Soil Environ. 45(2): xx-xx, 2026
DOI: doi.org/10.25252/SE/2026/243238
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Slow-release urea fertilizer and nitrification inhibitors affect greenhouse gas emissions and population of soil microorganisms in a saturated soil

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[Received:** September 26, 2023 **Accepted:** June 08, 2026 **Published Online:** June 29, 2026**]

Abstract

The application of urea-based fertilizers in water-saturated soils represents a significant source of greenhouse gas (GHG) emissions, particularly nitrous oxide (N_2O). This study evaluated the effects of zeolite-based slow-release urea combined with nitrification inhibitors on methane (CH_4), carbon dioxide (CO_2), and N_2O emissions, as well as nitrogen transformation and soil microbial populations under saturated conditions. A 49-day laboratory incubation experiment was conducted using five treatments: control (no nitrogen), granulated urea (U), urea + zeolite (UZ), urea + zeolite + neem (UZN), and urea + zeolite + dicyandiamide (UZD). Methane emissions declined rapidly after 14 days and remained low across all treatments, indicating dominant methane oxidation under saturated conditions. Zeolite-amended treatments increased CO_2 emissions, likely due to enhanced microbial respiration. Notably, the UZD treatment significantly reduced N_2O emissions compared to other urea treatments, while maintaining higher ammonium (NH_4^+) and lower nitrate (NO_3^-) concentrations throughout the incubation period. The reduction in N_2O emissions was not associated with changes in nitrite-oxidizing bacterial populations but rather with inhibition of ammonia monooxygenase (AMO), thereby suppressing nitrification and reduced substrate availability for N_2O emissions. These findings indicate that process-based controls on nitrogen transformation are more critical than microbial population size in regulating N_2O emissions. In contrast, neem-based treatments showed less consistent mitigation effects. Overall, the results demonstrate that combining zeolite-based slow-release urea with dicyandiamide is an effective strategy for mitigating N_2O emissions under saturated soil conditions. However, field-scale validation is required to confirm these findings under natural agricultural conditions.

Keywords: Nitrification inhibitor; Slow-release fertilizer; Nitrogen transformation; Ammonium dynamics; Greenhouse gas mitigation

Introduction

Flooded fields, which are a major condition of rice fields in Indonesia, are one of the primary sources of methane (CH_4) (Hafif *et al.*, 2024). As a greenhouse gas, CH_4 is second only to carbon dioxide (CO_2) in volume, but has 25 times greater global warming potential than CO_2 on a mass basis (IPCC, 2023). Methane is produced by the reduction of hydrogen and CO_2 in anaerobic conditions by methanogens (Anderson *et al.*, 2010). Atmospheric methane concentration have more than doubled over the last two centuries, increasing by 3% annually (Battle *et al.*, 1996) and is projected to potentially double due to wetland methane emissions between 2017 and 2100 due to increased availability of permafrost carbon (Lawrence *et al.*, 2015).

Together with CO_2 and CH_4, nitrous oxide (N_2O) is another important greenhouse gas associated with rice production. Nitrous oxide is 298 times more potent than CO_2 as a greenhouse gas and the atmospheric concentration of N_2O continues to rise at a rate of approximately 0.26% per year and has reached a concentration of 319 ppb (10-9 mol mol^{-1}). It is also involved in stratospheric ozone depletion (IPCC, 2023). Nitrous oxide is produced naturally in soil via denitrification and nitrification processes after fertilization,

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Cite This Paper: Jumadi, O., S. Wahyuni, Y.A. Djawad, N.A.S. Taufieq, St.F. Hiola, J. Mamangkey, W. Cheng and K. Inubushi. 2026. Slow-release urea fertilizer and nitrification inhibitors affect greenhouse gas emissions and population of soil microorganism in a saturated soil. *Soil Environ*. 45(1): xx-xx.

which substantially depend on anaerobic conditions and nitrate content (Zhu *et al.*, 2025). Therefore, the use of nitrogen-based fertilizer commonly increases N_2O emissions, and it is expected that 1% of the fertilizer is converted to N_2O emissions to the atmosphere on a global scale (Ma *et al.*, 2024).

The use of nitrification inhibitors has been shown to be an effective technique to reduce N_2O emissions and NO_3^- leaching from soils, when mineral or high organic-N fertilizers are applied. Dicyandiamide (DCD) is a widely used nitrification inhibitor that has been demonstrated to effectively reduce N_2O emissions under field conditions (Di *et al.*, 2007; Smith *et al.*, 2008; Jumadi *et al.*, 2019, 2020). Its mode of action involves the inhibition of ammonia monooxygenase (AMO), a key enzyme in ammonia-oxidizing microorganisms responsible for the oxidation of NH_4^+ to NO_2^- (Lei *et al.*, 2022). By suppressing this initial step of nitrification, DCD limits the formation of nitrogen intermediates that contribute to N_2O emissions.

