



# Zeolite-induced enhancement of soil cation exchange capacity reduces nitrogen gaseous emissions and improves nitrate retention under urea fertilization

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## ABSTRACT

Zeolite has great potential as a soil amendment due to its unique porous structure and high cation exchange capacity (CEC). While its use in soil pollution control and heavy metal remediation has been widely studied, its effects on soil carbon and nitrogen (N) cycling—particularly greenhouse gas emissions—remain insufficiently explored. NZone Max is an additive for N fertilizers that containing proprietary surfactant-based and calcium-complex compounds. It aims to improve N availability and uptake. However, only a limited number of studies have been conducted, and no clear conclusions have yet been reached regarding its effectiveness. To address this, an incubation experiment was designed using normal soil (S) with a CEC of 12 cmol  $\text{kg}^{-1}$  and soil amended with 10 % clinoptilolite zeolite (SZ, target CEC = 29.8 cmol  $\text{kg}^{-1}$ ), along with the addition of a no-fertilizer control (N0), urea (U), and urea + Nzone Max (UNZ) treatments.  $\text{NH}_3$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  were monitored continuously for one month, while soil mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), dissolvable organic C and microbial biomass C were measured at the end of the incubation. The addition of 10 % zeolite reduced  $\text{NH}_3$  and  $\text{N}_2\text{O}$  by approximately 50 %, while soil microbial respiration were stabilized, and the soil  $\text{NO}_3^-$  concentration at the end of the incubation was almost doubled in soil added with zeolite compared to no zeolite addition. In contrast, Nzone Max had no significant effect in any respect. These results highlight the potential of mineral-based amendments to regulate gaseous nitrogen losses, particularly  $\text{NH}_3$  and  $\text{N}_2\text{O}$ , and promote soil microbial and chemical stability. These findings may inform nutrient management strategies in a variety of soil conditions, particularly in regions with low native CEC or under intensive fertilizer use.

## 1. Introduction

The use of nitrogen (N) fertilizers in agriculture is one of the fundamental factors and indispensable in feeding the world's current populations (Eickhout et al., 2006; Smil, 1991). However, although the nitrogen use efficiency (NUE) was increasing in last decades, it remains lower than 50 % until now (Govindasamy et al., 2023; Yan et al., 2022). This inefficiency is not only cause economic loss

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during farming operations but also constrains environmental sustainability (Shi et al., 2024). Excess N fertilizer causes nitrate ( $\text{NO}_3^-$ ) leaching, which threatens water bodies, and contributes to eutrophication. Meanwhile, the presence of ammonium ( $\text{NH}_4^+$ ) and  $\text{NO}_3^-$  in soil can result in the release of various gaseous emissions, thereby exacerbating air pollution and climate change. Among these gaseous emissions, nitrous oxide ( $\text{N}_2\text{O}$ ) is particularly concerning because of its high global warming potential (IPCC, 2013) as a greenhouse gas and its role in depleting stratospheric ozone (Ravishankara et al., 2009). Urea-based fertilization is widely used because of its high N content and cost-effectiveness, but it also poses a significant risk of  $\text{NH}_3$  volatilization, especially under warm temperatures, high soil pH and low moisture conditions (Clay et al., 1990).  $\text{NH}_3$  emissions contribute to environmental problems such as air pollution, acidification and eutrophication of ecosystems (Götze et al., 2023; Martins et al., 2017). Consequently, there is an urgent need to improve the NUE in agriculture to mitigate these detrimental effects.

In recent decades, concerted efforts to increase NUE have resulted in several strategies, including precision agriculture, optimized fertilizer formulations, genetic breeding of crops and improved field management (Omara et al., 2019; Wu et al., 2025). Despite these advances, there are still space for improvement in NUE. Understanding and manipulating soil properties to match crop needs can significantly improve NUE, contributing to higher yields with less environmental impact. In this context, soil properties themselves play a very important role, particularly cation exchange capacity (CEC) - a key regulator of how soils store and deliver nutrients (Chen et al., 2023).

Cation exchange capacity (CEC) is a fundamental property of soil that describes its ability to hold and exchange positively charged ions (cations) such as  $\text{NH}_4^+$ , potassium and magnesium. A soil with a high CEC can temporarily store more nutrients, making them available to plants over a longer period of time and reducing the risk of these nutrients being leached or lost through gaseous emissions (Buragohain et al., 2019). Clay minerals and organic matter are traditionally associated with higher CECs, while sandy soils often have low CECs and are therefore more susceptible to nutrient leaching. Given the rising cost of fertilizer and the environmental impact of nutrient losses, strategies to engineer soil CEC have gained increased interest in recent years (Dastbaz et al., 2023; Khan et al., 2023).

One promising approach is the use of zeolites. Zeolites are a group of naturally occurring or synthetically produced, structurally stable aluminosilicate minerals with porous structure. This crystalline framework typically consists of interconnected tetrahedra of silica and alumina, creating a matrix of channels and cavities. These structural features give zeolites exceptionally high CEC values and the ability to adsorb various molecules and ions (Inglezakis and Zorpas, 2012). In agriculture, zeolites have been explored for a variety of purposes, including improving water retention in arid soils, removing heavy metals from contaminated sites, and serving as compost stabilizers (Cataldo et al., 2021; Montalvo et al., 2012; Mpanga et al., 2020). Their ability to hold and exchange  $\text{NH}_4^+$ , a major form of N in many soils, suggests that they could be an effective amendment for increasing NUE and reducing N losses (Sepaskhah and Yousefi, 2007; Torma et al., 2014).

In theory, incorporating zeolite into soil offers a number of potential benefits. First, the high CEC of zeolite helps to capture and retain  $\text{NH}_4^+$ , thereby reducing  $\text{NH}_3$  volatilization (Ali et al., 2022, p. 3; Ferretti et al., 2017). Second, by keeping  $\text{NH}_4^+$  bound to the zeolite surfaces, less  $\text{NH}_4^+$  is converted to nitrate ( $\text{NO}_3^-$ ). The less  $\text{NO}_3^-$  substrate and the more gradual conversion of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  and subsequently to  $\text{NO}_3^-$  reduced the  $\text{N}_2\text{O}$  spike from denitrification. In addition, the improved soil structure provided by the zeolite reduced the incidence of anaerobic microsites where denitrification thrives, thus potentially reducing  $\text{N}_2\text{O}$  emissions (Liu et al., 2022; Park et al., 2024). In addition, by retaining more mineral N in the root zone, zeolite-enriched soils provide a more consistent supply of nutrients to crops, which can reduce fertilizer requirements and result in improved plant growth and higher yields (Mondal et al., 2021; Ozbahce et al., 2018), supporting both economic and environmental objectives in agricultural systems. Although the benefits of zeolite amendments in soils has been recognized for over 20 years, much of the existing research has examined these benefits in an isolated manner, and there are relatively few comprehensive studies that monitor  $\text{NH}_3$ ,  $\text{N}_2\text{O}$ , and  $\text{CO}_2$  emissions simultaneously, track changes in soil mineral N, and assess shifts in microbial community activity.

