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RESEARCH ARTICLE

Combined effects of nitrification inhibitor and zeolite on greenhouse gas fluxes and corn growth

Oslan Jumadi[1] · Yusminah Hala[1] · R. Neni Iriany[2] · Andi Takdir Makkulawu[2] · Junja Baba[3] · Hartono[1] · St. Fatmah Hiola[1] · Kazuyuki Inubushi[3]

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Abstract

Field and incubation experiments were conducted to determine the emission rate of greenhouse gases, nitrogen change, populations of AOB, NOB, and fungi as well as growth of corn in response to amendment of urea granulated with and without nitrification inhibitors and zeolite. The application of urea with neem, urea with zeolite, urea with zeolite + neem, urea with zeolite + dicyandiamide, and urea with dicyandiamide (UD) decreased the N_2O emissions by 16.3%, 59.6%, 66.8%, 81.9%, 16.3%, and 86.7%, respectively. Meanwhile, patterns of CH_4 fluxes were mostly determined by small emissions. Increase in corn height, weight of cobs, biomass, and chlorophyll leaf contents were not significantly different between urea alone and urea with NIs and zeolite. In the incubation experiment, the highest concentration of NH_4^+ and N_2O production was detected during the first week and it remained high up to the second week of incubation in the combination of urea with NIs and zeolite treatments, although there was no significant difference compared with urea. During NH_4^+ decrease, the concentration of NO_3^- started to accumulate from the second to the third weeks. Production of CO_2 showed no significant differences among treatments. The static production of CO_2 could also explain that NIs and zeolite additions did not change AOB, NOB, and fungi activities after the fourth week of incubation.

Keywords Emission of N_2O and CH_4 · CO_2 Production · Dicyandiamide · Neem · Nitrification inhibitor · Zeolite

Introduction

Urea ($CO(NH_2)_2$ has been widely used by farmers as a major source of nitrogen fertilizer to support corn production which is the second most important cereal after rice in Indonesia. The worldwide demand for urea was forecasted to increase from 148 Mt in 2010 to 171.7 Mt in 2015, representing a growth of 3.2% per annum (IFA 2011). Nitrogen (N) is more substantial in a plant and needs larger quantities than other nutrients and it is estimated that only 30–40% of the application of N fertilizer is taken up by the crop. Most of it disappears by ammonia volatilization, nitrification, and denitrification. Therefore, the use of urea in the agriculture sector in order to increase the quantity and quality of agricultural food production can generate a negative impact on the environment, such as ozone layer depletion due to enhancement of greenhouse gas emissions, particularly nitrous oxide (N_2O) gas (Mosier and Kroeze 2000).

Emission of N_2O gas in agricultural land is determined by the nitrification process in aerobic soil conditions and formed nitrate (NO_3^-) that has mobility capacity to leach as a pollutant to the environment. The NO_3^- is susceptible to denitrification loss in anaerobic conditions in soil or water. In addition, that process is also a cause of low use nitrogen fertilizer efficiency in the agricultural sector (Mosier and Kroeze 2000). The process of release of N_2O from the soil into the atmosphere is influenced by diffusion processes in the soil and the capacity of soil to consume N_2O, which is determined by several factors such as the production footprint in the soil, soil organic matter, soil texture, and soil water content (Zhang et al. 2017; Majumdar et al. 2002; Jumadi et al. 2014).

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✉ Oslan Jumadi
oslanj@unm.ac.id

[1] Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar 90224, Indonesia

[2] Plant Breeding, Cereals Research Institute, Maros 90514, Indonesia

[3] Graduate School of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan

Efforts have been made to increase nitrogen use efficiency by additional substrates in nitrogen fertilizer, e.g., use of nitrification or urease inhibitors and slow release with coating technologies (Akiyama et al. 2013; Jumadi et al. 2008). The intent of these methods is to increase food production through an optimized fertilizer utilization rate to reduce the negative environmental impact of NO_3^- leaching and N_2O losses to the atmosphere. Hence, reduction of nitrogen losses to the environment is the main area of research to increase nitrogen use efficiency (Ruser and Schulz 2015). Nitrification inhibitors (NIs) retard the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) in soil for a certain time by inhibition of ammonia monooxygenase (AMO, the first enzymatic step of nitrification) (German-Bauer and Amberger 1989). Inhibition of nitrification can be utilized by the addition of synthetic nitrification inhibitors. However, these synthetic NIs are usually expensive and limited in availability in the market, particularly in Indonesia.