Despite its effectiveness, previous studies have reported that DCD does not significantly affect overall soil microbial biomass (Zaman *et al.*, 2009; Guo *et al.*, 2013), suggesting that its impact is primarily functional rather than structural. However, the adoption of DCD in low-input rice production systems remains limited due to its relatively high cost. As an alternative, plant-based nitrification inhibitors such as neem seed extracts have gained attention.

Neem seeds contain a range of bioactive compounds, including azadirachtin, nimbin, diepoxyazadiradione, dehydrogedunin, gallic acid, and gedunin (Jarvis *et al.*, 1999; Rahmadani *et al.*, 2023), which have been reported to influence soil microbial activity. In particular, azadirachtin has been shown to suppress populations of nitrifying microorganisms (Sarawaneeyaruk *et al.*, 2015; Das *et al.*, 2018), indicating its potential as a natural nitrification inhibitor. The use of zeolite in soil is widely recognized as a soil amendment due to its ability to slow down urea leaching and mineral release due to its high ion-exchange capacity (GirijaVeni *et al.*, 2021). This can slow the release of NH_4^+ from urea, thereby limiting the substrate for ammonia-oxidizing bacteria and reducing nitrification.

Rice cultivation in Indonesia predominantly adopts a continuously flooded field system after fertilization, keeping the soil to be submerged above a 6-cm water level (Jumadi *et al.*, 2012). Meanwhile, in global rice cultivation, the intermittent drainage strategy has been found to decrease the emissions of CH_4 (Nishimura *et al.*, 2020), suggesting that maintaining a saturated soil condition instead of a flooded condition may be a viable solution for reducing greenhouse gas emissions. However, the combined effects of nitrification inhibitors and slow-release fertilizers on greenhouse gas emissions under saturated soil conditions remain insufficiently explored.

While nitrification inhibitors and zeolite have been extensively studied under flooded paddy conditions, their combined effects under saturated (non-flooded) soil conditions remain poorly understood. This study provides novel insights into nitrogen transformation and greenhouse gas emissions under such conditions, with particular emphasis on process-based mechanisms linking nitrification inhibition and substrate availability. Therefore, this study aimed to evaluate the effects of urea amended with different nitrification inhibitors and slow-release formulations on trace gas emissions under saturated conditions. In addition, changes in soil mineral nitrogen (NH_4^+ and NO_3^-) and microbial populations were assessed to elucidate the underlying mechanisms governing greenhouse gas dynamics.

Materials and Methods

Soil preparation

The soil was taken from a rice paddy field in Maros district, South Sulawesi Province, Indonesia, which was classified as typic haplusterts (USA Department of Agriculture Natural Resources Conservation Service, 1998). The soil was identified as an alluvial soil type (Subardja *et al.*, 2014). The soil texture was classified as silt clay loam, consisting of 54% silt, 38% clay, and 8% sand. The soil pH was 6.7, and the C/N ratio was 9.76, with a total carbon content of 20.5 g–C kg^{-1} and total N content of 2.1 g–N kg^{-1} dry soil (Jumadi *et al.*, 2020). The water content before incubation was 65%. Five treatments in triplicate were carried out in a completely randomized design. Forty grams of soil were placed in 140 mL glass bottles and 5 mL of distilled water was given to each treatment to create saturated conditions. Different nitrogen fertilizer types acquired from Jumadi *et al.*, 2020 were applied as the treatments: Treatment C (without N application, set up as control), U (granulated urea with nitrogen content of 10%), UZ (granulated urea mixed with 10% zeolite), UZN (granulated urea mixed with 10% zeolite and 5% neem) and UZD (granulated urea mixed with 10% zeolite and 5% DCD). These fertilizers were prepared according to Jumadi *et al.*, 2020. The fertilizer was applied to the soil in the first week after the pre-incubation period. The average temperature during treatments was 25°C. A bottle was set up for gas measurement, which was closed tightly with a vial rubber stopper. A needle (Terumo, 23), to which a spinner had been attached, was inserted through the rubber as the link to collect the gas samples, while the spinner blocked air diffusion through the needle during incubation. The soil was incubated for 49 days (Figure 1).

