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Is the paper content original?	Yes
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Is the research methodology or the approach of the problem solving clearly described?	Yes
Do the data presentation and interpretation valid and reasonable?	Yes
Do the use of tables and figures help to clarify the explanation?	Yes
Have the discussion and/or analysis been relevant with the results of the study?	Yes
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Production of N_2O, CO_2 Gases and Microbe Responses in the Soil Amended with Urea Granulated Zeolite

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Abstract. This research study aims to determine the production of nitrous oxide (N_2O), carbon dioxide (CO_2), the rate of ammonium-nitrate, microbe responses in soil following the addition of urea coated by zeolite under soil incubated.The result showed that urea granulated with zeolite in soil sample had a significant effect on the production of $CO_2$and have no significant increase N_2O productions.Therefore, the addition of urea with zeolite seems to decrease the production of N_2O compared to urea alone.The concentrations of ammonium and nitrate during incubation time were significantly affected by the amended type of fertilizers.The availability of ammonium and nitrate in soil were increased by addition of urea zeolite 30%.The urea combinedzeolite increases the growth of the fungi population, while population of ammonium oxidizer bacteria seems appearedlower in soil that amended with fertilizer compared to the control.The study showed that urea granulated with zeoliteincreased the time availability of soil nitrogen.

1. Introduction

Nitrogen (N) is an essential nutrient for plants and deficiency will affect the plantsgrowth.The transformation of N in the soil is part of the N cycle and its dependent on the activity of microorganisms.Soil N is available for plants in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Crutzen 1995; IPPC 2007). The nitrification is aprocess oxidation of NH_4^+ to NO_3^-carried out by nitrifying microbial.This process willaffect the plant if their uptake of N slower than the nitrification because lack of efficiency and usage of N by plants, therefore that it can reduce crop production (Inubushi et al. 1996).The nitrification also has a negative impact, because it can produce secondary products in the form of nitrous oxide (N_2O) as greenhouse gases and NO_3^-as water pollution and also produces N_2O gas through denitrification which is one of the causes theglobal warming (Bouwman et al. 1995: IPPC 2007).Therefore, nitrification has an impacton environmental quality because the oxidation of NH_4^+ to NO_3^-, which dissolves easily as pollution in groundwater. High concentration of NO_3^- in water can spur the growth of microbes, algae, plankton, and water quality (Yanai et al. 2003).

Efforts to slow the release of N from urea fertilizer can increase the efficiency of nutrient absorption by plants and reduce environmental pollution (Akiyama and Tsuruta 2002). One the effort to reduce the loss of N in the soil is to designthe urea fertilizer in the form of *slow release fertilizer*(SRF).Urea in form *slow release* formmembranous zeolitecan optimize the absorption of N by plants, because SRF can control the release of N elements according to the time release and the amount needed by plants, and also maintain the presence of N in the soil (Ahmed et al. 2008). In addition, the amount of urea application with slow release in the field is smaller than the urea conventional (Jumadi et al, 2008). The aims of research are to determined rateof N_2O, CO_2 gases productionand rate of nitrification as well as the number of soil microbes which treated with ureagranulated with zeolite as a slow release fertilizer.

2. Material and Method

Granulating urea with zeolite was carried out using the inclined pan granulator method. Soil samples were taken from the maize field area in Indonesia Cereal Research Institute, Maros. Soil was taken at 0-15 cm depth and after removing the visible of debris and sieved through a 2 mm mesh and then kept at 55% of water holding capacity for 7 days preincubation. (Jumadi et al. 2005). A weight of 40 grams of soil placed into a150 ml bottle and then treated with:

1. Control (C) without nitrogen
2. Urea(U) =8,96 N-mg
3. 10% Zeolite Urea (UZ10%) =9.9 N-mg
4. 30% Zeolite urea (UZ30%) = Urea Zeolite N-11.7 mg
5. 50% Zeolite Urea (UZ50%) = Urea Zeolite N-20.2 mg