Several studies have also considered that organic NIs such as neem (*Azadirachta indica*) and karanja (*Pongamia glabra*) have NI properties to delay nitrification in nitrogen fertilizer (Opoku et al. 2014; Majumdar et al. 2000; Jumadi et al. 2019; Sharma and Prasad 1996; Kumar et al. 2007). Neem has been used in management of pests and disease in plants for a long time in India and besides having insecticidal properties, neem cake has been found to be an effective NI (Mohanty et al. 2008). Neem seeds contain secondary metabolites such as polyphenols, azadirachtin or certain unsaturated fats, and other tetranortriterpenoids that can act as inhibitors of nitrification and urease; therefore, it can improve the efficiency of urea fertilizer (Abbasi et al. 2011; Majumdar (2005)). Another strategy to increase the efficiency of nitrogen contained in the urea is the addition of polymer material to the urea fertilizer to slow release, such as polyolefin, polyethylene, and zeolite minerals (Ahmed et al. 2008; Azeem et al. 2014). Zeolites are naturally occurring aluminosilicate minerals that have a tridimensional porous structure with hollow channels; therefore, they have an extensive surface area that enables them to bind ammonium within the pore structure. In addition, zeolite can reduce ammonia volatilization because of its high CEC and affinity for NH_4^+. Furthermore, with small internal holes of zeolite (10^{-6} m), it will theoretically preserve NH_4^+ from responsible microbes that drive nitrification, e.g., ammonium oxidizer bacteria or ammonium oxidizer archaea (Kithome et al. 1998).

Methane monooxygenase (MMO) catalyzes methane (CH_4) gas as a substrate to form CO_2 gas and this process also can be driven by ammonium monooxygenase (AMO) (Hanson and Hanson 1996). A general characteristic of monooxygenase enzymes is a broad substrate range, and in this respect, AMO fits well in this group, as well as over 40 compounds which have been shown to be substrates of AMO which can competitively inhibit NH_3 oxidation. Hence, correlation mechanisms between AMO and MMO are affected by the amendment of nitrification inhibitor which can reduce the activity of both enzymes (McCarty 1999). Mohanty et al. (2008) pointed out that under incubation, experimental application of DCD at the time of soil incubation resulted in a substantial reduction in CH_4 production (31% over that of untreated control), but under field conditions, they suggested that repeat application of DCD with fertilizer N to flooded rice soils might not be effective in controlling CH_4 production. However, few studies have been conducted in field experiments to explore the effect of organic and chemical nitrification inhibitors combined with urea and zeolite on the rate of greenhouse gas fluxes, population of soil microbes, and growth of corn crops in tropical conditions.

The objective of this study is to evaluate the combined effect of urea granulated with organic and chemical nitrification inhibitors and zeolite on greenhouse gas fluxes under field conditions and production in incubation experiments. We also determined whether the growth of corn and soil microbial population is affected by additional urea granulated with natural slow release (zeolite) and nitrification inhibitors (neem cake and DCD).

Materials and methods

Soil sampling and field site

The cornfield experiment was located at the Indonesian Cereal Research Institute, South Sulawesi Province of Indonesia (4° 59′ 11.3″ S 119° 34′ 34″ E). Soil samples were taken from triplicate plots at 0–15-cm depth and sieved through a 2.00-mm sieve for soil properties analysis and incubation experiment purposes. The pH (1 M KCl 1:5), and total carbon (C) and nitrogen (N) were measured by electrode methods and C/N analyzer (MT 700. Yanaco), respectively. Data of daily temperature and precipitation were collected from the Meteorology, Climatology and Geophysical Agency. A field experiment was plotted in an area around of 400 m^2 and each microplot was measured 17.5 m^2 (2.5 m width × 7.0 m length). Three replicates of seven treatments were conducted with a completely randomized design from June 23, 2014–September 19, 2014. Corn var. B8 seeds were sown in a well-drained field and the distance between one corn crop to another was around 30 cm; therefore, there were around 153 corn crops in each plot. The nitrogen fertilizers were applied in the field by incorporation into soil with seven types of granulated fertilizer, namely (1) control (C; no addition of nitrogen); (2) urea (U; nitrogen content of 45%); (3) urea with neem (UN; neem (*Azadirachta indica* L) cake is a residual waste from the process of neem oil extraction. It was used as an

organic nitrification inhibitor and mixed with urea at a rate of 5% (w/w)); (4) urea with zeolite (UZ; zeolite is coarse natural zeolite from a local mine that is used as a natural slow-release fertilizer and mixed with urea at a rate of 10% (w/w)); (5) urea with zeolite and neem (UZN; zeolite and neem cake were mixed with urea at a rate of 10% and 5% (w/w), respectively); (6) urea with zeolite and dicyandiamide (UZD; zeolite and DCD were mixed with urea at a rate of 10% and 5% (w/w), respectively); and (7) urea with dicyandiamide (UD; DCD is chemical/synthetic nitrification inhibitor (Wako Ltd, Special Grade 90.0% and containing about 65% N) and mixed with urea at a rate of 5% (w/w)). The granulations of mixed fertilizers were done using an inclined pan granulator. The total rate of N applied in each treatment was 120 kg–N ha^{-1} applied in two splits (60 + 60) on July 1, 2014 ((1st fertilizer applied (FA)) and July 23, 2014 (2nd FA). At the second application of nitrogen fertilizer, each of the plot treatments also had an addition of KCl 100 kg ha^{-1} and SP-36 (super phosphate) 100 kg ha^{-1}. These rates and timing application of nitrogen treatments followed local farming practices.