In this experiment, we used conventional cropland soil as a control and soil amended with 10 % zeolite - an amount sufficient to more than double the CEC of the soil. In our study we also evaluated NZone Max™ (AgXplore International, USA), a proprietary nitrogen stabilizer containing surfactant-based and calcium-complex compounds. NZone Max is specifically designed to improve NUE and reduce  $\text{NO}_3^-$  leaching. Its benefits include maintaining N placement without disturbing soil biota, increasing N availability and uptake (Cascaldi et al., 2020; Castro, 2020). According to its labels, NZone Max opens exchange sites on soil colloids to which  $\text{NH}_4^+$  can attach. This is attributed to the surfactant-based and calcium-complex compounds in NZone Max, which enhance cation exchange and stabilize  $\text{NH}_4^+$  in the soil matrix.  $\text{NH}_4^+$  attached to an exchange site is protected against loss by volatilization, leaching and denitrification. By incorporating NZone Max into our trial framework, we aimed to compare its effectiveness in reducing N losses alongside zeolite amendments. We conducted a one-month incubation study in which we frequently measured  $\text{NH}_3$ ,  $\text{N}_2\text{O}$ , and  $\text{CO}_2$  emissions, followed by final measurements of soil mineral N and the abundance of key microbial genes involved in nitrification and denitrification. Through this approach, we aim to address several critical questions: 1), how does the addition of zeolite and NZone Max affect the magnitude of  $\text{NH}_3$ ,  $\text{N}_2\text{O}$ , and  $\text{CO}_2$  emissions compared to untreated soil? 2), Does the addition of zeolite and NZone Max contribute to a higher concentration of mineral N after one month, thereby improving N availability for potential plant uptake? 3), how does zeolite-enhanced CEC affect the abundance of microbial genes associated with the N cycle, and how might these shifts elucidate the underlying mechanisms of  $\text{NH}_3$  and  $\text{N}_2\text{O}$  reduction? By investigating these questions, we aim to evaluate whether zeolite amendments and NZone Max are a promising strategy for reducing greenhouse gas emissions, and enhancing the retention of soil mineral N.

## 2. Materials and methods

### 2.1. Soil properties and experimental design

In this study, we used natural cropland soil and soil incorporated with natural zeolite (Clinoptilolite) for the incubation. The natural soil was collected from the topsoil (0–25 cm) of the Reinshof agricultural research station, University of Göttingen, Lower Saxony, Germany (51°29'50.3 N, 9°50'59.9 E, 155 m asl). This depth captures the zone of active nutrient cycling and management impacts and falls within the standard 0–20–0–30 cm range used in European and international protocols. Mean annual precipitation of the experimental station is 632 mm and mean annual temperature is 9.7 °C (2003–2022, meteorological station at Göttingen, station ID: 1691, Germany's Meteorological Service). Soil was classified as luvisol (IUSS, 2015), with a texture of 61 % silt, 23 % sand and 16 % clay. Soil bulk density was 1.3 g cm<sup>-3</sup>, and soil pH was 7.0. Soil total carbon (C) concentration was 1.3 % and the total N concentration was 0.13 % (Römer et al., 2015). Soil was collected in autumn 2019 after the harvest of potatoes (*Solanum tuberosum* L.). CEC of the soil was 12 cmol kg<sup>-1</sup>. Another type of soil was Reinshof soil with 10 % of zeolite amendment (Soil+Zeolite). Zeolite powder (Clinoptilolite) was acquired from Samore GmbH (Bindlach, Germany). The used zeolite has a CEC of about 190 cmol kg<sup>-1</sup>. As a result, the soil mixed with zeolite has a CEC of 29.8 cmol kg<sup>-1</sup> (90 % \* 12 + 10 % \* 190 cmol kg<sup>-1</sup>).

We applied three different fertilizers to each of the two soils, for a total of six treatments. Treatments for different fertilizers are: (1) no fertilization (N0), (2) 120 kg N ha<sup>-1</sup> urea (U) and (3) 120 kg N ha<sup>-1</sup> urea+NZone Max (UNZ). NZone Max was added at 0.3 % w/w urea, as recommended by the manufacturer. For fertilizer application, urea is dissolved and added to the pots using a pipette gun. The treatments were listed in Table 1.

Before incubation, soil was air-dried to 2 % of water-filled pore space (WFPS) and sieved through 1 cm mesh for greater homogeneity. The soil was first adjusted to a WFPS of 30 % and then the soil was filled into the experiment pots with a 36 cm length, 29 cm width and 26 cm height. The amount of equivalent to 8.6 kg dried soil was filled to an 11 cm depth in the pot, therefore a 15 cm air-space was left for gas accumulation. Soil was pre-incubated at 50 % WFPS for one week and then raised to 60 % WFPS prior to fertilization. After fertilization, the soil were irrigated to reach 70 % WFPS, and then gradually lost water until reaching around 50 % WFPS. In practice, the soil were irrigated on days 6, 12, 20 and 27. Throughout the experiment, the incubation temperature were kept at 25 °C during the day and 15 °C at night. To minimize structural differences between the treatments, the soil was compacted to a bulk density (BD) of  $1.30 \pm 0.03$  g cm<sup>-3</sup>. The water-filled pore space (WFPS) was then calculated as follows:

$$\text{WFPS} = \frac{\theta_v}{1 - \text{BD}/\text{PD}} \quad (1)$$

Where  $\theta_v$  is the soil's volumetric water content, WFPS = PD is the particle density, and  $n = 1 - \text{BD}/\text{PD}$  denotes the total porosity. Unless otherwise stated, PD was set to 2.65 g cm<sup>-3</sup> (mineral soil). As all treatments had the same BD and were wetted identically, the nominal WFPS was equivalent across treatments. Using a lower PD for clinoptilolite (approximately 2.2–2.3 g cm<sup>-3</sup>) at 10 % (w/w) would only slightly alter the mixture PD and thus porosity by less than 1 percentage point (e.g. at BD = 1.30 g cm<sup>-3</sup>,  $n \approx 0.51$  for PD = 2.65 versus  $\approx 0.50$  for PD = 2.60). This is below our measurement precision and does not affect the interpretation.

### 2.2. Soil CEC measurement

Soil CEC was determined using a hexamminecobalt (III) chloride solution (CoHex solution) (ISO 23470: 2018(E)). Briefly, 2.229 g of hexamminecobalt (III) chloride (abcr GmbH, Karlsruhe Germany) was filled in a volumetric flask containing of 500 mL of distilled water as standard CoHex solution ( $\beta[\text{Co}(\text{NH}_3)_6]\text{Cl}_3 = 1.66$  cmol L<sup>-1</sup>). Then the solution was diluted to solution with concentration of 0 cmol L<sup>-1</sup>, 0.166 cmol L<sup>-1</sup>, 0.498 cmol L<sup>-1</sup>, 0.830 cmol L<sup>-1</sup>, 1.16 cmol L<sup>-1</sup> and 1.49 cmol L<sup>-1</sup> as test solution, then test solution was transferred into the spectrophotometer and recorded the optical absorption at 475 nm. An calibration curve was drawn using the concentration of CoHex solution as x-axis and the value of optical absorption as y-axis.

