

Effects of salt stress on nitrification and denitrification rates and N₂O emissions in soil using a ¹⁵N isotope tracing approach

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Abstract: Nitrous oxide (N₂O), 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 ¹⁵N stable isotope tracing technique were conducted to quantify the effects of salt stress on nitrification and denitrification rates and their contributions to N₂O 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 N₂O emissions under 60% water-filled pore space, respectively. These results indicate that nitrification represents the predominant source of N₂O 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 N₂O 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 N₂O emissions, stable isotope tracer

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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 (N₂O) 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 N₂O 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 N₂O emissions is crucial for achieving climate change mitigation goals.

Agricultural soils represent a primary anthropogenic source of N₂O 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 900×10⁶ hm² 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 N₂O 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 N₂O emissions to salinity remains a subject of intense scientific debate. For instance,

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Ghosh et al.^[16] observed a positive correlation between soil electrical conductivity (ECe, 0.44–7.2 dS/m) and N₂O emissions in laboratory incubations, whereas Zhang et al.^[17] reported a negative correlation between salinity and cumulative N₂O emissions over 15 d. Conversely, Jia et al.^[18] found that cumulative N₂O 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 N₂O emissions than both non-saline and high-salinity (5.0 dS/m) treatments, suggesting the existence of a threshold effect of salinity on soil N₂O emissions. A critical limitation of these existing studies is their reliance on total N₂O 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 N₂O emissions using a ¹⁵N stable isotope tracer technique. This approach enables direct partitioning of N₂O 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 N₂O 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/hm² 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/cm³. Each bottle was filled to a volume of 150 mL. After packing, the soil surface area was 58 cm². 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, NH₄¹⁵NO₃ (10 atom% ¹⁵N enrichment in NO₃-N) was dissolved in deionized water and applied uniformly to the soil surface using a syringe at a rate equivalent to 250 kg N/hm². 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 NH₄¹⁵NO₃ application was considered as day 0 of incubation. Gas samples for analyzing atom% ¹⁵N of N₂O-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% ¹⁵N of N₂O-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% ¹⁵N measurement was 0.5‰. The calculation formula for δ¹⁵N is shown in Equation (1):

$$\delta^{15}\text{N} = \frac{{}^{15}\text{R}_{\text{sample}}^i}{{}^{15}\text{R}_{\text{standard}}} \quad (1)$$

where, δ¹⁵N denotes nitrogen isotope ratio of N₂O; ¹⁵R_{sample}ⁱ denotes the value of ¹⁵N/¹⁴N of N₂O samples; ¹⁵R_{standard} denotes the value of ¹⁵N/¹⁴N of standard atmospheric N₂.

When the ¹⁵N atomic percentage of N₂O was measured, international standards for ¹⁵N atomic percentage provided by atmospheric N₂ 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 N₂O emission flux, mg/m²·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 N₂O (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, m³; A is the cross-sectional area of the soil in the incubation bottle, m²; and $\Delta c/\Delta t$ is the rate of change of N₂O 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 N₂O flux values using linear regression.

2.4 Soil sample collection and analysis

Soils were destructively sampled on days 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% ¹⁵N of NH₄⁺-N and NO₃⁻-N in the soil were determined using an IRMS (Delta V Plus-Precon, Thermo Fisher Scientific, Bremen, Germany). The atom% ¹⁵N of NH₄⁺-N ($\delta^{15}\text{N-NH}_4^+$) measurement was performed using the diffusion method to collect NH₄⁺, followed by analysis with an isotope ratio mass spectrometer and an elemental analyzer (EA-IRMS, isoprime100-vario PYRO cube, Elementar, Berlin, Germany) to measure $\delta^{15}\text{N}$ of NH₄⁺-N. The denitrifier method (*Pseudomonas chlororaphis subsp. aureofaciens* ATCC 13985) was used to convert NO₃⁻ to N₂O for determining $\delta^{15}\text{N}$ of NO₃⁻-N ($\delta^{15}\text{N-NO}_3^-$). The calibration standards for NH₄⁺ were IAEA-N-1 and IAEA-N-2, and for NO₃⁻ the calibration standards were USGS32, USGS34, and USGS35^[23].