Greenhouse gas emission and growth rate of corn measurements

The gas fluxes of N_2O and CH_4 were sampled using the closed chamber method (Jumadi et al. 2008). The concentration of N_2O and CH_4 in the samples was quantified using gas chromatographs (Shimadzu, GC 14B) equipped with an electron capture detector and a flame ionization detector, respectively. Percentage ratio of N_2O-N lost per amount of N applied as fertilizer into the field (EF, emission factor) and the percentages of the reduction of N_2O flux emitted from the fields with nitrification inhibitor or CRF was calculated according to Jumadi et al. (2008). The growth rate of the corn crop was sampled randomly using five corn plants from among the 153 corn crops of each plot. The growth parameters measured were plant height (cm) which was measured weekly until 50 days after seedling (DAS). Other plant growth parameters were also observed including leaf chlorophyll content (%) at 60 DAS, cob weight (kg), and the dry weight of corn stalk biomass, which were weighed after harvesting or at the end of the growth period.

Greenhouse gas production potential and microbial population

The incubation experiment was conducted to assess greenhouse gas production potential and viable microbial soil population with treatments identical to those used in the field experiment above. Forty grams of soil was incubated aerobically at 27 °C in sealed 120-mL bottles for a 4-week incubation in triplicate for each treatment. Production potential of N_2O and CO_2 gases and inorganic N as NH_4^+ and NO_3^- were determined for each soil sample. Every week, the gases in the headspace of each bottle were removed and the incubation bottles were then aerated and resealed for later sampling. Five grams of soil samples was weighed and immediately extracted with 25 mL of 2 M KCl (1:5). The amounts of NH_4^+ and NO_3^- were determined by nitroprusside and hydrazine reduction methods. Gas samples were determined by the concentrations of N_2O and CO_2 using GC. Determination of soil microbial population was sampled at the end of the soil incubation time of 28 days and then prepared for analysis. Total soil fungus was counted by plate count using potato dextrose agar media. The viable population of nitrifiers; i.e., ammonium oxidizer bacteria and nitrite oxidizer bacteria were estimated by the most probable number technique (Schmidt and Belser 1982). Standard deviations and means of all data were calculated. Each mean was compared with others using the Duncan ($P < 0.05$) value by SPSS software (Ver.20.0 for Windows, SPSS Inc., Chicago, USA).

Results and discussion

Soil properties and greenhouse gas fluxes

The soil used was an alluvial soil type. The texture of soil used was 8% sand, 54% silt, and 38% clay, which refers to texture class silty clay loam. The background field soil had a pH of 5.8, CEC of 28.15 cmolc/kg, P_2O_5 of 79.60 μg g^{-1} contained total carbon of 11.33 g-C kg^{-1} dry soil, total N of 1.40 g-N kg^{-1} dry soil, and C/N ratio of 8.09. The rainfall was monitored twice during cropping at 29 days after seedling (DAS) and 48 DAS giving measurements 24 mm and 3 mm, while soil moisture ranged from 5.0 to 60.12%. The temperature range during the experiments was 22–35 °C. The fluxes of N_2O showed a small increase at 20 DAS or 11 days after first nitrogen applied in urea zeolite (UZ) treatment and then decreased at the next measurement. The fluxes of N_2O rose after the second application of fertilizer on 26 DAS for U, UZ, and UN treatments. It was emitted longer with urea (U) treatment from 44 DAS to 60 DAS. The highest peaks of N_2O flux were measured as 1.36 mg-N m^{-2} h^{-1} with U treatment and 0.98 mg-N m^{-2} h^{-1} with UN treatment at 44 DAS. The N_2O fluxes for nitrogen additions outside of U treatment commenced a decrease at 48 DAS until the end of measurements and did not vary significantly from the control (Fig. 1).

The sum of N_2O fluxes in a season was highest with U treatment at 4.67 kg-N ha^{-1} $season^{-1}$, but statistically comparable with urea neem (UN) at 3.96 kg-N ha^{-1} $season^{-1}$. The