N_2O and CO_2 sample gases were taken on days of 7, 14, 21 and 28. Gas is taken from the bottle as much as 30 ml and then put into a vacuum bottle. Samples of gas in the vial then sent to the ChibaUniversity, Japan to be analyzed concentration of N_2O and CO_2 by gas chromatography (Shimadzu, GC14B) equipped with Electron Capture Detector (ECD) and Flame Ionization Detector (FID), respectively. Shortly after taking the gas, 10 gram of soil samples was extracted by adding 50 ml KCl 2M (1: 5) and then performed agitation for 30 minutes and filtered with paper AVANTEC 6. Analysis of NH_4^+ content was carried out by nitroprusside method (Anderson et al. 1989), while the content of NO_3^- was done by hydrazine reduction method (Hayashi et al. 1997). The population of ammonium oxidizing bacteria is calculated by most probable number (MPN) methods, while the fungal population is standard plate count (SPC). The research design was a completely randomized design and all determinations were carried out in triplicate. Data were statistically analyzed by Tukey ($p<0.05$) methods using SPSS ver.20

3. Results and discussion

Soil sample used in this experiment hasmedium acidic characteristics (between pH 4.8) and the type texture was clay-loamy with C/N ratio 10.The production rate of gas nitrous oxide(N_2O) showed that no any significant change during the incubation time up to the 28th days of incubation, but in cumulatively urea (U) was the highest N_2O production (0.015μg-N g dry soil^{-1})(Figure 1).

On the 28th incubation days the highest N_2O gas production in soil sample was urea (U) and urea with zeolite 50% (UZ50%) treatments as rate 0.004 μg-N g dry soil^{-1}, 0.002 μg-N g dry soil^{-1}, respectively. Meanwhile, the lowest gas production was in the control (C), urea zeolite 10% (UZ10%), urea zeolite30% (UZ30%), respectively.

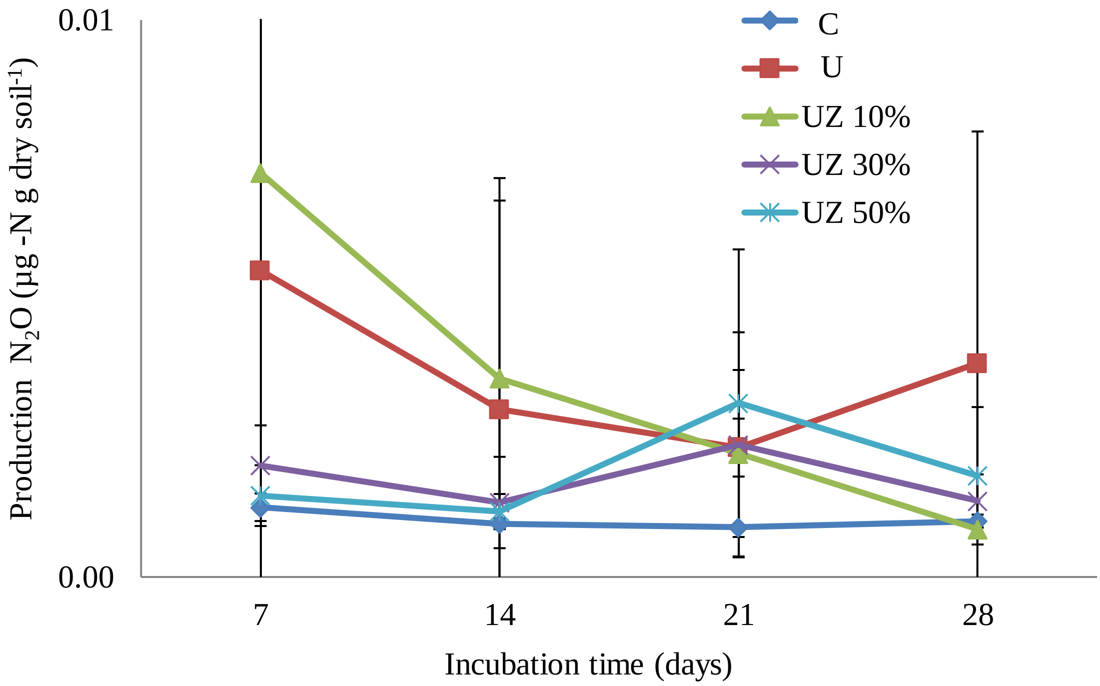

Figure 1. Production of N_2O gas treated with a combination of urea and zeolite.