2.5 Determination of the contribution of nitrification and denitrification to N₂O emissions

To quantify the amount of N₂O 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 NO₃⁻-N nitrogen pool) and f_n (nitrification pool, assumed to be equivalent to the NH₄⁺-N nitrogen pool) represent the atom% ¹⁵N in the denitrification pool and nitrification pool, respectively. It is assumed that the N₂O in the air at the headspace air of the incubation bottle prior to accumulation is negligible. f_m represents the atom% ¹⁵N of the mixed N₂O-N produced from the two different nitrogen pools. The contributions of nitrification and denitrification to N₂O emissions were calculated using Equation (3)^[24]:

$$f_m = d \times f_d + (1 - d) \times f_n \quad (3)$$

where, f_d represents the ¹⁵N atoms percentage in NO₃⁻-N ($\delta^{15}\text{N-NO}_3^-$); f_n represents the ¹⁵N atoms percentage in NH₄⁺-N ($\delta^{15}\text{N-NH}_4^+$); f_m represents the ¹⁵N atoms percentage in N₂O ($\delta^{15}\text{N-N}_2\text{O}$) produced from the two different nitrogen pools; d represents the contribution of denitrification to N₂O emissions; and $1-d$ represents the contribution of nitrification to N₂O 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 NH₄¹⁵NO₃ at the start of the formal incubation. Therefore, F_1 will represent primarily the nitrification rate. Since there is no leaching of NO₃⁻-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:

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

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

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 NO₃⁻-N content on day 1 and day n of incubation, respectively, mg/kg; A_1 and A_n represent the ¹⁵N atomic percent excess of NO₃⁻-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 N₂O 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 N₂O emissions. Statistical analysis was performed using SPSS 26.0 software (IBM SPSS Statistics, Chicago, USA).

3 Results

3.1 N₂O emission fluxes

Figure 1 presents the temporal variations in N₂O fluxes under different salinity levels. During the initial stage of incubation (days 1–3), N₂O 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, N₂O fluxes across all treatments exhibited a general decline with increasing salinity levels. On day 14, however, N₂O 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.

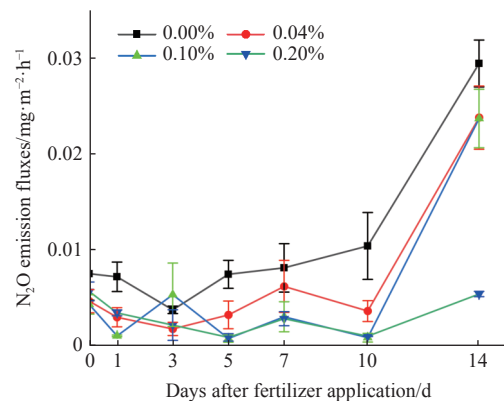
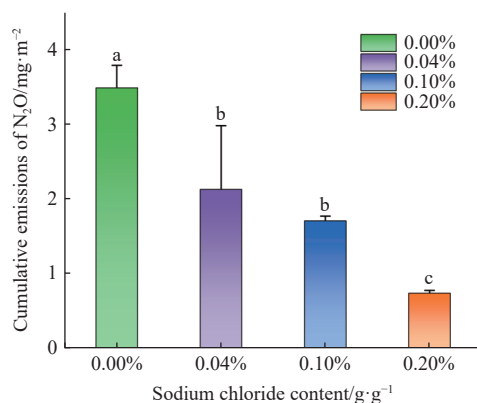


Figure 1 Temporal dynamics of N₂O fluxes under different salinity levels

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%; $\text{EC}_{1.5} \leq 1.101$ dS/m), both nitrification and denitrification rates were higher than those of the non-saline control. In contrast, strong salinity (NaCl =0.20%; $\text{EC}_{1.5} \geq 1.421$ 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.