1.5 g air-dried soil was added to a 50 mL falcon tube, then 25 mL CoHex solution was added to the tube. Tube was shaken for 1 h at  $20 \pm 2$  °C, then centrifuged for 10 min, clear filtrate or supernatant was carried out for determination of optical absorption on spectrophotometer under 476 nm (A<sub>1</sub>) and 380 nm (A<sub>2</sub>). The organic matter was absorbed at 380 nm and A<sub>2</sub> is the correction of the solutions.

$$A = 1.025A_1 - 0.205A_2 \quad (2)$$

**Table 1**

List of treatments and CEC of different treatments.

Soil type	Treatments	Fertilization	CEC (cmol kg <sup>-1</sup> )
Natural soil (S)	S+N0	0	12
	S+U	Urea 120 kg N ha <sup>-1</sup>	12
	S+UNZ	Urea+NZone Max 120 kg N ha <sup>-1</sup>	12
Soil+Zeolite (SZ)	SZ+N0	0	29.8
	SZ+U	Urea 120 kg N ha <sup>-1</sup>	29.8
	SZ+UNZ	Urea+NZone Max 120 kg N ha <sup>-1</sup>	29.8

The determinations were carried out in 24 h after the extraction. An extraction “blank” under the same conditions was also produced. Soil CEC was calculated using the form:

$$\text{CEC} = \frac{(A_0 - A) * V * 3}{b * m * W_{dm}} \quad (3)$$

- CEC: cation exchange capacity (cmol kg<sup>-1</sup>)
- A<sub>0</sub>: optical absorption of “blank”
- A: corrected optical absorption of sample
- V: Volume of CoHex solution, here 25 mL
- 3: Number of positive charges of [Co (NH<sub>3</sub>)<sub>6</sub>]<sup>3+</sup>.
- b: slope of extinction line, here b = 0.5582
- m: weight of soil sample (in g)
- W<sub>dm</sub>: percentage of dry matter (%)

### 2.3. NH<sub>3</sub>, CO<sub>2</sub> and N<sub>2</sub>O flux measurement

Soil NH<sub>3</sub> emissions were measured using the Dräger-Tube method (Pacholski et al., 2006), with an X-act 5000 pump (Dräger, Kiel, Germany). To collect the gases, two cylinders were inserted into the soil surface and the gases emitted from the soil were pumped through Dräger Ammonia Tubes (Dräger Safety, Lübeck, Germany). The yellow substance in the tube turns blue when it is in contact with NH<sub>3</sub>. The scale of blue color that emerges indicates the amount of NH<sub>3</sub> emitted. The measured concentrations were converted from ppm to kg N ha<sup>-1</sup> according to the protocol (Pacholski et al., 2006). Measurements were taken daily during the first week, then every two or three days thereafter. A total of 14 measurements were carried out over one month. Linear interpolation was used to calculate cumulative NH<sub>3</sub> emissions. We only measured the first three pots for each treatment because the results showed high repeatability and due to cost limitations.

For the CO<sub>2</sub> and N<sub>2</sub>O measurements, the pots were closed with caps prior to sampling. A 25 mL gas sample was then taken from the top of the sealed chamber and transferred directly to a pre-vacuumed 12 mL Exetainer vial (Labco, Lampeter, UK). Gas samples were taken at 0, 15, 30 and 45 min after chamber closure. Samples were taken daily for the first week and every two or three days thereafter. Sample analysis was carried out on a Bruker SCION™ 456 gas chromatograph (Bruker, Bremen, Germany). A flame ionization detector was used to determine the CO<sub>2</sub> concentration and an electron capture detector to analyze the N<sub>2</sub>O concentration. Flux rates were determined from the linear regression of gas concentration over time. Linear interpolation was applied to calculate cumulative N<sub>2</sub>O emissions.

### 2.4. Soil sample measurements

Soil samples were collected at the end of the trial. To determine soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations, about 15 g of subsample was weighted and added to 60 mL of 0.05 M K<sub>2</sub>SO<sub>4</sub>. According to our laboratory protocol, we use 0.5 M K<sub>2</sub>SO<sub>4</sub> for extraction only in forest soils with very high organic matter. A 0.05 M K<sub>2</sub>SO<sub>4</sub> solution has been proven reliable in our agricultural soils, which have much lower organic matter content than forest soils. The solution was shaken for 1 h, then filtered (MN615, 1/4; pore size, 4–12 µm; Macherey-Nagel, Düren, Germany) into two bottles, and then stored at –20°C until analysis. The NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations in the soil were quantified using a San+ + continuous flow analyzer (Skalar Analytical, Breda, The Netherlands). A further 10 g subsample was fumigated with chloroform for 24 h and then added to 40 mL 0.05 M K<sub>2</sub>SO<sub>4</sub> and extracted in the same way. Both non-fumigated and fumigated samples were analyzed for total C concentration using a 2100 TOC/TIC analyzer (Analytik Jena, Germany). The non-fumigated samples were used to measure dissolvable organic C (DOC) and the microbial biomass carbon (MBC) was calculated as the difference in extracted C between the non-fumigated and fumigated samples, using a conversion factor of 0.45 (Joergensen, 1996).

### 2.5. DNA extraction and qPCR analysis

Quantitative PCR (qPCR) analysis was conducted to determine the abundance of microbial genes associated with nitrogen cycling in soil samples collected at the end of the incubation experiment. For qPCR analysis, soil was freeze-dried for 72 hr. The freeze-dried soil was finely ground using a swing mill (MM400, Retsch, Haan, Germany). Soil total DNA was extracted from 0.4 g soil sample using a Quick Soil Isolation Kit (Huayueyang, China), and eight specific genes were targeted. Primers used for amplification were listed in Table S1. Real-time quantitative PCR (qPCR) was performed in 20 µL reaction volumes using ChamQ SYBR Color qPCR Master Mix (Nanjing Vazyme Biotech Co., Ltd.) on an ABI 7300 Real-Time PCR System (Applied Biosystems, USA). Each reaction contained 10 µL of 2 × Master Mix, 0.8 µL each of forward and reverse primers (5 µM), 0.4 µL of 50 × ROX reference dye, 2 µL of template DNA and 6 µL of nuclease-free water. The thermal cycling programme included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5 s, 58°C for 30 s, and 72°C for 1 min, with fluorescence signals collected at the end of each cycle. Melt curve analysis was then performed to confirm product specificity. Standard curves for absolute quantification were generated from a 10-fold serial dilution of plasmid DNA containing the target genes.