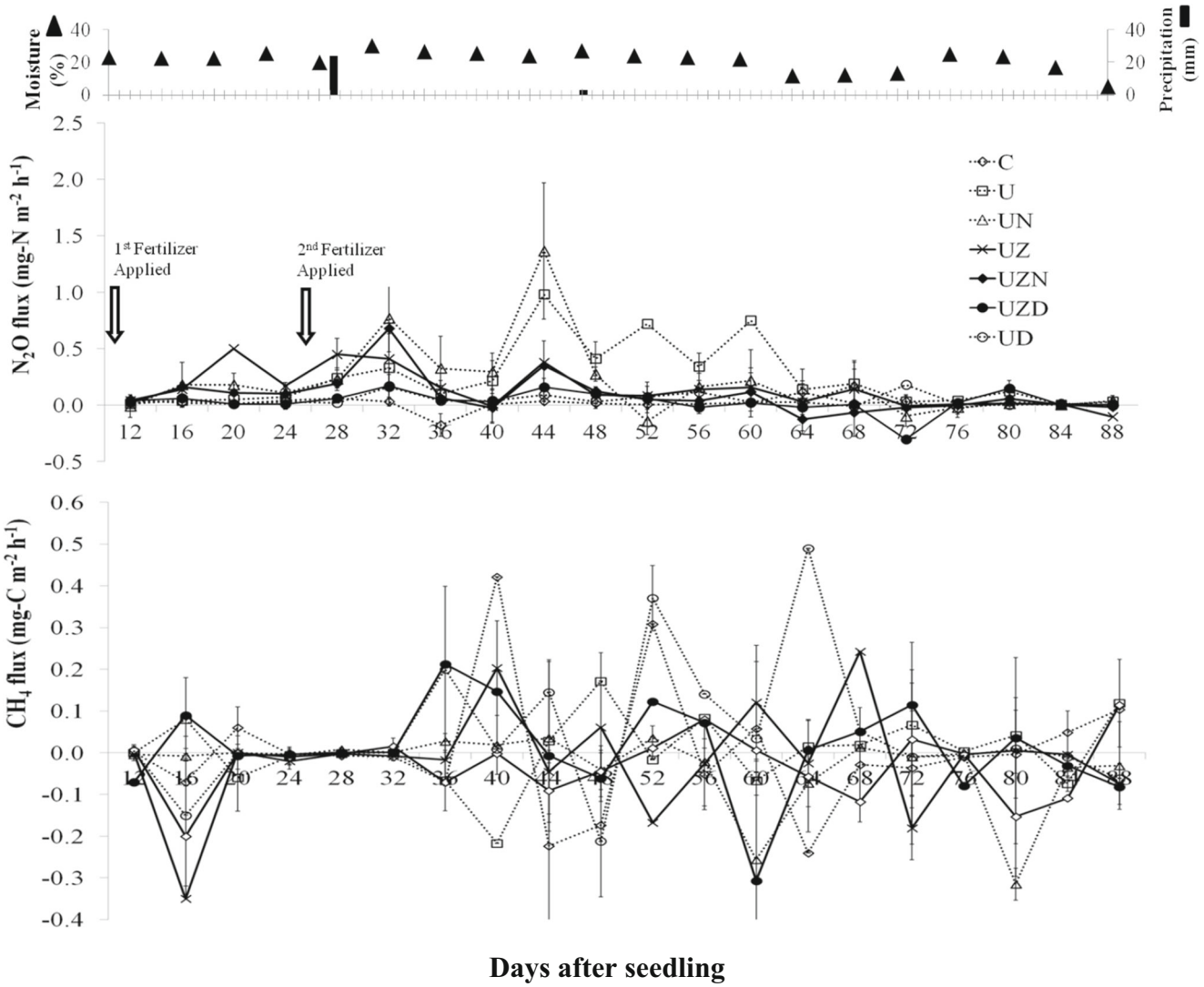

Fig. 1 Change of soil moisture, precipitation, N_2O, and CH_4 fluxes from cornfields affected by urea granulated with organic and chemical nitrification inhibitors and zeolite treatments during corn cropping season (June to September 2014). Vertical bars indicate ± standard deviations (see "Materials and methods" for abbreviations of symbols)

measurements for the other treatments were 2.07 kg-N ha^{-1} $season^{-1}$ for urea zeolite (UZ), 1.75 kg-N ha^{-1} $season^{-1}$ for urea zeolite neem (UZN), 0.89 kg-N ha^{-1} $season^{-1}$ for urea dicyandiamide (UD), and 1.10 kg-N ha^{-1} $season^{-1}$ for urea zeolite dicyandiamide (UZD) Meanwhile, control (C) plot was ambient at 0.31 kg-N ha^{-1} $season^{-1}$. The emission factor was also highest with U treatment at 2.1% compared with the other treatments: 0.8% (UZ), 1.8% (UN), 0.2% (UD), 0.7% (UZN), and 0.3% (UZD). Among treatments with nitrification inhibitor and zeolite, the lowest reduction of N_2O flux emitted from field corn was 16.3% with UN treatment. Reductions of N_2O flux with other treatments were 59.6% (UZ), 66.9% (UZN), 81.9% (UZD), and 86.7% (UD) (Table 1). The pattern of methane (CH_4) fluxes from the field was mostly determined by small release and uptake fluctuations across the measurements over one cropping season, except for UD treatment at 64 DAS which had the highest measurement (0.4 mg-C m^{-2} h^{-1}). The seasonal cumulative fluxes of CH_4 were as follows: − 0.29 (UZ), − 0.5 (UZN), − 0.63 (UN), 0.07 (C), 0.14 (UZD), 0.30 (U), and 0.9 (UD) kg-C h^{-1} $season^{-1}$ (Fig. 1).

