-salinity treatments led to a decline in the nitrification-derived N_2O fraction compared with the non-saline control, reflecting a shift in the relative dominance of N_2O production pathways toward denitrification.



Note: *, **, *** indicate significance at p≤0.05, 0.01, and 0.001, respectively (n=3). (The same below.)

Figure 5 Effects of salinity on the contribution of nitrification to N_2O production

In contrast, the denitrification-derived N_2O fraction generally decreased with increasing salinity during the early incubation stage (days 1-3), but plateaued between days 5 and 7 (Figure 6). During this phase, the slight- and moderate-salinity treatments enhanced the proportional contribution of denitrification to 33.7%-34.7% and 32.2%-34.5%, respectively, compared with 30.2%-32.4% in the non-saline control. The strong-salinity treatment consistently inhibited denitrification-derived N_2O production, implying that intense ionic stress restricts the functional activity of the denitrification process.

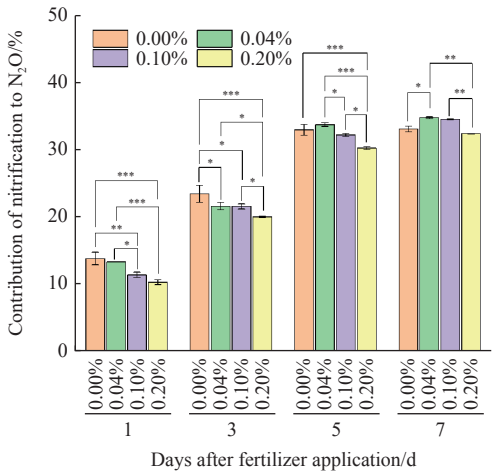


Figure 6 Effects of salinity on the contribution of denitrification to N_2O production

4 Discussion

4.1 Effect of salinity on the dynamics of N_2O emissions

As the incubation progressed, the inhibitory effect of salinity on N_2O fluxes became increasingly pronounced. N_2O emissions declined with increasing salinity, particularly during the day 5-14 period (Figure 1). Similarly, cumulative N_2O emissions decreased significantly under elevated salinity (Figure 2), aligning with the findings of Azam and Müller[26], who reported that NaCl additions suppressed N_2O emissions in controlled incubations. This inhibition can be attributed to osmotic stress and ion toxicity induced by high NaCl concentrations. Under constant soil moisture, increased salinity lowers the osmotic potential of the soil solution, compelling microorganisms to accumulate intracellular solutes to maintain osmotic equilibrium[27]. However, excessive intracellular solute concentrations can impair enzymatic activity and disrupt nitrogen transformation pathways[28], thereby reducing N_2O production. Moreover, since chloride ions are largely non-metabolizable and cannot be readily incorporated into microbial biomass, their accumulation may induce physiological stress or direct toxicity[29,30]. Thus, both osmotic imbalance and ion accumulation likely contributed to the observed suppression of N_2O emissions at higher salinity levels.

4.2 Non-linear responses of nitrogen transformation processes to salinity

Nitrification and denitrification rates exhibited a non-linear response to salinity. Based on isotope-dilution calculations, slight and moderate salinity (NaCl=0.04% and 0.10%, $EC_{1:5}$≤1.10 dS/m) stimulated nitrification, whereas strong salinity (NaCl=0.20%, $EC_{1:5}$≥ 1.42 dS/m) inhibited it. This non-linear pattern aligns with the findings of Zeng et al.[31], who observed that nitrification and denitrification rates peaked at $EC_{1:5}$≈1.13 dS/m and declined thereafter. Low salt concentrations are recognized to stimulate microbial activity[32] and can enhance the metabolic performance of ammonia-oxidizing and nitrite-oxidizing bacteria[33]. In contrast, high salinity levels ($EC_{1:5}$>5 dS/m) are known to suppress ammonia oxidation—the rate-limiting step of nitrification[34]. The negative correlation between salinity and nitrification observed in this study at $EC_{1:5}$≥1.42 dS/m may result from the narrow conductivity range used in the experiment, limiting microbial adaptation to strong osmotic gradients.

Denitrification rates exhibited a similar non-linear response to salinity. When $EC_{1:5}$≤1.10 dS/m, denitrification rates increased with salinity, whereas higher salinity ($EC_{1:5}$ ≥ 1.42 dS/m) suppressed the process. This observed pattern is consistent with the findings of Zeng et al.[31], who reported that denitrification first increased and then decreased as salinity rose. This phenomenon likely stems from the dual impact of salinity on microbial metabolism: Moderate salt levels may stimulate the activity of denitrifying enzymes, while excessive ionic stress inhibits N_2O reductase, reducing the conversion of N_2O to N_2[34,35]. Furthermore, denitrifying bacteria possess stronger osmotic tolerance and adaptive mechanisms than nitrifiers[36]. This may explain why denitrification rates exceeded nitrification rates under moderate and high salinity conditions (Figure 3). Collectively, these findings indicate that soil salinity modulates microbial nitrogen cycling through complex, non-linear physiological responses.