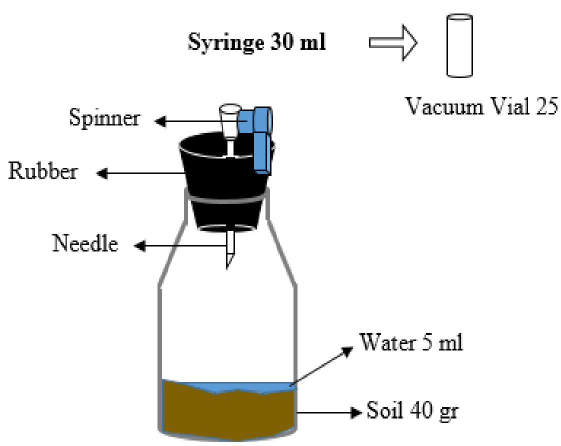

Figure 1: Illustration of the soil incubation setup for gas measurements. 10 grams of soil added with 5 mL of water was placed inside a 140 mL glass bottle. The bottle was closed by a rubber stopper. A needle which had been attached with spinner on the needle, placed through the rubber as the link to take the gas samples, while the spinner blocked the air diffusion through the needle while being incubated. ml should be corrected as mL in figure

Analytical methods for greenhouse gas emissions, NH_4^+, NO_3^-, and Microorganism

Population

Separate sets of incubation bottles were used for gas sampling and destructive soil sampling to avoid disturbance effects. Gas emissions were measured repeatedly from sealed bottles throughout the incubation period, while independent replicate bottles were destructively sampled at each time point (7, 21, 35, and 49 days after fertilization) for NH_4^+ and NO_3^- analysis. This design ensured that gas measurements were not affected by soil removal during mineral nitrogen analysis.

The gas samples were taken using disposable syringes and injected into evacuated air glass vials. The concentration of the gas was quantified using a gas chromatograph equipped with an electron capture detector (ECD), Flame Ionization Detector (FID) and Thermal Conductivity Detector (TCD) for N_2O, CH_4 and CO_2 gas determination, respectively (Jumadi *et al.*, 2019). Gas measurements represented net emissions (i.e., the balance between emissions and consumption processes) under controlled incubation conditions.

Following the gas measurement, incubated soil was also prepared for NO_3^- and NH_4^+ measurement every 14 Days after fertilization (daf) until 49 daf. Forty-five (45) bottles with the five fertilizer treatments were used for NO_3^- and NH_4^+ measurement at 7, 21, 35 daf. Furthermore, 15 bottles of soil for gas measurement were used to measure final NO_3^- and NH_4^+ at 49 daf after collecting the final gas sampling. Ten (10) grams of soil were taken from incubated soil and was weighed into a 75-mL plastic bottle with screw cap. The soil was mixed with 50 mL of 2M KCL by shaking for 45 minutes on a shaker with 200 rpm speed. The solution was filtered (Whatman No. 42). The concentrations of NH_4^+ were measured by the nitroprusside method (Anderson and Ingram, 1989), while concentrations of NO_3^- were measured by the hydrazine reduction method (Hayashi *et al.*, 1997). NH_4^+ and NO_3^- were measured at 7, 21, 35 and 49 daf.

Population of microorganisms in the incubated soil was observed at the 49 daf. Numbers of ammonia and nitrite-oxidizing bacteria in the soil were determined using the Most Probable Number (MPN) method (Bottomley *et al.* 2020). Ten grams of soil was mixed with 90 mL of sterile phosphate buffer as the first dilution (10^{-1}). Nine replicates of culture tubes were made for dilutions of 10^{-3} - 10^{-5}. Furthermore, populations of AOB were identified using Griess-Ilosvay reagent, while populations of NOB were identified using nitrate spot reagent. Populations of fungi were estimated by the total plate count method (Bottomley *et al.*, 2020).

Data are presented as means ± standard deviations. Statistical analyses were performed using SPSS Statistics for Windows (Version 19.0; IBM Corp., Armonk, NY, USA). Differences among treatments were evaluated using analysis of variance, followed by the least significant difference (LSD) test at a significance level of $p < 0.05$.

Results

Methane emissions

Methane emissions from all treatments showed a similar pattern. There was a sharp decline in methane emissions at 14 daf for all treatments (Figure 2a). Treatment UZN appeared to have the highest values for the first three weeks compared with other urea treatments and was approximately 60% higher than in the U treatment at 7 daf.

At 7 daf, methane emissions were highest in the control and UZN treatments (4.82 and 4.59 µg g^{-1} soil, respectively), followed by UZD and UZ, while the lowest emissions were observed in the U treatment (0.28 µg g^{-1} soil). The emissions of all treatments then plunged to a range of 0.03-0.05 µg g^{-1} soil at 14 daf. Overall, differences among treatments were evident only at the initial stage, after which emissions converged to similarly low levels across all treatments.