## 2.6. Statistics

We conducted a two-way ANOVA (soil type x N fertilizer) to analyze cumulative  $\text{NH}_3$ ,  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emissions, soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents, soil DOC and MBC, and functional genes. For gas and soil parameters, residual normality (Shapiro-Wilk test; Q-Q plots) and homogeneity of variance (Levene test) showed no significant violations for any of the parameters except for  $\text{NO}_3^-$ . Thus, no transformation was applied to these parameters. The  $\text{NO}_3^-$  data were log-transformed to assess the effects of soil type and N type. For each gene, we fitted models on both the raw and log10-transformed scales and reported the preferred result based on residual normality (Shapiro-Wilk test). Significant interactions (soil type x N type) were followed by simple effects tests with a Tukey HSD adjustment. If the interaction was not significant, main effects were interpreted with Tukey post hoc tests for N fertilizer, with  $p < 0.05$  used as the criterion for statistical significance. All data analyses were performed using the IBM SPSS Statistics 21.0 software package.

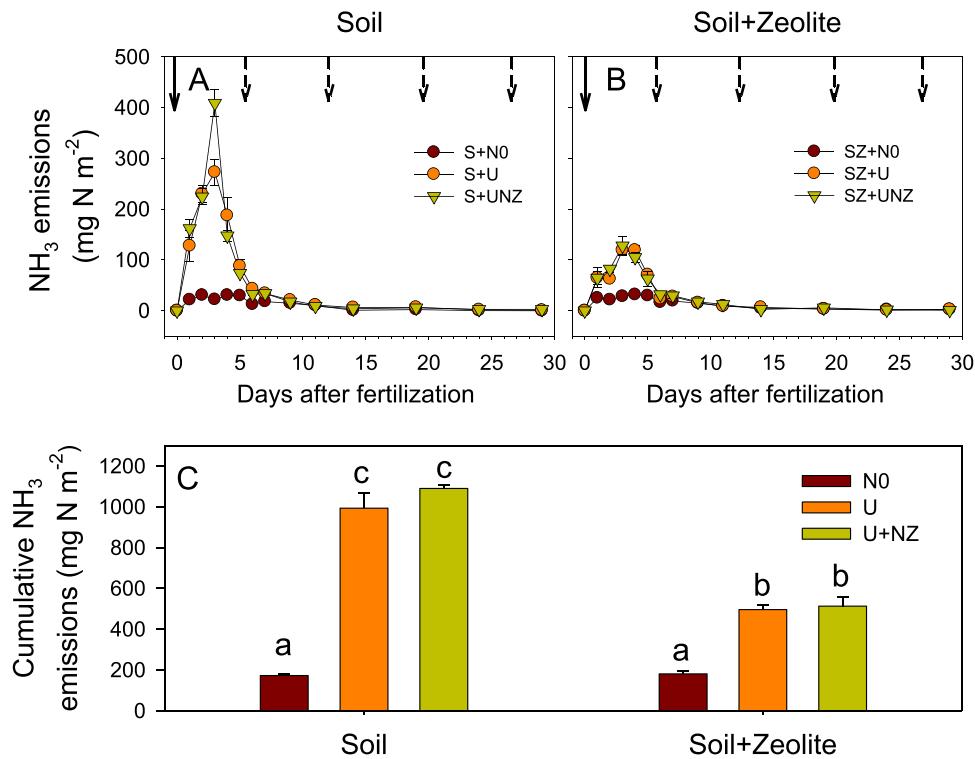
## 3. Results

### 3.1. soil $\text{NH}_3$ emissions

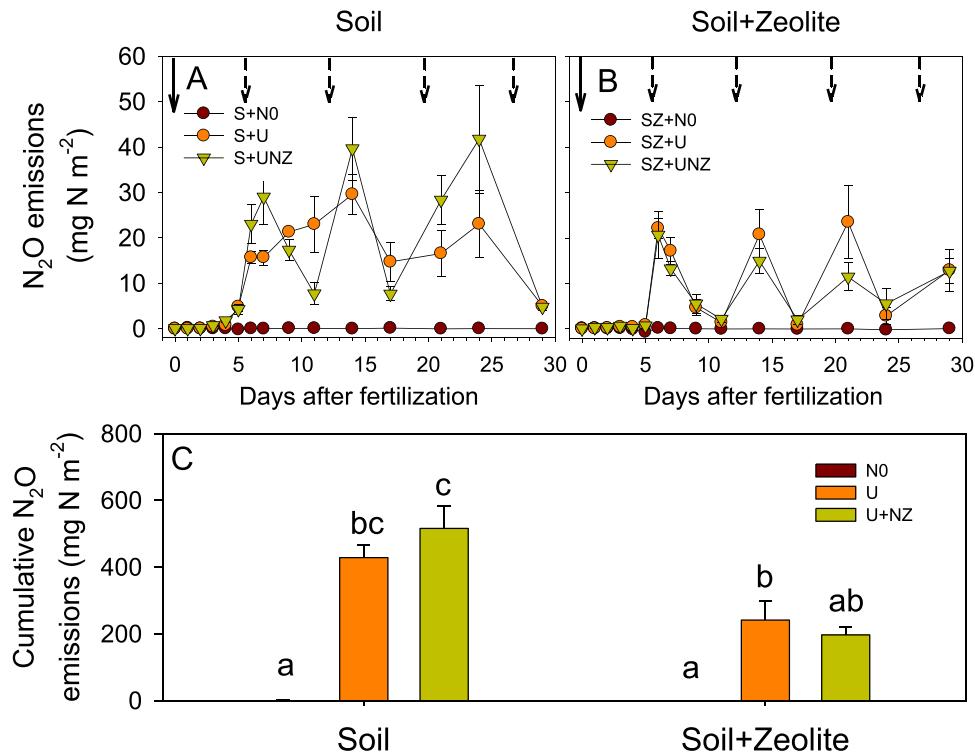
$\text{NH}_3$  emissions increased rapidly after fertilizer application, reaching their highest levels on the third day and then declining to near zero after approximately one week (Fig. 1A, B). Among the treatments, S+UNZ recorded the highest peak of about  $400 \text{ mg N m}^{-2} \text{ d}^{-1}$ , followed by S+U with about  $290 \text{ mg N m}^{-2} \text{ d}^{-1}$  (Fig. 1A). In contrast, SZ+U and SZ+NZ exhibited significantly lower peaks of around  $120 \text{ mg N m}^{-2} \text{ d}^{-1}$  (Fig. 2B). Cumulative  $\text{NH}_3$  emissions followed a similar pattern, with S+U and S+UNZ treatments emitting a total of  $1000\text{--}1100 \text{ mg N m}^{-2}$  in total, whereas SZ+U and SZ+NZ released only about  $520 \text{ mg N m}^{-2}$  (Fig. 1C). Statistical analysis revealed that SZ was significantly higher than S ( $p < 0.001$ ,  $\eta^2=0.874$ ). NZone Max had negligible effects on reducing  $\text{NH}_3$  emissions.

