Methane and nitrous oxide productions affected by natural nitrification inhibitors under different soil types

H L Susilawati1*, A Wihardjaka1 and P Setyanto2

1 Division of Greenhouse Gas Emission and Absorption, Indonesian Agricultural Environment Research Institute, Jl. Jakenan-Jaken KM 5 Jakenan, Pati, Central Java, 59182, Indonesia
2 Directorate General of Horticulture. Jl. AUP No. 3 Pasar Minggu, South Jakarta, 12520, Indonesia

* Corresponding author: helenalina_s@yahoo.com

Abstract. Inefficient used of urea fertilizer contributes to producing methane (CH$_4$) and nitrous oxide (N$_2$O) emissions. One of the options to mitigate CH$_4$ and N$_2$O emissions is through the use of nitrification inhibitors (NI). The objective of this study was to evaluate the effectiveness of some plant-based NI (NNI) on CH$_4$ and N$_2$O production under two different soils types. An incubation experiment was arranged using 2 x 3 x 3 factorial designs. All treatments were conducted with three replicates. The first factors were paddy soils (Inceptisols and Vertisols), the second were NNI (coconut husk, coffee waste, and Ageratum conizoides) and the third were the rate of the NNI (10, 20 and 30 ppm). The results showed that CH$_4$ production was found lowest at the rate of 30 ppm of coconut husk application. Coconut husk at the rate of 20 ppm produced the lowest N$_2$O production. Based on the global warming potential (GWP), the lowest production was the application of Ageratum conizoides at the rate of 30 ppm. Applying a higher rate of natural NI resulted in lower productions of CH$_4$ and N$_2$O. Chemical contents of natural NI influenced microbial activities that produce CH$_4$ and N$_2$O through nitrification and denitrification processes.

1. Introduction
Recently, the application rate of synthetic fertilizer in the farmer fields is increasing to enhance crop production. Nitrogen (N) is the most essential nutrient for all crops because it is needed by plant in largest quantities of any essential nutrient. However, N use efficiency is quite low because of the rapid loss of N fertilization by erosion and runoff, leaching, and gaseous emissions. The losses cause low N uptake of the plant and cause serious environmental issues [1].

Nitrous oxide (N$_2$O) and methane (CH$_4$) are the two important sources of greenhouse gases (GHG) from paddy rice field implicated in global climate warming [2]. Ineffective used of N fertilization contributes to N$_2$O and CH$_4$ production in the soil. The major contributor to N$_2$O emissions comes from ammonia (NH$_3$) volatilization and denitrification. N$_2$O is by-product of microbial nitrification and denitrification [3]. Ammonium based fertilization influences the growth and activity of methane oxidizers that produce CH$_4$ [4]. Moreover, the existence of NH$_4^+$ in the soil stimulates CH$_4$ production because of the competition of NH$_4^+$ for the oxidation with CH$_4$ by methanotrophs [5].

N uptake of the plant can be promoted and N losses can be reduced by various techniques e.g., using nitrification inhibitors (NI). The usage of NI is able to optimize N use efficiency, suppress nitrification and N losses [6]. Moreover, NI is capable of reducing N$_2$O emissions from agricultural soils [7].
However, some of the synthetic chemical NI are high cost lack of availability in the market and not popular with the farmers. There is a need to investigate local and cheap materials which can retard nitrification and mitigate greenhouse gas (GHG) emission. Some herb and plant materials can be used to delay nitrification process because the chemical compound on the them inhibit the microbial activities in the soil and then retard the process of nitrification by slowing down conversion rate of ammonium to nitrite e.g., polyphenols, quinines, terpenes and fatty acid [8]. Fatty acid has 98% effect to reduce nitrification loss [9]. Herb and plant materials that used for NI (NNI) are mostly eco-friendly because the products are cheap, biodegradable and easily find in the nature.

Several research studies have confirmed that some plants are effective for delaying the nitrification process as well as reducing N\textsubscript{2}O emissions e.g. neem (\textit{Azadirachta indica} Adr. Juss.), mint (\textit{Mentha spicata} L.), \textit{Brachiaria humidicola} (Rendle) Schweick [10]. Karanjin was a plant material that reduced 92-96% N\textsubscript{2}O emission and inhibited 62-75% nitrification [11]. Extract of neem increased apparent N recovery around 25-30% [12]. This paper is the first study that reports on N\textsubscript{2}O and CH\textsubscript{4} productions after urea application in combination with natural products in proposed as nitrification inhibitors. The purpose was to suggest a possible strategy to have a minimum N\textsubscript{2}O and CH\textsubscript{4} emissions from two different soils through application of natural products as NI.