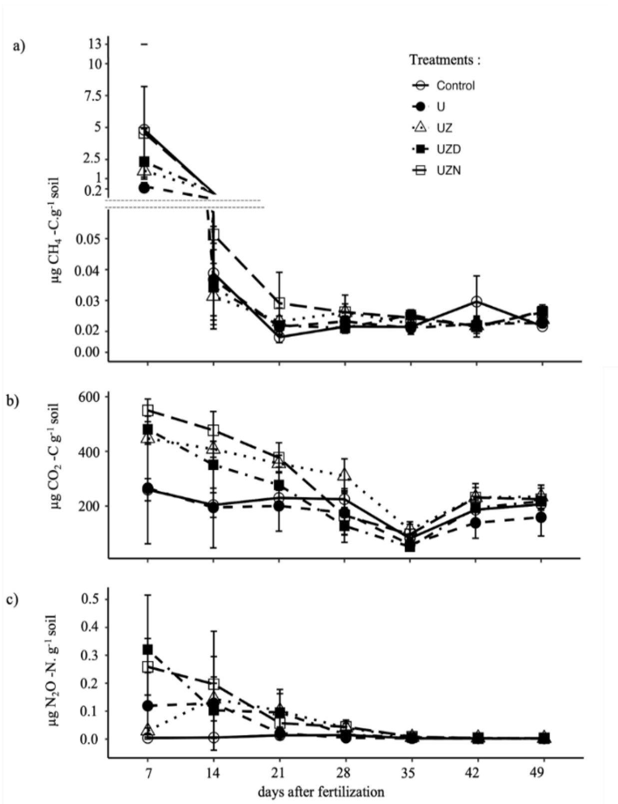

Figure 2: Changes of gas emissions in saturated soil during 49 days after fertilization (daf) varied between treatments. 40 grams of soil were incubated with an addition of 5 mL water to create a saturated soil condition. Soil was treated with 5 treatments: C (without N application), U (granulated urea), UZ (granulated urea mixed with zeolite), UZD (granulated urea mixed with zeolite and DCD) and UZN (granulated urea mixed with zeolite and neem). Gas emissions were measured at 7, 14, 21, 28, 35, 42 and 49 daf . a) Changes in CH_4 emissions b) Changes in CO_2 emission. c) Changes in N_2O emission. Error bars represent standard deviation (n = 3)

Carbon dioxide emissions

Carbon dioxide emissions from five fertilization treatments reached a peak at the initial measurement (Figure 2b). Emissions of CO_2 at 7 daf were 55.01, 48.06, 44.75, 26.55 and 25.99 µg g^{-1} soil from treatment UZN, UZD, UZ, U and control, respectively. Higher CO_2 emissions were observed in treatments UZ, UZN and UZD at 7, 14, and 21 daf. The CO_2 emissions from all treatments then gradually declined and reached the lowest level at 35 daf, approximately 5.0–11.5 µg g^{-1} soil. Following this, the CO_2 emissions in all treatments then gradually increased at 42 and 49 daf. Treatments UZ and UZN consistently produced higher CO_2 emissions than other treatments between 35 and 42 daf. Meanwhile, treatment C and U emitted less CO_2 compared with the other treatments. This trend was observed from 7 to 49 daf. Overall, zeolite-amended treatments (UZ, UZN, and UZD) exhibited consistently higher CO_2 emissions compared to non-zeolite treatments.

Nitrous oxide emissions

The changes in nitrous oxide emissions are illustrated in Figure 2c. During the 49-daf observation, the N_2O emissions tended to decrease in all treatments. Emissions of N_2O in C was very low during 7 to 49 daf, only about 0.001 to 0.02 µg g^{-1} soil. These low N_2O emissions in control was due to the absence of nitrogen in the fertilizer in that treatment. This also demonstrates the reliability of the measurements, where the absence of nitrogen fertilizer was expected to produce lower N_2O emissions.

The UZN and UZD treatments showed higher N_2O emissions at 7 daf which were 0.26 and 0.32 µg g^{-1} soil respectively. Treatment U and UZ produced 0.12 and 0.03 µg g^{-1} soil respectively, while C only produced 0.004 µg g^{-1} soil. All treatments produced less N_2O at 14 daf, with the exception of UZ which increased to 0.14 µg g^{-1} soil which then decreased in the following measurements. No significant difference was found at 14-21 daf. Following this, UZ and UZN tended to produce higher N_2O emissions at 28 to 49 daf. This amount ranged from 0.04 µg g^{-1} soil at 28 daf to 0.03 µg g^{-1} soil at 49 daf in both treatments. Among the treatments, UZD consistently showed lower N_2O emissions during the later stages of incubation (21–49 daf), indicating its effectiveness in suppressing nitrification-derived N_2O emissions.