4.3 The contributions of nitrification and denitrification to N_2O emissions

Throughout the incubation period, the relative contributions of nitrification and denitrification to N_2O production shifted markedly with incubation time and salinity (Figures 5 and 6). As the experiment progressed, the nitrification-derived N_2O fraction gradually decreased, whereas the denitrification-derived fraction increased. This transition likely reflects the progressive depletion of oxygen and the subsequent expansion of anaerobic microsites within the soil matrix. Reduced oxygen availability favors denitrifying microorganisms[37], while osmotic stress and nutrient limitation restrict nitrifier activity[38]. Because denitrifiers are generally more salt-tolerant than nitrifiers[36], they become the dominant N_2O producers under prolonged incubation and increasing salinity. During the initial stage of incubation (days 1-3), the contribution of nitrification to N_2O production increased with salinity (Figure 5). This transient stimulation can be attributed to the partial inhibition of nitrite-oxidizing bacteria, which triggers nitrite accumulation and subsequently promotes N_2O formation through incomplete nitrification[39]. However, during days 5-7 of incubation, both slight and moderate salinity suppressed nitrification-derived N_2O production. This suppression was likely driven by oxygen depletion: Nitrifiers consume oxygen more rapidly under higher NaCl concentrations[38], thereby inducing microaerobic conditions that impede the nitrification process.

In contrast, the denitrification-derived N_2O fraction was initially suppressed by salinity during days 1-3, but subsequently increased under slight and moderate salinity stress during days 5–7 of the incubation (Figure 6). This temporal pattern suggests that the denitrifying community requires a transient acclimation period to adjust to saline conditions before regaining functional activity. This aligns with Li et al.[35], who observed that NaCl concentrations of 0.5% enhanced denitrification and nitrogen removal, whereas higher levels (4%) exerted an inhibitory effect. It has been reported that moderate salinity levels can diversify and expand the populations of denitrifiers harboring functional genes like *nirK*, *nirS*, and *nosZ*[40]. In contrast, extreme ionic stress is known to undermine cellular health and restrict electron transport[41]. As a result, it is observed that slight and moderate salinity fostered denitrification-derived N_2O emissions, while strong salinity led to their suppression.

A primary limitation of this study is that N_2—the potential end-product of denitrification—was not quantified, which may lead to an underestimation of gross denitrification rates. Given that N_2O can be further reduced to N_2, the relative contribution of denitrification to net N_2O fluxes might be overestimated if this sink is ignored. Furthermore, the results obtained under constant

laboratory conditions (60% WFPS, 25°C) represent potential rates under stabilized microbial activity, which may differ from actual emissions in salt-affected farmlands, where temperature and moisture regimes fluctuate dynamically. Notably, the 14-day incubation period may have been insufficient to fully capture the complete progression of N_2O emissions, as evidenced by the sustained fluxes observed toward the end of the monitoring window. Therefore, future studies should employ extended observation periods to accurately characterize emission peaks and long-term dynamics. Additionally, while the microbial physiological responses and adaptation strategies were inferred based on nitrogen transformation rates, direct molecular evidence—such as functional gene abundances (e.g., *amoA*, *nirS/K*, and *nosZ*) or microbial community composition—was not collected in this study[42]. Consequently, the findings should be interpreted as mechanistic insights into potential transformation pathways rather than direct estimates of total annual field emissions or exhaustive biological characterizations.

5 Conclusions

This study utilized ^{15}N stable isotope tracing techniques to quantify the effects of soil salinity on nitrogen transformation processes and to distinguish the relative contributions of nitrification and denitrification to N_2O emissions. The results demonstrated that the cumulative N_2O emissions decreased significantly with increase in soil salinity. Slight and moderate salinity (NaCl=0.04% and 0.10%; $EC_{1:5}$≤1.10 dS/m) increased both nitrification and denitrification rates, whereas strong salinity (NaCl=0.20%; $EC_{1:5}$≥1.42 dS/m) suppressed both processes. During the one-week incubation period, nitrification was the dominant source of N_2O, accounting for approximately 65%-70% of the total emissions, while denitrification contributed 30%-35%. These findings indicate that nitrification represents the predominant source of N_2O production in saline soils in the first week of incubation. The findings suggest that implementing nitrogen management to inhibit the nitrification process (e.g., the addition of nitrification inhibitors along with fertigation) may effectively mitigate N_2O emissions in saline farmland.

Acknowledgements

This research was supported by the Science and Technology Program of Inner Mongolia Autonomous Region (Grant No. NMKJXM202208, 2024BYNECAU016) and the Key Research & Development Program of Ningxia Hui Autonomous Region (Grant No. 2023BCF01017).

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