### 3.2. soil $\text{N}_2\text{O}$ emissions

$\text{N}_2\text{O}$  fluxes began to rise five days after fertilization, with multiple peaks following each irrigation event. At all peaks, the soil without zeolite exhibited higher  $\text{N}_2\text{O}$  fluxes than the zeolite-amended soil (Fig. 2A, B). However, for the soil without zeolite, S+UNZ produced higher emission peaks but also showed lower valley peaks compared to S+U (Fig. 2A). In contrast, in the zeolite-treated soil, SZ+U showed higher peaks than SZ+NZ, and the valley levels were close to zero (Fig. 2B). Cumulative  $\text{N}_2\text{O}$  emissions revealed that



**Fig. 1.** Soil  $\text{NH}_3$  fluxes for (A) soil without zeolite (S) and (B) soil with zeolite (SZ), under different fertilization treatments (NO: no N, U: urea, and UNZ: urea + NZone Max). Solid arrows indicate fertilization events and dashed arrows indicate irrigation. Panel (C) shows cumulative  $\text{NH}_3$  emissions throughout the incubation. Error bars represent the standard error of the mean for each treatment ( $n = 3$ ). In (C), different letters indicate significant differences ( $p < 0.05$ ) between treatments (Turkey HSD test).



**Fig. 2.** Soil N<sub>2</sub>O fluxes for (A) soil without zeolite (S) and (B) soil with zeolite (SZ), under different fertilization treatments (N0: no N, U: urea, and UNZ: urea + NZone Max). Solid arrows indicate fertilization events and dashed arrows indicate irrigation. Panel (C) shows cumulative N<sub>2</sub>O emissions throughout the incubation. Error bars represent the standard error of the mean for each treatment ( $n = 4$ ). In (C), different letters indicate significant differences ( $p < 0.05$ ) between treatments (Turkey HSD test).

the impact of various N treatments depends on soil type ( $p < 0.05$ ,  $\eta^2 = 0.39$ ). Significant difference were observed between soil and soil added with zeolite ( $p < 0.01$ ,  $\eta^2 = 0.52$ ). Specifically, the addition of 10 % zeolite reduced cumulative N<sub>2</sub>O emissions by about 50 % ( $p < 0.05$ ) compared to soils without zeolite.

### 3.3. Soil CO<sub>2</sub> emissions

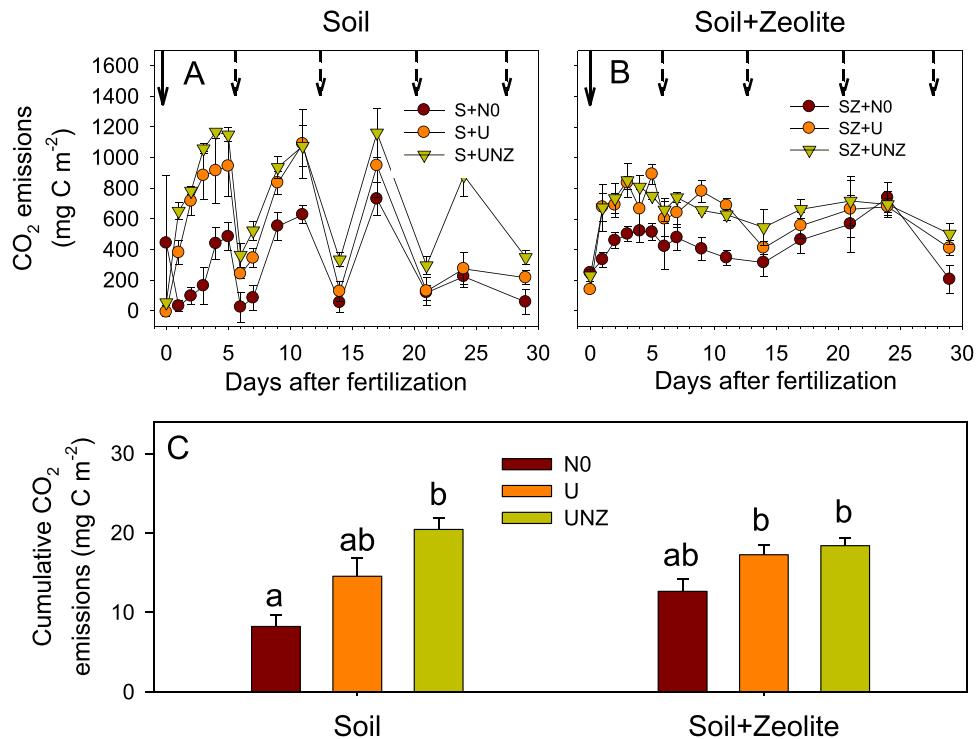
CO<sub>2</sub> fluxes in the soil without zeolite showed multiple peaks after fertilization and each irrigation event, with S+UNZ consistently showing higher peaks than S+U (Fig. 3A). Although CO<sub>2</sub> fluxes also increased in the zeolite-amended soil after fertilization and irrigation, the overall peaks were substantially lower and the valleys were higher (Fig. 3B). Cumulative CO<sub>2</sub> emissions showed no difference between S and SZ soils ( $p = 0.07$ ). N fertilizer increased CO<sub>2</sub> emissions ( $p < 0.05$ ), but no difference between U and UNZ ( $p = 0.17$ ) (Fig. 3C). Numerically, S+UNZ emitted about 28 % more CO<sub>2</sub> than S+U, while SZ+UNZ showed only a 4 % increase over SZ+U, but both of these differences were not statistically significant ( $p = 0.24$  and  $p = 0.99$ , respectively).

### 3.4. soil mineral N and organic C

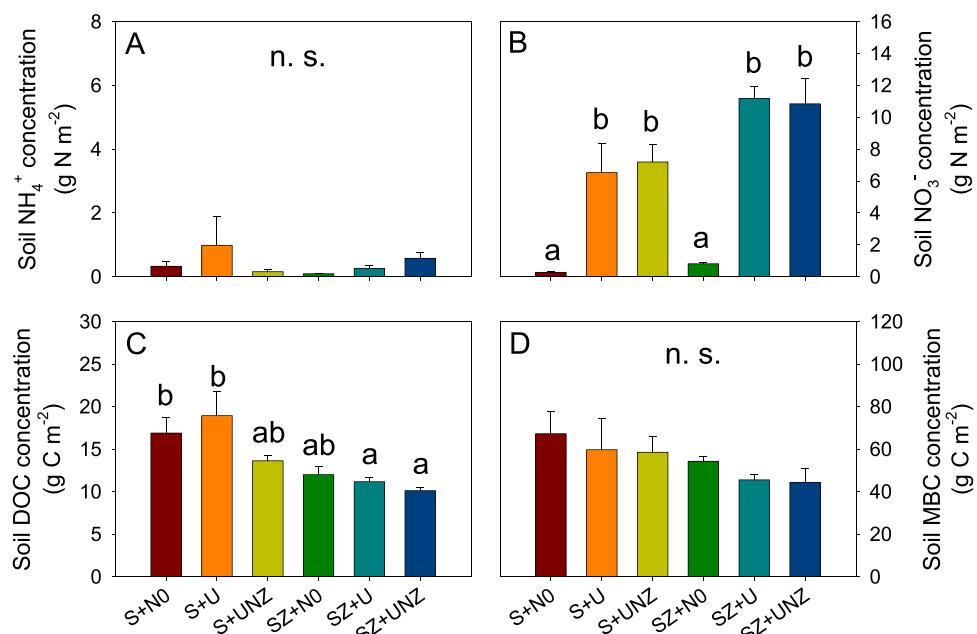
At the end of the experiment, soil NH<sub>4</sub><sup>+</sup> concentrations remained below 1 g N m<sup>-2</sup> in all treatments (Fig. 4A). In contrast, in the fertilized treatments, zeolite-amended soils (SZ) presented significantly higher NO<sub>3</sub><sup>-</sup> concentrations than soils without zeolite (S) ( $p < 0.001$ ,  $\eta^2 = 0.68$ ), and the addition of NZone Max had no distinguishable effect (Fig. 4B). Specifically, SZ+U and SZ+UNZ reached about 11 and 10.5 g N m<sup>-2</sup>—close to the applied fertilizer level—while S+U and S+UNZ were only around 7 g N m<sup>-2</sup>. For soil DOC, SZ+U and SZ+UNZ were significantly lower than S+N0 and S+U ( $p < 0.05$ ). There were no statistically differences in MBC among the treatments (Fig. 4C,D). However, zeolite-amended soil (SZ) had significantly lower DOC and MBC than unamended soil (S) ( $p = 0.0002$ ,  $\eta^2 = 0.538$  and  $p = 0.011$ ,  $\eta^2 = 0.32$ , respectively). The fertilized treatments did not differ statistically from the unfertilized controls ( $p = 0.1$  and  $p = 0.07$  for DOC and MBC, respectively).