2. Materials and methods

Soil samples were collected from two different soil types in Pati, Central Java. The soil samples were taken from the surface layer (0 to 15 cm) at all sites. The soils were classified as Inceptisols and Vertisols. The soil samples were brought to the Greenhouse Gas Laboratory of Indonesian Agricultural Research Institute (IAERI), Jakenan, Central Java, Indonesia.

A laboratory experiment was arranged using 2 x 3 x 3 factorial in randomized completely designs. The treatments of incubation were conducted with three replicates. The first factors were paddy soils (Inceptisols and Vertisols), the second were NNI (coconut husk, coffee waste, and Ageratum conizoides) and the third were the rate of the NNI (10, 20 and 30 ppm). This study did not include the NNI rate of 0 ppm because previous experiment from this study observed that the application of urea increased immediately N\textsubscript{2}O production in the soil compared to the application of urea and NNI. Thus application of urea and NNI resulted lower GHG productions.

Air-dried soil samples were ground to pass through a 2 mm stainless steel sieve and removed from plant residues. The samples of 20 g soil were placed in a 100 ml incubation bottles and 40 ml of distilled water was added to keep the soil saturated [13]. The magnetic bar was kept inside the incubation bottle to homogenize the soil just before headspace air sampling. The incubation bottles were closed tightly and equipped with a pH-Eh electrode, a place for gas samples collection, gas inlet, and gas outlet.

Incubation was started by stirring the soil suspension and flushing the headspace with N\textsubscript{2} through the gas inlet-outlet at a flow rate of 300 ml min\textsuperscript{-1}, then the beakers were closed and the gas samples were taken using a 5 ml syringe. After 24 hours, the soil suspensions were stirred and gas samples were retaken [14]. Gas samples were analyzed on the same day using a gas chromatograph equipped with flame ionization detector (FID) and electron capture detector (ECD). During the incubation period, the beakers were kept in incubator and maintained at temperature of 30°C [13]. The N\textsubscript{2}O and CH\textsubscript{4} production were computed using the following formula [15]:

\[
E = \left(\frac{C_{24}}{20 g} - \frac{C_{0}}{20 g}\right) \times \frac{V_{h} \times m_{W}}{m_{V} \times 273.2} \times \frac{273.2}{(273.2 + \theta)}
\]

- \(E\) : Production of CH\textsubscript{4} or N\textsubscript{2}O (mg g\textsuperscript{-1} soil d\textsuperscript{-1})
- \(C_{0}\) : Concentration of CH\textsubscript{4} or N\textsubscript{2}O during gas sampling of time 0 (ppm)
- \(C_{24}\) : Concentration of CH\textsubscript{4} or N\textsubscript{2}O after 24 hours of gas sampling (ppm)
- \(V_{h}\) : Volume of headspace in the beaker (ml)
- \(m_{W}\) : Molecular weight of CH\textsubscript{4} or N\textsubscript{2}O (g)
- \(m_{V}\) : Molecular volume of CH\textsubscript{4} or N\textsubscript{2}O
- \(\theta\) : Temperature of incubator (°C)
3. Results and discussions