Ammonium (NH_4^+) and Nitrate (NO_3^-) contents of soil

The changes in NH_4^+-N and NO_3^- contents in soil are shown in Figure 3a-b. The amount of NH_4^+-N at the initial measurement was approximately 0.070-0.080 µg g^{-1} soil in all treatments, except for control, which was 0.023 µg g^{-1} soil. This amount then increased at 21 daf in treatments that received urea, then drastically declined at 35 daf. Application of urea in four treatments (U, UZ, UZD and UZN) significantly increased NH_4^+ content at 7 and 14 daf compared with control. Also, different concentration levels occurred between treatments that received urea and control. The application of UZD at 35 daf and followed by UZ at 49 daf increased NH_4^+ content compared with control by a further 0.027 µg g^{-1} soil, while control was 0.015 µg g^{-1} soil.

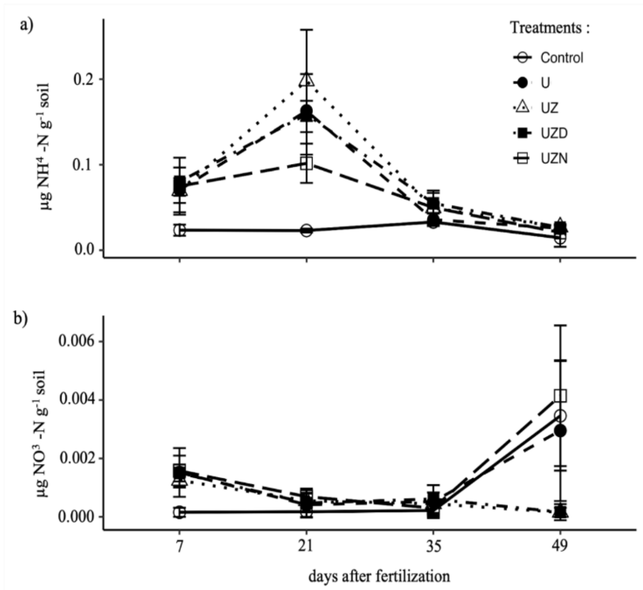

Figure 3: Changes of mineral nitrogen concentration in saturated soil during 49 days after fertilization (daf) varied between treatments. 40 grams of soil were incubated with an addition of 5 ml water to create a saturated soil condition. Soil was treated with 5 treatments: C (without N application), U (granulated urea), UZ (granulated urea mixed with zeolite), UZD (granulated urea mixed with zeolite and DCD) and UZN (granulated urea mixed with zeolite and neem). Nitrogen concentration in the form of NH_4^+ and NO_3^- was measured at 7, 21, 35 and 49 daf. a) Changes in NH_4^+ concentration b) Changes in NO_3^- concentration. Error bars represent standard deviation (n = 3)

Coincident with the increase of NH_4^+, the NO_3^- in all urea treatments decreased at 35 daf. At initial measurement, all urea treatments contained larger amounts of NO_3^- than control due to the absence of urea in control. There was a substantial increase in NO_3^- by 0.0035, 0.0030 and 0.0041 µg g^{-1} soil for control, U and UZN at 49 daf respectively, while UZ and UZD were noticeably lower by 0.0002 µg g^{-1} soil. Notably, the UZD treatment maintained higher NH_4^+ concentrations and lower NO_3^- levels compared with other treatments, indicating effective inhibition of nitrification.

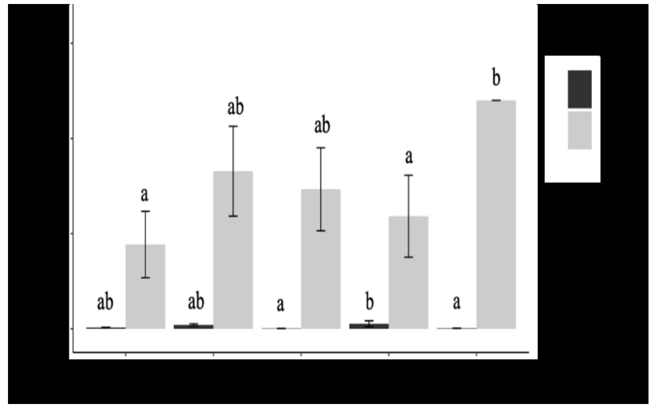

Figure 4: Population of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in soil at 49 days after fertilization (daf). Soil was treated with 5 treatments: C (without N application), U (granulated urea), UZ (granulated urea mixed with zeolite), UZD (granulated urea mixed with zeolite and DCD) and UZN (granulated urea mixed with zeolite and neem). Different letters denote comparison between treatments based on *LSD* test $p < 0.05$. Error bars represent standard deviation (n=3)

Population of ammonia-oxidizing vacteria (AOB) and Nitrite-oxidizing bacteria (NOB)

Figure 4 presents the populations of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), quantified using the most probable number (MPN) method at 49 days after fertilization (daf), when the soil microbial community was considered to have reached a relatively stable state. Overall, NOB populations were substantially higher than those of AOB.