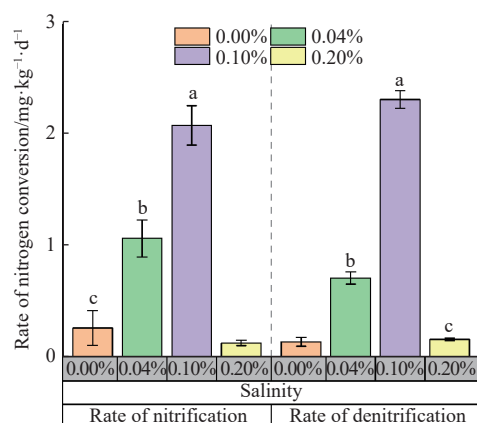
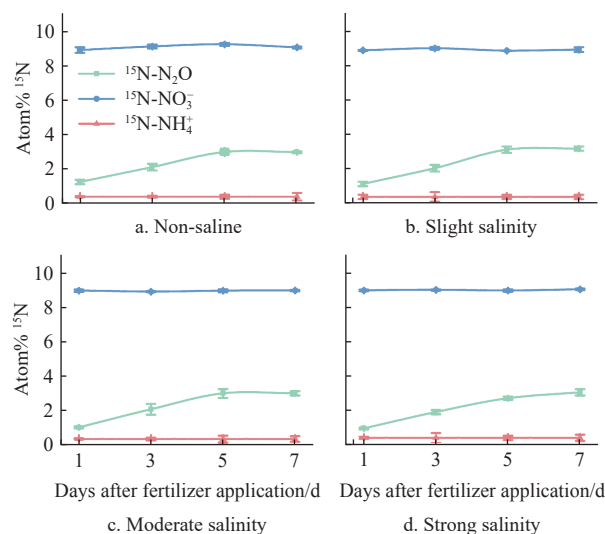


Figure 3 Effects of salinity on nitrification and denitrification rates (calculated from day 1 and day 7 data)

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

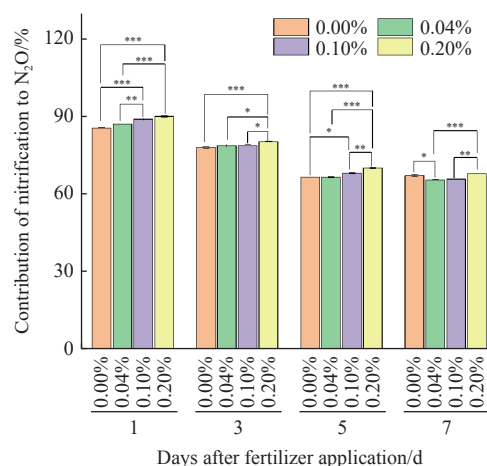
0.3701 atom% across all treatments, which was closely aligned with the natural abundance (0.3663 atom%). This indicates that N_2O was simultaneously derived from nitrification (the NH_4^+ pool) and denitrification (the NO_3^- pool).



Note: Vertical bars represent $\pm \text{SE}$ ($n=3$).

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 $p \leq 0.05$, 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 N₂O production, implying that intense ionic stress restricts the functional activity of the denitrification process.

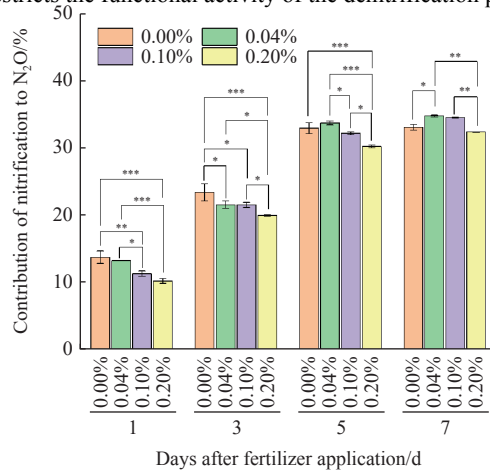


Figure 6 Effects of salinity on the contribution of denitrification to N₂O production