Figure 1 shows the pattern of cumulative CH$_4$ production after NNI applications from two different soil types, namely Inceptisols and Vertisols. Different rates and types of NNI had a significant influence on total cumulative CH$_4$ production during 31 days of incubation. Application of coconut husk at rates of 10 ppm in Inceptisols resulted in the highest cumulative CH$_4$ production and followed by 20 ppm and 30 ppm were approximately around 2.61, 2.03 and 0.11 mg CH$_4$ g soil$^{-1}$, respectively. Cumulative CH$_4$ production in Vertisols were 3.81, 3.98 and 2.67 mg CH$_4$ g soil$^{-1}$ for coconut husk application at rate of 10, 20 and 30 ppm, respectively. The trend of cumulative CH$_4$ production after Ageratum conizoides application in Inceptisols and Vertisols were similar with coconut husk application. While cumulative CH$_4$ production in Inceptisols from waste of coffee at 10, 20 and 30 ppm rate were 0.06, 0.08 and 0.07 mg CH$_4$ g soil$^{-1}$, whereas in Vertisols 3.66, 2.99 and 3.56 mg CH$_4$ g soil$^{-1}$ for waste of coffee application at rate of 10, 20 and 30 ppm, respectively. The highest rate of coconut husk and Ageratum conizoides resulted in highest CH$_4$ reduction in the Inceptisols as well as in the Vertisols. While application of coffee waste increased CH$_4$ production. NNI have the effect to CH$_4$ production most likely because the chemical contents from the NNI e.g., tannin, flavonoid, sulphur, etc. The chemical contents of NNI have the inhibitory effect on methanogenic bacteria in CH$_4$ production.

There is conflicting report that mention microbial immobilization of NO$_3$-N and keep hold NH$_4$ in the soil that lead to increase the nitrifiers population and resulted on reduction of CH$_4$ oxidation [16]. The range of CH$_4$ production in the Inceptisols after applying coconut husk, waste of coffee and Ageratum conizoides were approximately around 0.007–2.608; 0.041–0.076 and 0.056–0.096 mg CH$_4$ g soil$^{-1}$, respectively. While CH$_4$ production in the Vertisols after applying coconut husk, waste of coffee and Ageratum conizoides were approximately 0.013–3.979; 0.005–3.658 and 0.007–2.608 mg CH$_4$ g soil$^{-1}$, respectively. This finding showed that CH$_4$ production was found lowest at the rate of 30 ppm of coconut husk and Ageratum conizoides application in the Inceptisols and Vertisols during incubation period.

Figure 2 shows that the production of N$_2$O in Inceptisols from the third NNI ranged from 0.870-90.685 µg N$_2$O g soil$^{-1}$. The highest reduction of N$_2$O production in the Inceptisol was resulted from Ageratum conizoides application. Cumulative N$_2$O production in the Vertisols was lowest from the coconut husk application. The range of the cumulative N$_2$O production from the coconut husk application in the Vertisols were approximately around 0.978–13.316 µg N$_2$O g soil$^{-1}$. Coconut husk at the rate 20 ppm produced lowest N$_2$O production in the Vertisols. While the highest amount of N$_2$O production in the Vertisols resulted from the application of Ageratum conizoides approximately 1.051–518.951 µg N$_2$O g soil$^{-1}$. Chemical contents of NNI inhibit the breakdown of NH$_4$-N, retard the formation of NO$_2$ and NO$_3$ during nitrification and reduce the availability of NO$_3$ for denitrification [17]. N$_2$O is by product of nitrification and denitrification. Chemical contents of NNI affect microbial activities and give the impact to process of nitrification and denitrification.

NNI influences the production of CH$_4$ as well as N$_2$O from both of the soil types. Cumulative of CH$_4$ production showed variations among the different soils likely because soil properties such as soil organic carbon, NO$_3$-, NH$_4$ and total Fe significantly affected the CH$_4$ production [18]. Calculation of global warming potential (GWP) is an index that needed to compare the effectiveness of GHG to trap heat in the atmosphere. Coconut husk and Ageratum conizoides application were effective in reducing GWP emission (Figure 3). Applying a higher rate of NNI resulted in lower GWP. While higher rate of coffee waste application increased GWP. The lowest GWP was found from the application of Ageratum conizoides at the rate of 30 ppm. The rate of 20-30 ppm coconut husk and Ageratum conizoides application could reduce 16–57% and 41–72% of GWP, respectively. Chemical contents of NNI influenced microbial activities that produce CH$_4$ and N$_2$O through methanotrophic activity, nitrification and denitrification processes.
Figure 1. Cumulative CH₄ production from a) Inceptisols and b) Vertisols after application of natural nitrification inhibitors.
Figure 2. Cumulative N$_2$O production from a) Inceptisols and b) Vertisols after application of natural nitrification inhibitors.
4. Conclusions

Ineffective used of N fertilization is one of the sources of greenhouse gas emission and it could be reduced by applying natural nitrification inhibitors. The highest rate of coconut husk and *Ageratum conizoides* resulted the highest CH$_4$ reduction in the Inceptisols as well as in the Vertisols. The highest reduction of N$_2$O production was resulted from *Ageratum conizoides* application. Applying 30 ppm rate of *Ageratum conizoides* resulted in lowest global warming potential (GWP).