Growth rate of corn crop

All corn crop growth parameters were enhanced by amendment of urea with and without NIs and zeolite compared with control (Table 2). The corn crop growth parameters such as height, cob weight, and chlorophyll rates were comparable for all nitrogen fertilizers (U, UZ, UN, UD, UZN, and UZD), and significantly different from control (C). The dry weight of stalk and leaf (biomass) of the corn crop was also greater with U, UZ, UN, UD, UZN, and UZD treatment compared with C. However, the weight of biomass with UZ (1.02 kg) was not

Table 1 The emission factor (EF %) and N_2O emission reduction in a corn crop season. Means followed by the same letter are not significantly different at ($P < 0.05$) by the Duncan test

Treatments	Total N_2O (kg-N ha^{-1} $season^{-1}$)	EF (%)	Reduction (%)
C	0.31[a]		
U	4.67[d]	2.10	
UN	3.96[cd]	1.80	16.3
UZ	2.07[bc]	0.80	59.6
UZN	1.75[b]	0.70	66.8
UZD	1.10[a]	0.30	81.9
UD	0.89[a]	0.20	86.7

significantly different from C (0.68%). The treatments of UZ seem to result in lower values of harvest parameters compared with other nitrogen additions. The corncob weight with U (1.42 kg) and UD (1.40 kg) treatments were significantly different from that of UZ (1.27 kg), but not significantly different from weights with other amendments, namely 1.30 kg (UZN), 1.37 kg (UN), and 1.39 kg (UZD). Despite that, UZ treatment has more chlorophyll content (53.64%) compared with other treatments: 38.24% (C), 45.72% (UN), 47.05% (UZN), 49.20% (UD), 50.06% (UZD), and 52.46% (U) (Table 2).

N_2O and CO_2 production, nitrogen change, and microbial soil abundance in incubation soil

The cumulative production of N_2O in the first week increased by application of U to 0.321 ± 0.099 μg g^{-1} dray soil (ds) but was not significantly different from other nitrogen applications except UZN (0.095 ± 0.003 μg g^{-1} ds) and C (0.047 ± 0.003 μg^{-1} ds). In the second week of incubation, the N_2O production of UZN (0.180 ± 0.09 μg g^{-1} ds) increased significantly from C (0.013 ± 0.002 μg^{-1} ds), but it was statistically comparable with other treatments. In the third week of incubation, the treatments of UZ produced N_2O up to 0.185 ± 0.044 μg g^{-1} ds, while other treatments slightly decreased afterward to the end of the incubation time (Fig. 2). With regard to soil respiration, no statistically significant differences were observed among soils treated with urea plus additional NIs and zeolite and control, but amendment of UD seems to stimulate the production of CO_2 up to the second week of incubation and then slightly down to the same rate of CO_2 production as the other nitrogen treatments (Fig. 2).

Soil NH_4^+ concentrations were considerably higher when the soil was treated with all types of nitrogen fertilizers compared with C during the first week of incubation and stayed constant in the second week. In the third week of incubation, the concentrations of NH_4^+ in soils treated with nitrogen were slightly reduced and dramatically decreased in the fourth week of incubation and were not significantly different from C. Soil NO_3^- concentrations in soil treated with nitrogen were low in the first week and not significantly different from C at 0.041 ± 0.02 μg g^{-1} ds except for UN at 0.71 ± 0.02 μg g^{-1} ds. The concentration of NO_3^- was substantially larger beginning in the second week until the end of incubation (Fig. 3). The abundance of ammonium oxidizer bacteria (AOB) ranged from 1.4×10^3 to 8.5×10^4 cell per dry gram soil (ds), whereas nitrite oxidizer bacteria (NOB) population ranged from 9.2×10^2 cell ds^{-1} to 8.2×10^4 cell ds^{-1} in all the soil samples. Soil fungi population in all treatments was not far from 2.6×10^3 cell ds^{-1} to 4.7×10^4 cell ds^{-1} (Table 3).