4 Discussion

4.1 Effect of salinity on the dynamics of N₂O emissions

As the incubation progressed, the inhibitory effect of salinity on N₂O fluxes became increasingly pronounced. N₂O emissions declined with increasing salinity, particularly during the day 5-14 period (Figure 1). Similarly, cumulative N₂O emissions decreased significantly under elevated salinity (Figure 2), aligning with the findings of Azam and Müller^[26], who reported that NaCl additions suppressed N₂O 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 N₂O 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 N₂O 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 N₂O reductase, reducing the conversion of N₂O to N₂^[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 N₂O emissions

Throughout the incubation period, the relative contributions of nitrification and denitrification to N₂O production shifted markedly with incubation time and salinity (Figures 5 and 6). As the experiment progressed, the nitrification-derived N₂O 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 N₂O producers under prolonged incubation and increasing salinity. During the initial stage of incubation (days 1-3), the contribution of nitrification to N₂O 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 N₂O formation through incomplete nitrification^[39]. However, during days 5-7 of incubation, both slight and moderate salinity suppressed nitrification-derived N₂O 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 N₂O 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 N₂O emissions, while strong salinity led to their suppression.

A primary limitation of this study is that N₂—the potential end-product of denitrification—was not quantified, which may lead to an underestimation of gross denitrification rates. Given that N₂O can be further reduced to N₂, the relative contribution of denitrification to net N₂O 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 N₂O 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 ¹⁵N 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 N₂O emissions. The results demonstrated that the cumulative N₂O 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 N₂O, accounting for approximately 65%–70% of the total emissions, while denitrification contributed 30%–35%. These findings indicate that nitrification represents the predominant source of N₂O 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 N₂O emissions in saline farmland.

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[References]