The population of AOB was higher in treatments UZD and U which were about 5.0 x 10^3 and 4.0 x 10^3 cells g^{-1} soil respectively (Figure 4). Meanwhile, treatments UZ and UZN reduced the populations of AOB compared to control. About 5.0 x 10^2 and 3.0 x 10^2 cells g^{-1} soil were observed in treatments UZN and UZ respectively. On the other hand, the population of NOB was higher than AOB, Hence, it is important to notice that the use of urea fertilizer increased NOB population compared with control. However, these differences in microbial abundance did not directly correspond to observed N_2O emission patterns.

Population of fungi

Fungal populations were quantified at 49 days after fertilization (daf) using the total plate count method (Figure

5). All fertilized treatments showed reduced fungal abundance compared with the control. The control treatment exhibited the highest fungal population (1.83 × 10^4 cells g^{-1} soil), although the differences were not statistically significant. Application of urea alone reduced the fungal population to 1.30 × 10^4 cells g^{-1} soil. The UZ treatment showed a moderate reduction in fungal abundance compared with the control, but higher values than those observed in inhibitor-amended treatments. The greatest reductions were observed in the UZD and UZN treatments, both showing fungal populations of 1.17 × 10^4 cells g^{-1} soil.

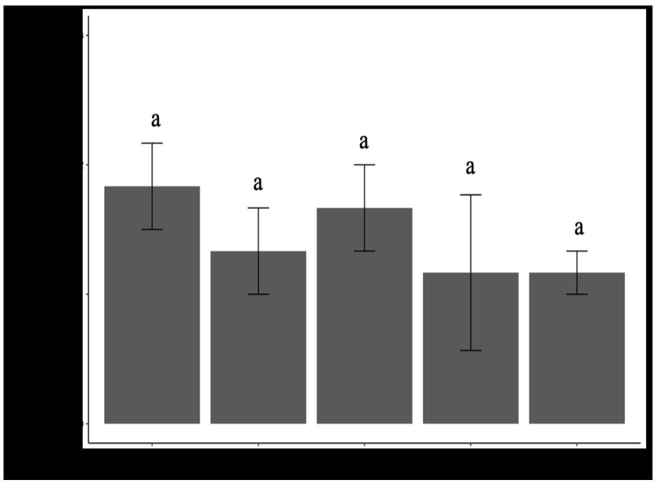

Figure 5: Population of fungi in soil at 49 days after fertilization (daf). Soil was treated with 5 treatments: C (without N application), U (granulated urea), UZ (granulated urea mixed with zeolite), UZD (granulated urea mixed with zeolite and DCD) and UZN (granulated urea mixed with zeolite and neem). Different letters denote comparison between treatments based on LSD test *p* < 0.05. Error bars represent standard deviation (n=3)

Discussion

Nitrification inhibitors and Zeolite affect the changes in carbon-based greenhouse gases

Methane emissions significantly decreased at 14 daf of measurement and tended to be stable after 21 daf in all treatments. In accordance with Wang *et al.* (2020) and Wu *et al.* (2013), their study also reported a substantial decline in CH_4 emissions from rice fields during the initial stages after planting. This significant decrease can be caused by methanotroph activity in the oxidation of the produced CH_4. As CH_4 emissions are the result of methanogen activity in the anoxic layer of the soil, an oxic layer forms above the anoxic soil layer (Ward *et al.*, 2020). The oxic layer is where methanotrophs live, which are responsible for oxidizing CH_4 into CO_2.

According to Ward *et al.* (2020), CH_4 oxidation is a very effective process in limiting CH_4 emissions from wetlands inundated with a shallow layer of oxic water. Furthermore, the setup of our experiment is in a controlled incubation environment, so there was less chance for disturbance to oxic soil layers that had been formed. Therefore, the shallow layer of oxic water can successfully reduce CH_4 emissions despite different treatments being applied. Another explanation for the observed decline in CH_4 emissions is that the presence of readily oxidizable organic compounds (ROC) became depleted after 7 daf, consequently, the emissions of CH_4, which is strongly linked to the amount of ROC, also decreased (Yagi and Minami, 1990; Wang *et al.*, 2020).