Discussion

The measurements of N_2O flux show the application of urea with nitrification inhibitors (NIs) and zeolite reduces the N_2O emission range from 16.3 to 86.7% through a corn planting season (Table 1). Our study shows that urea with dicyandiamide (DCD) as a chemical NI has substantial repression on N_2O flux from cornfields compared with organic NI (neem cake) particularly urea with neem (UN) treatment with the lowest inhibition of 16.3%. This quantity of reduction is very similar to the finding by Akiyama et al. (2013) who used meta-analysis data by integrating results of field measurements showing that NI from neem products could repress N_2O emission by an average of 14% with the range of

Table 2 The average of plant height at 50 days after seedling (DAS), weight of cobs, dry weight of corn plants, and chlorophyll content at 60 DAS. Means followed by the same letter are not significantly different at ($P < 0.05$) by the Duncan test

Treatments	Plant height (cm)	Cob weight (kg)	Dry weight of corn plants (stalk and leaf) (kg/plant)	Chlorophyll content (%)
C	182.13[a]	0.75[a]	0.68[a]	38.24[a]
U	226.13[b]	1.42[c]	1.25[b]	52.46[bc]
UN	229.00[b]	1.37[bc]	1.33[b]	45.72[b]
UZ	220.80[b]	1.27[b]	1.02[ab]	53.64[c]
UZN	226.87[b]	1.30[bc]	1.10[b]	47.05[bc]
UZD	226.73[b]	1.39[bc]	1.14[b]	50.06[bc]
UD	218.00[b]	1.40[c]	1.20[b]	49.20[bc]

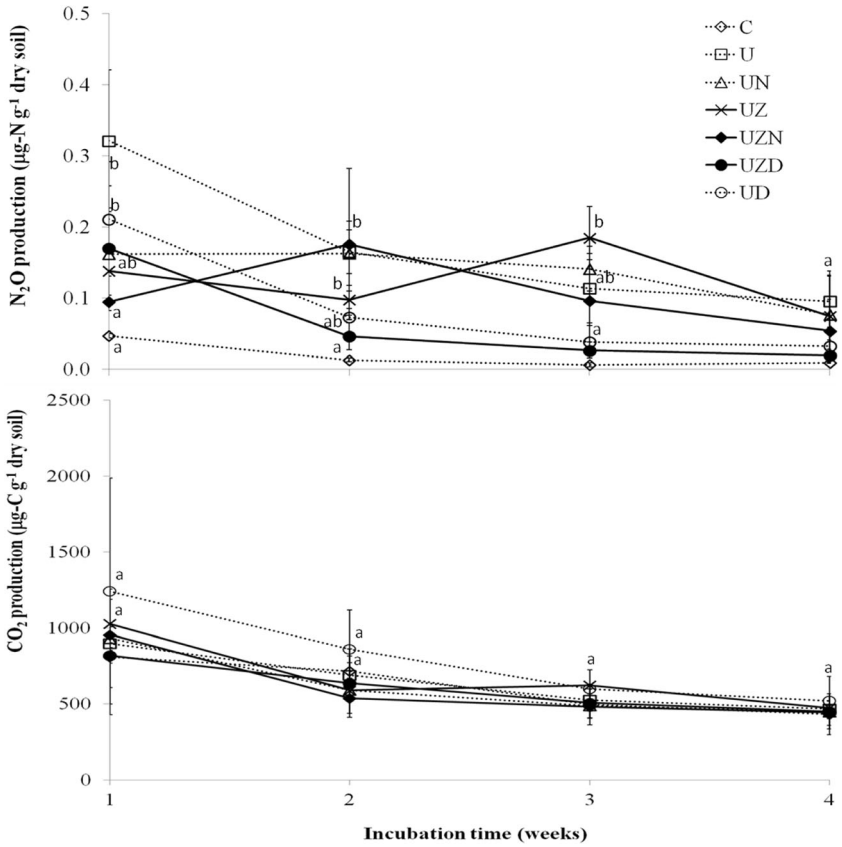

Fig 2 Change in the concentration of N_2O and CO_2 production during incubation time in weeks. Means followed by the same letter are not significantly different ($P < 0.05$)

reduction being 25 to 7%. In the same tropical field experiment, it was observed that there was a substantial repression of N_2O flux from cornfields in soils with a combination of nitrogen fertilizer with DCD which reduced N_2O flux by 55.8% compared with urea and also by 11.7% compared with control release fertilizer (LP-30) (Jumadi et al. 2008). This study suggests that the addition of zeolite with urea (UZ) also reduces 59% of N_2O emission compared with urea alone. Hence, zeolite is apparently a slow-release nitrogen stabilizer and more effective combination with organic NI (neem cake).

Another experiment that used neem oil as an organic NI, Opoku et al. (2014), resulted in various repressions of N_2O production, observed a superior inhibitory effect of neem seed oil at 58% at day 30 relative to a lower dose of DCD at 29%, and concluded that neem seed oil, which is less expensive and could be obtained locally in the tropics, has a similar potency as DCD. In addition, organic NI neem could also have activities that inhibit urease in acidic soil and maintain higher concentration of urea for 2 weeks, but it does not occur in typical neutral and alkaline soil (Mohanty et al. 2008). The soil used had a pH of 5.8, which is moderately acid category, which possibly acts to inhibit activity of urease.