- [1] Intergovernmental Panel on Climate Change (IPCC). Climate Change 2021: The Physical Science Basis. 2021. Available: <https://www.ipcc.ch/report/sixth-assessment-report-working-group-i/>. Accessed on [2026-04-09].
- [2] Yang L Q, Zhang X J, Ju X T, Wu D. Oxygen-depletion by rapid ammonia oxidation regulates kinetics of N₂O, NO and N₂ production in an ammonium fertilised agricultural soil. *Soil Biol Biochem*, 2021; 163: 108460.
- [3] Tian H Q, Xu R T, Canadell J G, Thompson R L, Winiwarter W, Suntharalingam P, et al. A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, 2020; 586: 248–256.
- [4] Li S X, Wang Z H, Hu T T, Gao Y J, Stewart B A. Nitrogen in dryland soils of China and its management. *Adv Agron*, 2009; 101: 123–181.
- [5] Voss M, Baker A, Cornell S, Bange H, Conley D, Deutsch B, et al. The European nitrogen assessment: Sources, effects and policy perspectives. Cambridge University Press. 2011.
- [6] Zhang X, Davidson E A, Mauzerall D, Searchinger T D, Dumas P, Shen Y. Managing nitrogen for sustainable development. *Nature*, 2015; 528: 51–59.
- [7] Lassaletta L, Billen G, Grizzetti B, Garnier J, Leach A M, Galloway J N. Food and feed trade as a driver in the global nitrogen cycle: 50-year trends. *Biogeochemistry*, 2014; 118: 225–241.
- [8] Cassman K, Dobermann A. Nitrogen and the future of agriculture: 20 years on. *Ambio*, 2022; 51: 17–24.
- [9] Hassani A, Azapagic A, Shokri N. Global predictions of primary soil salinization under changing climate in the 21st century. *Nat Commun*, 2021; 12: 6663.
- [10] Food and Agriculture Organization of the United Nations (FAO). Salt-affected soils are a global issue. 2021. Available: <https://openknowledge.fao.org/handle/20.500.14283/cb4809en>. Accessed on [2026-04-09].
- [11] Stavi I, Thevs N, Priori S. Soil salinity and sodicity in drylands: a review of causes, effects, monitoring, and restoration measures. *Front Environ Sci*, 2021; 9: 712831.
- [12] Devkota K P, Devkota M, Rezaei M, Oosterbaan R. Managing salinity for sustainable agricultural production in salt-affected soils of irrigated drylands. *Agric Syst*, 2022; 198: 103390.
- [13] Lodhi A, Arshad M, Azam F, Sajjad M H. Changes in mineral and mineralizable N of soil incubated at varying salinity, moisture and temperature regimes. *Pak J Bot*, 2009; 41: 967–980.
- [14] Iwaoka C, Imada S, Taniguchi T, Du S, Yamanaka N, Tateno R. The impacts of soil fertility and salinity on soil nitrogen dynamics mediated by the soil microbial community beneath the halophytic shrub tamarisk. *Microb Ecol*, 2018; 75: 985–996.
- [15] Bateman E J, Baggs E M. Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biol Fertil Soils*, 2005; 41: 379–388.
- [16] Ghosh U, Thapa R, Desutter T, He Y B, Chatterjee A. Saline-sodic soils: potential sources of nitrous oxide and carbon dioxide emissions? *Pedosphere*, 2017; 27(1): 65–75.
- [17] Zhang L H, Song L P, Wang B C, Shao H B, Zhang L W, Qin X C. Co-effects of salinity and moisture on CO₂ and N₂O emissions of laboratory-incubated salt-affected soils from different vegetation types. *Geoderma*, 2018; 332: 109–120.
- [18] Jia J, Bai J H, Wang W, Yin S, Zhang G L, Zhao Q Q, et al. Salt stress alters the short-term responses of nitrous oxide emissions to the nitrogen addition in salt-affected coastal soils. *Sci Total Environ*, 2020; 742: 140124.
- [19] Li Y W, Xu J Z, Liu S M, Qi Z M, Wang H Y, Wei Q, et al. Salinity-induced concomitant increases in soil ammonia volatilization and nitrous oxide emission. *Geoderma*, 2020; 361: 114053.
- [20] Huang Q Z, Xu X, Lyu L J, Ren D Y, Ke J Y, Xiong Y W, et al. Soil salinity distribution based on remote sensing and its effect on crop growth in Hetao Irrigation District. *Trans CSAE*, 2018; 34(1): 102–109.
- [21] Chen Z J, Zhou T L, Huang G H, Xiong Y W. Soil microbial community and associated functions response to salt stresses: Resistance and resilience. *Sci Total Environ*, 2024; 954: 176475.
- [22] Li C J, Xiong Y W, Zou J Y, Dong L, Ren P, Huang G H. Impact of biochar and lignite-based amendments on microbial communities and greenhouse gas emissions from agricultural soil. *Vadose Zone J*, 2021; 20(2): e20105.
- [23] Lin W, Ding J J, Li Y Z, Zhang W, Ahmad R, Xu C Y, et al. Partitioning of sources of N₂O from soil treated with different types of fertilizers by the acetylene inhibition method and stable isotope analysis. *Eur J Soil Sci*, 2019; 70(5): 1037–1048.
- [24] Stevens R J, Laughlin R J, Burns L C, Arah J R M, Hood R C. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biol Biochem*, 1997; 29: 139–151.
- [25] Di H J, Cameron K C, McLaren R G. Isotopic dilution methods to determine the gross transformation rates of nitrogen, phosphorus, and sulfur in soil: a review of the theory, methodologies, and limitations. *Aust J Soil Res*, 2000; 38: 213–230.
- [26] Azam F, Müller C. Effect of sodium chloride on denitrification in glucose amended soil treated with ammonium and nitrate nitrogen. *J Plant Nutr Soil Sci*, 2003; 166(5): 594–600.
- [27] Gonzalez-Silva B M, Jonassen K R, Bakke I, Østgaard K, Vadstein O. Nitrification at different salinities: biofilm community composition and physiological plasticity. *Water Res*, 2016; 95: 48–58.
- [28] Skujins J J, McLaren A D. Enzyme reaction rates at limited water activities.