References

[1] Ju X T, Xing G X, Chen X P, Zhang S L, Zhang L J, Liu X J, Cui Z L, Yin B, Christie P, Zhu Z L and Zhang F S 2009 *Proc Nat Acad Sci.* **106** 3041–6

[2] IPCC 2007 Changes in atmospheric constituents and in radiative forcing in: Solomon S, Qin D, Manning M, et al. (Eds.) *Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge, UK/New York: Cambridge University Press) pp 498–540

[3] Mosier A and Kroeze C 2000 *Chemosphere—Global Change Sci.* **2** 465–73.

[4] Bodelier P L E, Roslev P, Henckel T and Frenzel P 2000 *Nature* **403** 421–4

[5] Knowles R 1993 Methane: processes of production and consumption *Agricultural Ecosystem Effects on Trace Gases and Global Climate Changes* ASA Special Publication 55 (Madison, USA: American Society of Agronomy) pp 145–56

[6] Misselbrook T H, Cardenas L M, Camp V, Thorman R E, Williams J R, Rollett A J and Chambers B J 2014 *Environ. Res. Lett.* **9** 115006 (11pp) doi:10.1088/1748-9326/9/11/115006

[7] de Klein C A M and Eckard R J 2008 *Aust. J. Exp. Agric.* **48** 14–20

[8] Souri M K 2016 *Open Agriculture* **1** 144–50

[9] Subbarao G V, Nakahara K and Ishikawa T 2008 *Plant Soil* **313** 89-99

[10] Kiran U and Patra D D 2002 *Commun. Soil Sci. Plant Anal.* **33** 1375–88

Subbarao G V, Nakahara K, Hurtado M P, Ono H, Moreta D E, Salcedo A F, Yoshihashi A T, Ishikawa T, Ishitani M, Ohnishi-Kameyama M, Yoshida M, Rondon M, Rao I M, Lascano C E, Berry W L and Ito O 2009 *Proc. Nat. Acad. Sci.* **106** 17302–7

Prasad R, Sharma S N, Singh S, Devakumar C, Saxena V S and Shivay Y S 2002 *Fert. News* **47** 63–67
[11] Majumdar D 2002 *Chemosphere* **47** 845–50
[12] Vyas B N, Godrej N B and Mistry K B 1981 *Fert. News* **2** 19–25
[13] Mitra S, Wassmann R, Jain MC and Pathak H 2002 *Nutr. Cycl. Agroecosyst.* **64** 169–82
[14] Wang B, Xu Y, Wang Z, Li Z, Ding Y and Guo Y 1999 *Biol. Fertil. Soils* **29** 74–80
[15] Lantin R S, Aduna J B and Javellana A M J 1995 *Methane measurements in rice fields. Instruction manual and methodologies, maintenance and troubleshooting guide* A joint undertaking by: International Rice Research Institute (IRRI), United State Environmental Protection Agency (US-EPA) and United Nation Development Program (UNDP)
[16] Malla G, Bhatia A, Pathak H, Prasad S, Jain N and Singh J 2005 *Chemosphere* **58** 141–7
[17] Majumdar D, Kumar S, Pathak H, Jain M C and Kumar U 2000 *Agric. Ecosys. Environ.* **81** 163–9
[18] Pathak H and Nedwell D B 2001 *Water Air Soil Poll.* **129** 217–28
[19] Inubushi K, Umebayashi M and Wada H 1990 Methane emission from paddy fields *Transactions of 14th International Congress of Soil Science (Kyoto)* vol 2 (Kyoto, Japan: International Society of Soil Science) pp 249–54
[20] Kimura M 1992 Methane emission from paddy soils in Japan and Thailand *Proceeding of an International Workshop Organized in the Framework of the Dutch National Research Programme on Global Air Pollution and Climate Change (Wageningen)* ed N H Batjesa and E M Bridges WISE Rep. 2 (Wageningen, Netherlands: ISRIC) pp 43-79