The application of urea fertilizers mostly did not have a significant effect on CH_4 emissions. However, the addition of neem + zeolite resulted in higher CH_4. A possible reason behind the higher CH_4 emissions is that neem seed contains some major bioactive compounds, such as Terpinen-4-ol, 1,2,4-Trithiolane, 3,5-diethyl, etc., which are known for antimicrobial activity (Swapna Sonale *et al.*, 2018). These bioactive compounds might affect the activity of the methanotroph in the oxic layer, therefore, affecting the CH_4 oxidation activity. Meanwhile, the use of another nitrification inhibitor, DCD, contributed to higher CH_4 emissions at 35 and 49 daf. Previous studies have shown similar result of using nitrification inhibitor DCD tends to produce higher CH_4 emissions in paddy field (Datta and Adhya, 2014).

Furthermore, CO_2 emissions in the first three weeks was significantly higher in the use of urea fertilizer with zeolite in three treatments (UZ, UZN and UZD). The source of CO_2 emissions in soil is mainly from microbial activity, for example the decomposition of organic material, or also by the conversion of CH_4 to CO_2 through CH_4 oxidation by methanotroph. The use of zeolite has been known for improving soil health condition, including soil microorganism composition (Prisa, 2021). Therefore, it may enhance the growth of decomposing microbes which led to a higher CO_2 emission.

Effect of nitrification inhibitor and Zeolite on N_2O emissions and Mineral nitrogen concentrations

The emissions of N_2O in granulated urea only lasted for the first two weeks (0.12– 0.13 µg g^{-1} soil), then declined to a low level at the third measurement. There were no further emissions after 28 daf, although some small emissions might have been expected as an intermediary product of nitrification. A small amount of N_2O would be expected as the product of nitrification (about 1% of NH_4^+ processed by nitrification), approximately 0.01 µg N_2O g^{-1} soil day^{-1} listed as a low level. N_2O emissions from nitrification rarely exceed 2% (Carneiro, *et al*., 2010).

The use of zeolite + granulated urea (UZ) produced significantly lower NO_2 emissions in the initial measurement, but then later increased in the following week. The addition of zeolite in urea seems to give a delay effect in the emissions of N_2O. However, this delaying effect did not last long because the amount of N_2O at 28 daf- 49 daf was found to be higher compared with other treatments. Rather than having delayed N_2O emissions, Liu *et al.* (2020) found that the zeolite application significantly mitigates N_2O emissions. This discrepancy regarding the effectiveness of zeolite in literature might be due to differences in experimental setup and zeolite application rate. Furthermore, zeolite application produces a lower NO_3^- concentration at 49 daf, suggesting that less NH_4^+ being converted at that time compared with granulated urea treatment.

Furthermore, the use of DCD + zeolite tended to reduce N_2O emissions. Although it increased N_2O emissions in the initial measurement, the N_2O was produced significantly lower after that. This was also followed by the lower amount of NO_3^- at 21 until 49 daf, while the ammonium concentration was still higher during the 49-daf measurement. This indicates that DCD reduces N_2O emissions by inhibiting the conversion of NH_4^+ into NO_3^-. Even though the number of AOB at 49 daf was higher compared with others, it did not affect the NO_3^- emissions, as the concentration of the NO_3^- was lower compared with other treatments.

The addition of neem and zeolite tended to promote a higher N_2O emissions compared with the granulated urea treatment throughout the 49-days measurement. However, this finding is different from Bordoloi *et al.* (2020) which argued that neem-coated urea is able to lower the N_2O emissions. This difference in this finding can be caused by different experimental setup and duration of experiment. Moreover, the use of neem was not significantly different from U for the NH_4^+ content in the beginning, but then produced significantly lower NH_4^+ content at 49 daf and higher NO_3^- content at the same time. The use of neem seed extract as a nitrification inhibitor may not be as effective as DCD, but rather works at a later stage. Therefore, it might be useful to prolong the duration of future experiments to investigate more the effectiveness of neem seed extract as a nitrification inhibitor in saturated soil conditions.

Overall, the UZD treatment showed the most consistent effect in reducing N_2O emissions while maintaining higher NH_4^+ and lower $NO_3^−$ concentrations, indicating its effectiveness in regulating nitrogen transformation under saturated conditions.