- Science, 1967; 158(3808): 1569.
- [29] Adviento-Borbe M A A, Doran J W, Drijber R A, Dobermann A. Soil electrical conductivity and water content affect nitrous oxide and carbon dioxide emissions in intensively managed soils. *J Environ Qual*, 2006; 35(6): 1999–2010.
- [30] Mahmood M Z, Bibi S, Shahzad M, Fakhar A, Rafique M, Qayyum A. Mechanisms of microbes to combat salinity in soil by producing secondary metabolites. *Arab J Geosci*, 2022; 15: 45.
- [31] Zeng W Z, Xu C, Wu J W, Huang J S, Ma T. Effect of salinity on soil respiration and nitrogen dynamics. *Ecol Chem Eng S*, 2013; 20(3): 519–530.
- [32] Windey K, Bo I D, Verstraete W. Oxygen-limited autotrophic nitrification–denitrification (OLAND) in a rotating biological contactor treating high-salinity wastewater. *Water Res.*, 2005; 39(18): 4512–4520.
- [33] Aslan S, Simsek E. Influence of salinity on partial nitrification in a submerged biofilter. *Bioresour Technol*, 2012; 118: 24–29.
- [34] Li J C, Cai L, Lu H F, Ma B, Chen G S, Kong D D, et al. Effects of ion combinations and their concentrations on denitrification performance and gene expressions of an aerobic strain *marinobacter hydrocarbonoclasticus* RAD-2. *Environ Microbiol*, 2023; 11: 1867.
- [35] Wang X Y, Zhu H, Yan B X, Shutes B, Bañuelos G, Cheng R. Response of the microbial community to salt stress and its stratified effect in constructed wetlands. *Environ Sci Pollut R*, 2021; 28: 18089–18101.
- [36] Halverson L J, Jones T M, Firestone M K. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Sci Soc Am J*, 2000; 64: 1630–1637.
- [37] Schlüter S, Lucas M, Grosz B, Ippisch O, Zawallich J, He H, et al. The anaerobic soil volume as a controlling factor of denitrification: A review. *Biol Fert Soils*, 2024; 61: 343–365.
- [38] Bai J H, Gao H F, Xiao R, Wang J J, Huang C. A review of soil nitrogen mineralization as affected by water and salt in coastal wetlands: issues and methods. *Clean Soil Air Water*, 2012; 40(10): 1099–1105.
- [39] Wei Q, Li X T, Xu J G, Dai H X, Li B, Xu J Z, et al. Responses of soil N₂O and CO₂ emissions and their global warming potentials to irrigation water salinity. *Atmosphere*, 2022; 13(11): 1777.
- [40] Miao Y, Liao R H, Zhang X X, Liu B, Li Y, Wu B, et al. Metagenomic insights into salinity effect on diversity and abundance of denitrifying bacteria and genes in an expanded granular sludge bed reactor treating high-nitrate wastewater. *Chem Eng J*, 2015; 277: 116–123.
- [41] Chen J H, Han Y, Wang Y M, Gong B Z, Zhou J, Qing X X. Start-up and microbial communities of a simultaneous nitrogen removal system for high salinity and high nitrogen organic wastewater via heterotrophic nitrification. *Bioresour Technol*, 2016; 216: 196–202.
- [42] Xue D M, Li J, Yu H, Jin K, Wang Z L. Effects of oxytetracycline on denitrification and anammox processes in riparian buffer zone soil. *Int J Agric & Biol Eng*, 2024; 17(6): 286–295.