The highest carbon dioxide (CO_2) gas production was determined in UZ30% as 651,925 µg-C g dry soil^{-1} then followed by UZ 10%, U, C and UZ 50% as 579.8, 553.2, 479.2 and 298.7 µg-C g dry soil^{-1}, respectively (Figure 2).

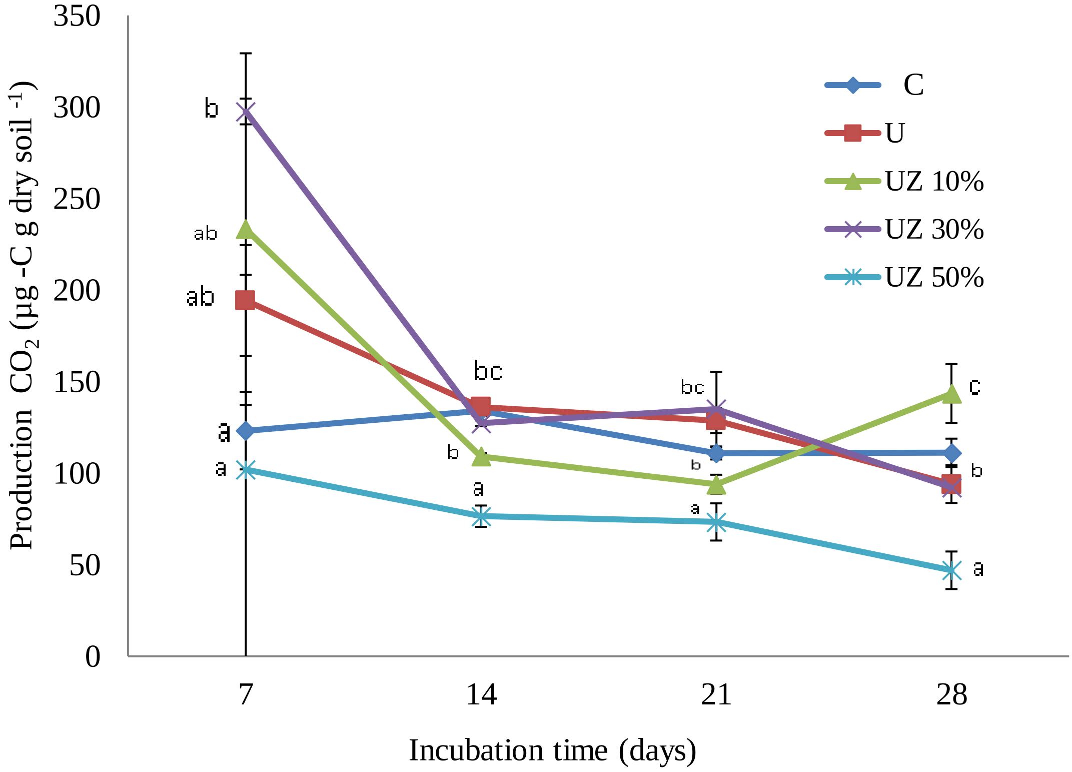

Figure 1. Production of CO_2 gas treated with a combination of urea and zeolite.

Addition of zeolite on urea was not having a significant effect on N2O gas production during incubation time. The highest total N2O gas production was determined at urea treatment without zeolite (U). It is possible that urea without zeolite does not resist the release of NH4+ as results of ammonification from urea, so that the nitrification process takes place with sufficient NH4+, therefore, resulting in the high production of N2O (Ahmed et al. 2008). The lowest N2O gas production was measured in UZ30% and UZ50% treatments; this indicates that the addition of slow release material in the form of zeolite could reduce N2O gas production. The presences of zeolite material temporary prevent the release of NH4+. This occurs because zeolite is binding NH4+. The lowest N_2O gas production was found in soil samples with control treatment. It was well understood that without nitrogen substrata the nitrification usually deceased, therefore N_2O gas was determined lowest at soil without amended of nitrogen (Jumadi et al. 2014).