Different responses from soil microorganism population

The use of urea fertilizer affects the population of AOB and NOB in soil. Therefore, the population of AOB and NOB is expected to be higher in all urea treatments. Our result on AOB population shows that the use of zeolite and zeolite + neem slightly reduced the AOB population. The population of AOB can be affected by the amount of readily available NH_4^+ in the soil. The physicochemical and surface charges properties in zeolites have the potential to act as binding agents for NH_4^+ (Sarkar and Naidu, 2015). Therefore, although the concentration of NH_4^+ was high in urea + zeolite, there was less NH_4^+ readily available for AOB to be converted into NO_3^-. This is also supported by our result of a lower NO_3^- concentration in zeolite treatment at that time of measurement. Meanwhile, the low abundance of AOB in zeolite + neem treatment might be caused by the fact that neem contains high tannin and the other secondary metabolite compounds such as azadirachtin, diepoxy Azadiradione, dehydrogedunin, and gedunin that can reduce the activity of nitrifiers (Rahmadani *et al.* 2023). Therefore, reducing AOB population.

Furthermore, the study shows contrasting responses of NOB abundance under neem and DCD treatments; however, these differences do not directly explain the observed patterns in nitrogen transformation and N_2O emissions. In the DCD treatment, N_2O reduction cannot be attributed to a decrease in NOB population, as the microbial data do not show a consistent suppression of NOB abundance. Instead, the lower N_2O emissions observed in the DCD treatment are more plausibly explained by the inhibition of ammonia monooxygenase (AMO), which suppresses the oxidation of NH_4^+ to $NO_2^−$. This inhibition limits the formation of $NO_2^−$ and subsequently $NO_3^−$, thereby reducing substrate availability for both nitrification and denitrification processes.

This interpretation is supported by the lower $NO_3^−$ concentrations and higher NH_4^+ levels observed in the DCD treatment compared to other urea treatments. Thus, the reduced N_2O emissions are primarily driven by constrained nitrogen transformation pathways rather than changes in NOB population size. In contrast, the neem treatment showed a tendency toward higher NOB abundance, but this did not correspond to a consistent reduction in N_2O emissions, suggesting that neem may exert a weaker or less direct inhibitory effect on nitrification processes under saturated conditions.

The application of urea fertilizer reduced fungal population due to the high toxicity of urea with respect to fungal growth. Urea can decrease the growth of Oomycota, saprophytic and pathogen fungi (Veverka, 2007). As a result, the growth of fungi in all urea treatments was slightly lower compared with the control. Moreover, treatments neem + zeolite and DCD + zeolite reduced fungi population 1.17×10^4 cells g^{-1} soil in both treatments. The lower fungi growth in the use of neem + zeolite may relate to the triterpenoid compounds in neem seed that can inhibit the population of fungi. Ospina *et al.* (2015) reported that neem can reduce the activity of *T. Tubrum, T. Mentagrophytes* and Microsporum. Furthermore, dicyandiamide compound in treatments has also been proven to be an inhibitor of fungi growth. Carneiro (2010) found that application of dicyandiamide to soil can change the microbial composition structure of soil, which increases bacteria population and reduces fungal activity. On the other hand, the addition of zeolite + urea affects the soil fungi population less compared with other urea treatments. This shows the benefit of using zeolite for improving soil microorganism composition (Prisa, 2021).

This study was conducted under controlled laboratory conditions, which may not fully capture the complexity of field environments, including plant-soil interactions, fluctuating redox dynamics, and spatial heterogeneity. Therefore, extrapolation to field-scale systems should be made with caution, and future studies should validate these findings under realistic agronomic conditions.

Conclusion

The present study demonstrates that the combined application of zeolite-based slow-release urea and nitrification inhibitors significantly regulates greenhouse gas emissions and nitrogen transformation under saturated soil conditions. Among the treatments, zeolite combined with dicyandiamide (DCD) was the most effective in reducing N_2O emissions, maintaining higher NH_4^+ levels, and suppressing NO_3^- formation. This mitigation effect was not linked to changes in nitrite-oxidizing bacterial (NOB) populations but rather to the inhibition of ammonia monooxygenase (AMO), indicating that process-based controls on nitrification are more critical than microbial abundance in regulating N_2O emissions. In contrast, neem-based treatments showed less consistent effects, suggesting lower or delayed inhibitory efficiency. Zeolite application enhanced CO_2 emissions, likely due to increased microbial respiration, while having limited influence on CH_4 emissions.

Overall, the combination of zeolite and DCD represents an effective strategy for mitigating N_2O emissions in saturated soils. However, further validation under field conditions is required to account for environmental variability and plant–soil interactions. These findings provide important insights for improving nitrogen use efficiency and reducing greenhouse gas emissions in water-saturated agricultural systems.

Acknowledgment

This work was supported by the Ministry of Higher Education, Science and Technology of Indonesia. The authors sincerely acknowledge the dedicated technical support and contributions of the laboratory team during the experimental work and data collection.

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