### 3.5. soil microbial gene abundances

Soil DNA was extracted from the samples at the end of incubation (29 days after fertilization) and qPCR was performed. There was no statistical difference in the copy number of bacterial 16 s rRNA, fungal 18 s rRNA, AOA, *narG*, *napA*, *nirS*, *nirK*, *nosZ* and *nifH* genes,

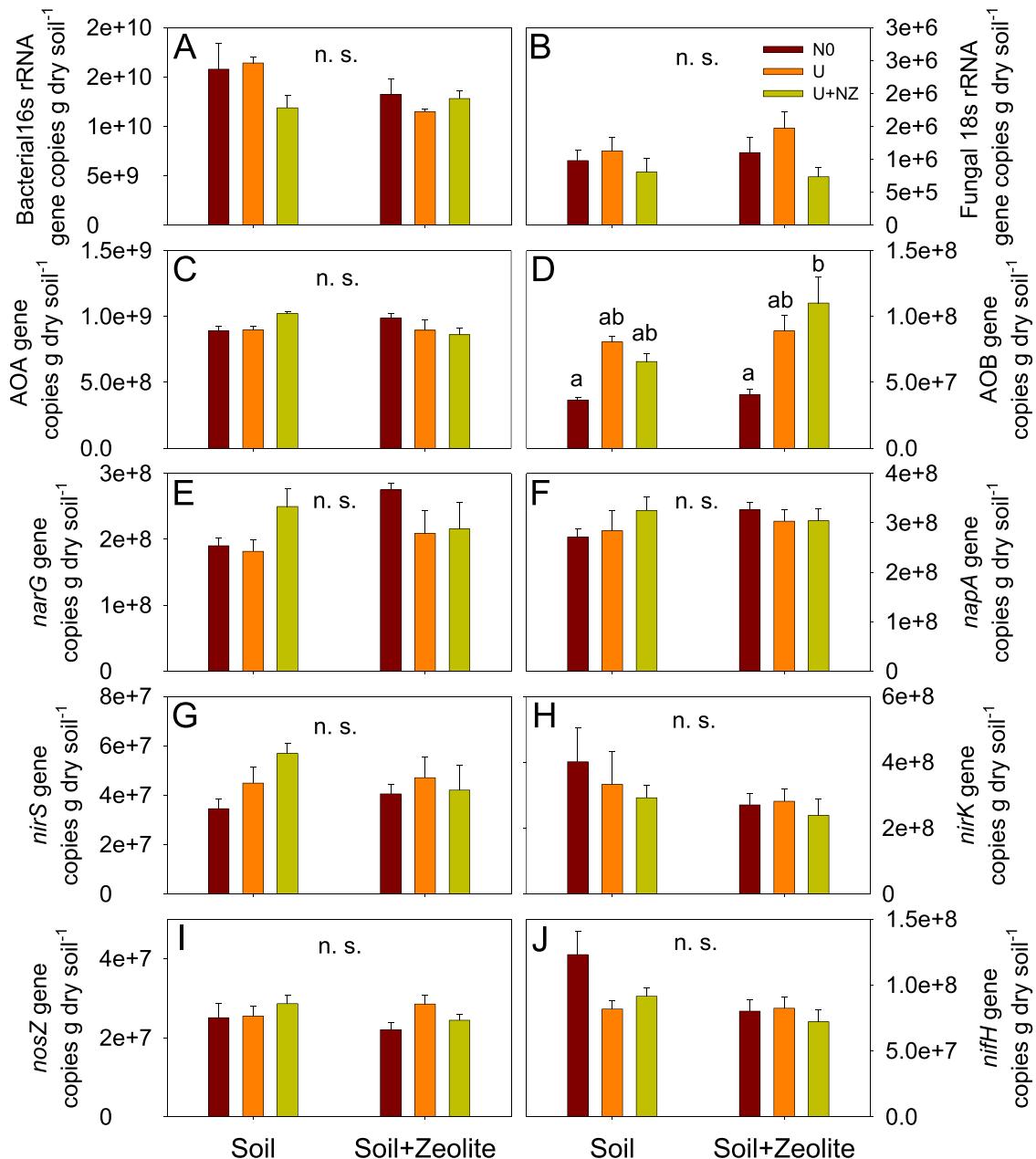


**Fig. 3.** Soil  $\text{CO}_2$  fluxes for (A) soil without zeolite (S) and (B) soil with zeolite (SZ), under different fertilization treatments (NO: no N, U: urea, and UNZ: urea + NZone Max). Solid arrows indicate fertilization events and dashed arrows indicate irrigation. Panel (C) shows cumulative  $\text{CO}_2$  emissions throughout the incubation. Error bars represent the standard error of the mean for each treatment ( $n = 4$ ). In (C), different letters indicate significant differences ( $p < 0.05$ ) between treatments (Turkey HSD test).