On28 of incubations, the nitrate concentration decreased in the urea treatment, this was in line with the increase in N2O gas production on the same treatment. The possibility of this change is also possible due to denitrification that converts nitrate to N2O gas (Jumadi et al 2005).Park and Komarneni (1997) reported that zeolite erionite, clinoptilolite, chabazite, and phillipsite types have the capacity to store nutrients in the form of KNO_3 and NH_4NO_3 and thus have the capacity as slow-release fertilizers. Although in this study of the type of zeolite used has not been characterized. Urea coated with zeolite 30% or 50% can be proposed as composites for slow release fertilizer and reduce the production of N_2O gas in the soil, because it produces a total N_2O gas production lower than other treatments. Therefore, it is necessary in the future to do research on the type of zeolite used. Hence, zeolite used in this study has the capacity to suppress N_2O gas production and maintain NH_4^+ in soils.

The research showed the highest NH_4^+ concentration and N_2O production were detected on the 7th day of incubation both on urea with or without zeolite. These also testify that urea hydrolysis and the nitrification process occurred on the first week after incubation. The rate of change in CO_2 gas production as results of the contribution of soil microbial respiration.Although, the treatment seems induced the production of CO_2 production. According to Ahmed et al (2008) zeolite can reduce CO_2gas,due to it has property capacity to absorbed CO_2gas.

The change of ammonium concentration in soil during incubation can be seen in table 1, which is showing that the seventh and fourteenth day the ammonium concentration showed a significant difference, the twenty-first and twenty-eighth day there were no significant changes. The highest ammonium concentration in 10% of urea zeolite (UZ10%), followed by urea (U) and 50% of urea zeolite (UZ50%), and urea zeolite30% (UZ30%). Application of urea with zeolite as a slow-release material gives a significant effect on NH_4^+ and NO_3^- concentrations, the highest NH_4^+ concentration was found on the seventh day of incubation of soil samples treated with zeolite. This is probably due to nitrification was just startingprocess and the availability of NH_4^+. In the next weeks incubation, NH_4^+ concentration decreased at 28 days of incubation time, while $NO3^-$concentration continued toraise up, particularly in urea (U) treatment. Therefore, the resultsalso showed that the fast change of $NO_3^-$from NH_4^+delivered by nitrification process. Nitrification is an aerobic process in which NH_4^+ is oxidized to NO3-, this process occurs naturally in the environment and is carried out by groups of ammonium and nitrite oxidizing microbe (Li et al. 2002). Nitrification is an important step in the nitrogen cycle, where NH_4^+ is the initial substrate of nitrification (Inubushi et al. 1996).

The results of the analysis showed that 30% UZ could control the rate of NH_4^+ seen in the second week, NH4 + concentration was still the highest compared to other treatments or other zeolite levels. According to Hadi et al 2008, the addition of nitrifying inhibitors and slow release agents such as poliflyn material on various types of clay soil showed effective in repressing the release of N_2O into the atmosphere and NO_3^- in the soil.

The study results also showed that UZ30% could control the rate of NH_4^+ which seen in the second week that the NH_4^+ concentration was highest compared to other treatments. According to Jumadi et al. 2008; Hadi et al 2008, the addition of nitrifying inhibitors and slow release agents such as poliflyn material on various types of clay resulted effectively in repressing the release of N_2O into the atmosphere and NO_3^-in soil.

Changes of NO_3^- concentration can be seen in table 2, which shows during the incubation period NO_3^- content in soil imposed by the addition of nitrogen. It was significantly different with soil without nitrogen amendment. Urea with Zeolite 10% as the highest concentration of NO_3^-in soil compared to other treatments (Table. 2).