The organic NI used in this study was obtained from neem cake, typically a waste product from extracted neem seed oil. It seems to be less effective as a nitrification inhibitor due to the low concentration of secondary metabolites as a function of substrate substitution to AMO of nitrifying bacteria. In addition, the results that neem cake was less effective for inhibiting nitrification could probably be due to the organic matter including secondary metabolites in neem cake undergoing rapid degradation or being utilized by soil microbes for ammonification to obtain NH_4^+. Afterward, it continued to transform with nitrification, and thus produced N_2O as well as NO_3, which was leached from the soil if the efficacy of nitrogen uptake of the plant was less.

Park and Komarneni (1997) tested four types of natural zeolite namely erionite, clinoptilolite, chabazite, and phillipsite that have capacity to store nutrients (KNO_3 and NH_4NO_3) and are potential candidates as slow-release fertilizers. Hence, further characterization is required to define the type of natural zeolite used in this study; nevertheless, zeolite has capacity for lowering the emission of N_2O up to 50% in cornfields. During the experiment, following the addition of urea with and without NIs and zeolite, the highest concentration of NH_4^+ and N_2O production was detected in the first week, indicating that urea was hydrolyzed and nitrification began within the first week of incubation. The concentration of NH_4^+ remained higher up to 2 weeks of incubation with the combination of urea with NIs and zeolite treatments although there were no significant differences compared with urea. During the NH_4^+ decrease, the concentration of NO_3^- started to accumulate from the second week to the fourth week, which

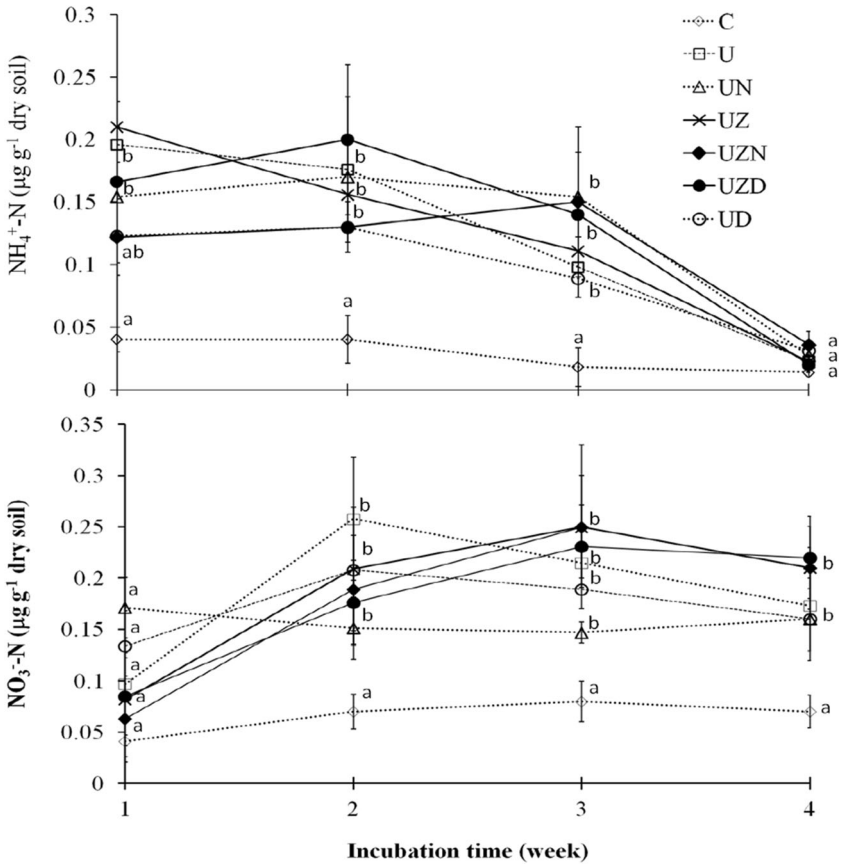

Fig 3. Change in the concentrations of NH_4^+-N and NO_3^--N during incubation time in weeks. Means followed by the same letter are not significantly different at ($P < 0.05$)

indicates that nitrification was occurring until the end of incubation. Therefore, it showed NIs and zeolite hampered N_2O production under incubation conditions. Similarly, Ahmed et al. (2008) pointed out that applying urea with TSP and zeolite had a significant advantage over urea alone by increasing the formation of NH_4^+ over NH_3 and retaining more NH_4^+ in soil incubation and suggested that NH_4^+ exchanged in zeolite type clipnoptilolite not only served as a slow-release fertilizer in medium-textured porous soil but also reduced volatilization of NH_3 when urea and zeolite were added to an alkaline coarse texture soil. The retention of NH_4^+ on the cation exchange sites of the zeolite may also partly explain how the urea with zeolite mixture in granule form can reduce N_2O production compared with urea without zeolite and nitrification inhibitors.