**Fig. 4.** Soil  $\text{NH}_4^+$  (A),  $\text{NO}_3^-$  (B), dissolved organic carbon (DOC, C), and microbial biomass carbon (MBC, D) concentrations under each treatment: S (soil without zeolite), SZ (soil with 10 % zeolite), NO (no N), U (urea), and UNZ (urea + NZone Max) at the end of the incubation period (after 29 days). Error bars represent the standard error of the mean ( $n = 4$ ). Different letters above the bars indicate significant differences among all treatments, while "n.s." denotes no significant difference ( $p < 0.05$ ).

only AOB showed a higher abundance in the fertilized treatments ( $p < 0.001$ ,  $\eta^2 = 0.55$ ) (Fig. 5), and *nifH* in SZ showed lower abundance than S ( $p < 0.05$ ,  $\eta^2 = 0.20$ ). In addition, there is a tendency that UNZ decreased the fungal 18 s rRNA gene copy number without statistical difference. Furthermore, the ratio of (*nirS+nirK*)/*nosZ* of S+N0, S+U, S+UNZ, SZ+N0, SZ+U and SZ+UNZ were 17.4, 14.8, 12.2, 14.1, 11.5 and 11.5, respectively. We assessed the correlation between (*nirS+nirK*)/*nosZ* and  $\text{N}_2\text{O}$ . Overall, the relationship was not significant (Pearson  $r = -0.25$ ,  $p = 0.24$ ; Spearman  $p = -0.23$ ,  $p = 0.29$ ). However, when we stratified the data by soil type, we observed a moderate negative correlation in SZ ( $r = -0.59$ ,  $p = 0.04$ ), whereas no association was detected in S ( $r = 0.07$ ,  $p = 0.84$ ).



**Fig. 5.** bacterial 16 s rRNA (A), fungal 18 s rRNA (B), AOA (C), AOB (D), *narG* (E), *napA* (F), *nirS* (G), *nirK* (H), *nosZ* (I) and *nifH* (J) gene copy number per dry soil in soil and soil with zeolite under different fertilizers (N0, U and UNZ). Error bars represent the standard error of the mean ( $n = 4$ ). Different letters above the bars indicate significant differences among all treatments, while “n.s.” denotes no significant difference ( $p < 0.05$ ).

## 4. Discussions

### 4.1. soil gaseous emissions

The observed spike in  $\text{NH}_3$  emissions within the first three days after fertilization is consistent with the well-documented pattern of urea hydrolysis (Dawar et al., 2011; Fisher et al., 2016), indicating that the main source of  $\text{NH}_3$  volatilization is the elevated  $\text{NH}_4^+$  concentration from urea hydrolysis. The gradual decline to near zero  $\text{NH}_3$  fluxes after one week reflects the depletion of this readily transformable N pool (Dawar et al., 2011; O'Toole et al., 1982). The presence of 10 % zeolite significantly reduced  $\text{NH}_3$  peaks and cumulative emissions, most likely due to the high CEC provided by zeolite and its adsorption of  $\text{NH}_4^+$ , thus less free  $\text{NH}_4^+$  available for volatilization. (Ahmad et al., 2021; Ferretti et al., 2017; Sun et al., 2019). In contrast, the addition of NZone Max showed minimal effect on  $\text{NH}_3$  fluxes and cumulative  $\text{NH}_3$  emissions in both soils, which is consistent with our previous study (Wang et al., 2020b). The most likely reason is that our soil already had high clay content (16 %), which overshadowed the effect of NZone Max in stabilizing  $\text{NH}_4^+$  in the soil at the recommended addition rate (0.3 % w/w to urea).

$\text{N}_2\text{O}$  emissions began to rise only after  $\text{NH}_3$  volatilization had passed its peak, meaning that the peak periods of urea hydrolysis and  $\text{NH}_4^+$  nitrification had largely ended. As a result, denitrification likely became the primary source of  $\text{N}_2\text{O}$ , with a large amount of  $\text{NH}_4^+$  being converted to  $\text{NO}_3^-$ . This inference is further supported by the repeated agreement of  $\text{N}_2\text{O}$  emission peaks with irrigation events. Although soil water-filled pore space (WFPS) was targeted at 60 %, actual values varied between 50 % and 70 %. Each irrigation temporarily increased the WFPS to about 70 %, which then decreased to about 50 % before the next irrigation. The soil moisture after the irrigation favors the denitrification (Cardenas et al., 2017; Firestone et al., 1989), and thus explains the timing of the observed peaks of  $\text{N}_2\text{O}$ .

In recent years, several studies have documented that zeolite addition can reduce  $\text{N}_2\text{O}$  emissions (Ferretti et al., 2017; Park et al., 2024; Zaman and Nguyen, 2010), although there are exceptions (Park et al., 2014). The reduced  $\text{N}_2\text{O}$  level indicates more complete denitrification of  $\text{N}_2\text{O}$  to  $\text{N}_2$  in soils with added zeolite. First, zeolite adsorbs  $\text{NH}_4^+$  and moderates its transformation to  $\text{NO}_3^-$ , preventing nitrification-induced  $\text{N}_2\text{O}$  and the large  $\text{NO}_3^-$ -induced denitrification peak. Second, Liu et al. (2022) suggest that the reduction of  $\text{N}_2\text{O}$  emissions is more effective when the soil experienced drying and rewetting. This is attributed to improved aeration and moisture distribution, which prevents abrupt shifts to highly anaerobic conditions. Such shifts often lead to incomplete denitrification and  $\text{N}_2\text{O}$  accumulation. Moreover, Liu et al. (2024) infer that the addition of zeolite promotes the growth and stability of denitrifiers carrying *nosZ*-gene, reduces the *nirS+nirK/nosZ* ratio, and thus tending to more complete  $\text{N}_2$ , which also applies to our study. However, in our study, we further infer that in our with 10 % zeolite engineered soils, zeolite provide much more available pores than normal soil, even at 70 % WFPS, thus providing  $\text{N}_2\text{O}$  from denitrification more time to stay in the pores, and thereby more likely to be further denitrified to  $\text{N}_2$ .

So far, only one of our previous studies evaluated the effect of NZone Max on  $\text{N}_2\text{O}$  emissions (Wang et al., 2020b), which is consistent with this study that no reduction in  $\text{N}_2\text{O}$  emissions was observed. The limited effectiveness of NZone Max observed in this study is likely due to the soil's high clay content. In fine-textured soils, the abundance of natural cation exchange sites may mask the additive's ability to increase  $\text{NH}_4^+$  retention. Furthermore, the densely structured and low-diffusivity characteristic of clay soils can restrict NZone Max's movement and interaction with soil N and soil microorganisms. Together, these conditions hinder its effectiveness in stabilizing N and reducing gaseous losses or leaching. Interestingly, in normal soils, peaks and cumulative  $\text{N}_2\text{O}$  emissions of UNZ were always higher than U, on the contrary, their peaks of UNZ in soil with zeolite was lower than U (Fig. 2A and B). We infer that in normal soil without plant, organic C was a limiting factor for denitrification (Wang et al., 2020a). NZone Max provided additional organic C for denitrification. However, in zeolite-engineered soils, although the additional C promoted denitrification, it also promoted more complete  $\text{N}_2\text{O}$ -to- $\text{N}_2$  denitrification due to the large amount of pore space provided by the zeolite.