On the 21st after incubation, the highest population of ammonium oxidizing bacteria (AOB) was found in the soil without amended nitrogen (C) which was 9.97 x 10^4cfu g dry soil^{-1}. While, soil sample wasadded urea zeolite 5% (UZ 50%) has the highest population of fungi as 3.1 x 10^4cfu g dry soil^{-1} (Table 3).

Table 1. Average concentration of ammonium (NH_4^+ µg-N g dry soil^{-1})with acombinationurea and zeolite treatment.

Treatments	Incubationtimes				Total Production
	7	14	21	28	
Control (C)	0.0001^a	0.0000^a	0.0001^a	0.0001^a	0.0003
Urea (U)	0.0013^{ab}	0.0008^b	0.0004^a	0.0001^a	0.0026
Zeolite Urea (UZ10%)	0.0018^b	0.0007^b	0.0002^a	0.0001^a	0.0028
Urea Zeolite	0.0010^{ab}	0.0006^{ab}	0.0004^a	0.0001^a	0.0021

(UZ30%)					
Zeolite Urea (UZ50%)	0.0012 [ab]	0.0008 [b]	0.0004[a]	0.0002[a]	0.0026

The numbers followed by the same letter mean that there is no significant difference in the level of α <0, 05 Tukey.

Table 2. Average concentration of nitrate (NO_3^- µg-N g dry soil^{-1}) with combinationurea and zeolite treatments

Treatments	Day after incubation				Total Production
	7	14	21	28	
Control (C)	0.0000[a]	0.0002[a]	0.0000[a]	0.0002[a]	0.0004
Urea (U)	0.0005[b]	0.0013[b]	0.0011[b]	0.0005[ab]	0.0034
Zeolite Urea (UZ10%)	0.0004[b]	0.0012[b]	0.0011[b]	0.0012[bc]	0.0039
Urea Zeolite (UZ30%)	0.0006[b]	0.0013[b]	0.0013[b]	0.0015[c]	0.0047
Zeolite Urea (UZ50%)	0.0004[b]	0.0010[ab]	0.0011[b]	0,0015[c]	0.0014

The numbers followed by the same letter mean that there is no significant difference in the level of α <0, 05 Tukey.

The population of ammonium oxidizing bacteria in soil treated with urea and zeolite in 21days after incubation was lower than control (C), while the number of soil fungi in the control (C) was lower than soil that amended with urea and zeolite (Table.3). Addition of nitrogen to the soil seems to enhance fungal growth compared to the ammonium oxidizing bacteria themselves, although the population of AOB soil was not different. This result seems not support from the previous observation studies which showed that the addition of nitrogen increased the number of bacterial cells belonging to chemoautotroph including ammonium oxidizing bacteria (Jumadi et al. 2008b).

Addition of zeolite to granule urea has NH_4^+retention potential and also suppresses N_2O gas production. Therefore, the study resultsshowed an efficient release of $NH_4^+$to soil and it has a potential as nutrient use-efficiency by plants. Although, its efficiency justification still needs a further research both laboratory and field scales.

Table 3. The population of Ammonium Oxidizing Bacteria (AOB) and soilfungi insoil with a combination of urea and zeolite treatments (21st days after incubation).

Treatments	Ammonium Oxidizing Bacteria (cfu g dry soil^{-1})	Total Fungi (cfu g dry soil^{-1})
Control (C)	9.9 x 10^4	1.8 x 10^4
Urea (U)	2.1 x 10^4	1.5 x 10^4
Zeolite Urea (UZ10%)	1.8 x 10^4	2.1 x 10^4
Urea Zeolite (UZ30%)	5.8 x 10^4	2.5 x 10^4
Zeolite Urea (UZ50%)	5.7 x 10^4	3.1 x 10^4

4. Conclusions

Application urea with zeolite can suppress N_2O gas production, while the production of CO_2 gas enhances in soil samples. The combination of urea and zeolite could delaythe release of ammonium and nitrate. The addition of urea and zeolite in soil at 21 daysafter incubation enhanced the total population of fungi. However, the population of ammonium oxidizing bacteria seems decreased.

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