Comparing the influence of urea with NIs and zeolite on N_2O production, our study found no significant difference in CO_2 production between nitrogen and NIs with zeolite additions and control (Fig. 2). The pattern of CO_2 produced recorded a slight decrease in the second week of incubation and was constant thereafter. In the first week of incubation with UD treatment, a little more CO_2 production was detected compared with the second week, which was possibly due to the contribution from the decomposition of urea and DCD. Soil respiration is a reflection of soil microbial metabolism or biological activity in soil; hence, we speculate that the static production of CO_2 in this study can also be explained in that the addition of NIs and zeolite to the soil sampled did not cause much change in microbial abundance (AOB, NOB, and fungi) after 4 weeks of incubation (Table 3). Another study shows that the addition of neem cake can reduce the population of nitrifying bacteria with a prolonged time of incubation. Santhi et al. (1986) observes that application of urea mixed with neem cake and neem leaf increases the total microbial count in wetland soil up to the 15th day after the application, but after that, up to the 30th day of incubation,

Table 3 Microbial population of AOB (ammonium oxidizer bacteria), NOB (nitrite oxidizer bacteria; MPN, cell g^{-1} dry soil), and fungi (CFU, cell forming unit, g^{-1} dry soil) at 4-week incubation

Treatments	AOB	NOB	Fungi
C	1.4×10^3	9.2×10^2	3.3×10^3
U	2.0×10^3	1.1×10^3	4.0×10^3
UN	1.9×10^3	3.4×10^3	4.6×10^3
UZ	3.6×10^3	8.2×10^4	3.3×10^3
UZN	2.0×10^3	1.1×10^3	2.6×10^3
UZD	8.5×10^4	7.1×10^4	3.9×10^3
UD	4.0×10^4	9.6×10^2	4.7×10^4

the population of *Nitrosomonas*, *Nitrosococcus*, and *Nitrobacter* exhibited a declining trend.

Patra et al. (2006) also reported that natural NIs and DCD retarded *Nitrosomonas*, *Nitrobacter*, and total bacterial and Actinomycete populations. However, the result of this study agrees with Singh et al. (2008) which observed no impact of DCD on soil respiration and microbial biomass in the three soils investigated, while Jumadi et al. (2008) observed microbial soil population (AOB, NOB, and fungi) enhanced by additional urea coated with DCD in the first week and third week of incubation were low with fluctuations decreasing. This is also partly explained by the fact that the mode of action of DCD is a complex reaction which temporarily inhibits the protein bond of AMO to the cell membrane. Amberger (1986) found a specific inhibition of nitrite formation at concentrations of 100 to 300 ppm of *Nitrosomonas europaea* and suggested that DCD is a bacteriostatic agent and not a bactericidal agent. That might also possibly be the mode of action of neem cake as the organic nitrification used in this study.

The initial measurements of the concentrations of CH_4 fluxes were occasionally at a lower level of emission until 32 DAS of the cropping season which much might be influenced by field soil being in aerobic condition. However, there was a presence of CH_4 flux after rainfall on 29 DAS and 48 DAS. With UD treatment, CH_4 flux exhibited a temporary positive peak at 52 and 64 DAS (Fig. 1). Urea with organic NIs (neem—UN and UZN) seems to oxidize CH_4, while urea and urea combined with DCD (UD and UZD) emitted CH_4 from the field with averages of 0.30, 0.9, and 0.14 kg-C h^{-1} $season^{-1}$, respectively.

Several studies observed that NH_4^+ or inorganic fertilizer usually inhibits atmospheric CH_4 oxidation due to the competition at the level of nitrification (Le Mer and Roger 2001). Different results by Datta and Adhya (2014) suggested that application of urea + Nimin significantly increased the methanotrophic bacterial population in the soil compared with urea + karanjin, urea + DCD, and control; therefore, it may be attributed to low CH_4 emission released from field. While, Dai et al. (2013) observed methanotroph population abundance and CH_4 uptakes were not significantly affected by the application of DCD and urea-N or animal urine N at different rates. Our observation also confirmed that all urea fertilizers combined with neem cake uptake the CH_4 fluxes in the field while urea with DCD generated CH_4 emission.

Nitrogen is one of the essential nutrients for crop growth and development. Nitrogen can be made available by amending nitrogen in urea with NIs and zeolite. All corn growth parameters observed had no significant differences between urea with and without NIs and zeolite (Table 2). Sharma and Prasad (1996) suggest that the use of nitrification inhibitors (neem and DCD) in split time resulted in a greater increase of nitrogen efficiency in maize-wheat rotations compared with a single application. Nitrogen is a constituent component of leaf chlorophyll and about 60% functions as enzymes and derivative membrane proteins in plant cells. Therefore, additional nitrogen increases the photosynthetic efficiency of crops which might have resulted in enhancement of corn growth such as plant height, yield, and biomass. Our study results suggest that applying urea fertilizer with organic nitrification inhibitors with and without zeolite in a corn crop of a tropical area has the potential to mitigate N_2O emission; however, further research is needed on the long-term application of recent type nitrogen fertilizers to better quantify greenhouse gas fluxes from various types of fields.

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