There has been limited literature investigating how zeolite amendment to soils affects  $\text{CO}_2$  emissions. Although there are two studies showing that zeolite addition reduced  $\text{CO}_2$  emissions (Ferretti et al., 2017; Galamini et al., 2025), while another study (Mühlbachová and Simon, 2003) shows that zeolite amendment reduced  $\text{CO}_2$  emissions from a luvisol and no reduction from a cambisol, whereas their soil pH was 8.3–8.5 and 6.3–6.9, respectively. We didn't find any differences in cumulative  $\text{CO}_2$  emissions between soils with and without zeolite (Fig. 3C), but very interestingly, our high-resolution monitoring of  $\text{CO}_2$  emissions showed clearly different patterns: In soils without zeolite,  $\text{CO}_2$  emissions decreased much more sharply after irrigation and then increased more drastically, but the zeolite-amended soil showed a much more stabilized pattern of  $\text{CO}_2$  fluxes (Fig. 3A and B). This is a very clear evidence that zeolite stabilized soil microbial community and its respiration. The stability could be due to two primary mechanisms: first, zeolite improves soil structure and water-holding capacity (Nakhli et al., 2017; Ramesh and Reddy, 2011; Talebnezhad and Sepaskhah, 2013), which in turn stabilizes soil moisture regimes from drastic changes after irrigation, resulting in more stable microbial activity and consistent  $\text{CO}_2$ . Second, the high CEC of zeolite provided a steady nutrient supply (Al-Busaidi et al., 2008; Sarkar and Naidu, 2015), protecting the microbial communities from "boom-and-bust" respiration cycles. In addition, although not statistically significant, the increased  $\text{CO}_2$  peaks and cumulative emissions of the UNZ from soils without zeolite also indicate that the NZone Max provided additional organic C sources for microbes.

### 4.2. soil N and C cycles

Despite the expectation that zeolite can retains large amount of  $\text{NH}_4^+$ , soil  $\text{NH}_4^+$  concentrations remains near zero in all treatments (Fig. 4A), suggesting that most of  $\text{NH}_4^+$  were volatilized or nitrified to  $\text{NO}_3^-$  at the end of the incubation, resulted in high  $\text{NO}_3^-$  levels in all treatments (Fig. 4B). However, the significantly higher  $\text{NO}_3^-$  levels in zeolite amended soils indicate that zeolite retained more  $\text{NH}_4^+$

and more gradually undergone nitrification, rather than being lost through volatilization or leaching (Omar et al., 2015; Torma et al., 2014). Moreover, although we did not include plants, some studies have also shown that zeolite addition can increase crop yields (Liu et al., 2022; Sha et al., 2022; Zheng et al., 2024). Meanwhile, DOC and MBC did not differ significantly among treatments (Fig. 4). A reducing trend in DOC and MBC might be caused by the dilution of 10 % zeolite into the soil. The results suggest that neither fertilization nor zeolite addition triggered notable shifts in available organic substrates or microbial biomass. The relatively short incubation period, along with potentially abundant background C, may have buffered against detectable changes (Ferretti et al., 2018).

We found no differences in bacterial gene abundance in all treatments, but a potential reduction in fungal gene abundance in urea + NZone Max in both soils (Fig. 5 A and B), which may indicate that NZone Max potentially inhibits the fungal microbial community. We also found that ammonia-oxidizing bacteria (AOB) was more responsive to N fertilizer than ammonia-oxidizing archaea (AOA), which is consistent with other studies (Li et al., 2021; Tao et al., 2017). Zeolite promoted AOB was also documented in other studies (Wu et al., 2020; Yang et al., 2024; Zhang et al., 2022), while in our results with only insignificant tendency (Fig. 5 C and D). We did not find statistical differences in our denitrification-related gene abundances (Fig. E, F and G), however, Liu et al. (2024) demonstrated that the addition of zeolite reduced the *nirS+nirK/nosZ* ratio, which is partially in agreement with our results, which could be a potential reason for the reduced  $\text{N}_2\text{O}/\text{N}_2\text{O}+\text{N}_2$  ratio from denitrification. However, more robust conclusions require more direct evidence, such as enzyme activity or functional expression, e.g. nitrification/denitrification rates.

#### 4.3. Implications and limitations

Our laboratory application rate was much higher than the typical field rate of 5–10 t ha<sup>-1</sup> (Liu et al., 2022; Park et al., 2024). Our objective was to double the cation exchange capacity (CEC) in the short term. A 10 % (w/w) dose exceeded what would likely be required to reach that target, but produced clear, pronounced effects. The next step is to test lower zeolite rates to determine if similar outcomes can be achieved more efficiently. Possible strategies to reduce material use and increase efficiency also require verification. These strategies include co-applying zeolite with N fertilizer (e.g., as a carrier or blend) rather than mixing it into the soil in bulk, as well as prioritizing high-value cropping systems (e.g., orchards or greenhouse vegetables), where the return per unit of zeolite is higher.

While zeolite may alter the water-holding capacity (WHC) of soils, our incubations were conducted in closed, non-draining conditions with constant WPFS, which governs oxygen diffusion and nitrogen transformations. Therefore, any differences in WHC would not affect the comparisons of treatments in this study. However, field conditions involving drainage warrant further evaluation. Although CO<sub>2</sub> efflux had not fully stabilized by day 30, the repeating pattern of lower N<sub>2</sub>O and more stable CO<sub>2</sub> in zeolite-amended soils suggests that extending the incubation period would not change the observed effects. However, longer-term incubations and field studies are recommended to improve confidence levels and evaluate persistence under seasonal dynamics.

## 5. Conclusions

This study demonstrates that enhancing soil CEC through zeolite amendment significantly reduces NH<sub>3</sub> and N<sub>2</sub>O emissions from urea-fertilized soils. In addition, zeolite improved nitrate retention and stabilized microbial respiration, indicating improved NUE and microbial resilience. From a sustainability perspective, such mineral-based amendments hold promise for contributing to climate and air quality targets by simultaneously improving nutrient retention and reducing dependence on synthetic fertilizers. These findings provide scalable insights for climate-smart nutrient management in intensifying agricultural systems. Future work should focus on optimizing application rates and evaluating long-term agronomic and environmental outcomes, particularly in low-CEC soils or intensively managed systems.

## Authors' contributions

HW and KD designed the experiment. LJK conducted the incubation experiment with assistance from HW. PS provided experimental materials and resources, and contributed to the writing of the discussion section. XQ and ZL carried out the microbial measurements and performed microbial data analysis. HW drafted the initial manuscript. All authors reviewed and revised the manuscript and approved the final version for submission.

## CRediT authorship contribution statement

**Haitao Wang:** Writing – original draft, Validation, Methodology. **Lea Johanna Krug:** Writing – review & editing, Methodology, Investigation, Data curation. **Xin Qian:** Writing – review & editing, Resources, Methodology, Investigation. **Klaus Dittert:** Writing – review & editing, Supervision, Conceptualization. **Zongxin Li:** Writing – review & editing, Resources, Methodology, Investigation. **Panxu Sun:** Writing – review & editing, Resources, Project administration, Data curation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2025.104567](https://doi.org/10.1016/j.eti.2025.104567).

## Data availability

Data will be made available on request.

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