Effect of Biofertilizer Addition on Nitrous Oxide Emission

Endah Retnaningrum[1], Irfan D. Prijambada[2,*], Sukarti Moeljopawiro[1] and Budi S. Daryono[1]

[1]Fac. of Biology, Universitas Gadjah Mada, JL. Teknika Selatan Sekip Utara, Yogyakarta 55281, Indonesia

[2]Dept. of Agricultural Microbiology, Fac. of Agriculture, Universitas Gadjah Mada, JL. Flora, Bulaksumur, Yogyakarta 55281, Indonesia

Abstract: Application of nitrogen fixing biofertilizer, such as *Azotobacter,* has a potential for reducing nitrous oxide (N_2O) emission. The aim of this study was to examine the effect of nitrogen fixing biofertilizer addition to common practices of urea and fresh cattle manure usages for maize (*Zea mays* L.) growing on N_2O emission. The field experiment was conducted at Gunung Kidul, Yogyakarta, Indonesia. The treatments were addition of fresh cattle manure (M), fresh cattle manure added with nitrogen fixing biofertilizer (MB), urea (U), urea added with nitrogen fixing biofertilizer (UB), and control (no N fertilizer added). Nitrogen contents of the added urea and fresh cattle manure were adjusted to be equal. Urea and fresh cattle manure were given three times throughout the experiment period, i.e. 12, 30, and 48 days after planting (DAP). Urea was given at a rate of 44, 29, and 15 kg.ha^{-1}, respectively while fresh cattle manure was given at a rate of 6000, 4000, and 2000 kg.ha^{-1}, respectively. The emitted N_2O was collected using a closed-chamber method at 24, 42, 60, and 72 DAP and were determined using Gas Chromatograph. Soil properties including available N (NH_4^+-N and NO_3^--N) and organic C contents were also analyzed. On the harvesting time, the harvest index and the grain yield were determined. Biofertilizer addition influence decomposition process of cattle manure and urea that led to mineralization and nitrification of residual organic matter and hence to cause soil NH_4^+N in the order concentration of M treatment > MB > U > UB > C, and soil NO_3^--N of MB treatment > M > U > UB > C. Reduction of NO_3^-N was resulted in the highest N_2O emission of M > U > MB > UB > C ($P < 0.01$). The grain yield, and harvest index of maize were resulted in the order value of MB > UB > U > M> C treatments. Available mineral N and soil organic C contents strongly affected N_2O emission ($P < 0.01$). The results suggested that biofertilizer addition to common agricultural practices reduce N_2O emission and simultaneusly increased grain yield, and harvest index of maize.

Keywords: *Azotobacter*, urea, cattle manure, nitrous oxide.

INTRODUCTION

Concentration of N_2O in the troposphere is currently increasing at a rate of 4.6% from 2006 to 2008. Soil has been identified to be dominant source of N_2O, contributing about 15.8% of the total N_2O emission. Nitrous oxide has a long lifetime of about 114 years in comparison to CH_4 and CO_2. Therefore, it has a more important role in the destruction of stratosphere ozone and contributes to global warming [1].

From our community services activities, it was noticed that most of farmers at Gunung Kidul, Yogyakarta use N fertilizer in the form of fresh cattle manure and urea for growing agricultural commodities. Application of fresh cattle manure and urea for grassland, rice and maize [2-4] may lead to the production of N_2O in soils and its emission [5]. Skiba and Smith [6] suggested that soil N_2O emission is not simply affected by the availability of mineral N, but also affected by organic carbon.

Biofertilizer applications, particularly N_2 fixing bacteria such as *Azotobacter,* have been reported to increase crop yields while reducing the amount of applied N fertilizer [7]. Biofertilizer applications are more environmentally sound and their applications may mitigate the onset of global warming due to N_2O emission. However, most of farmers at Gunung Kidul Regency, Yogyakarta have not taken the advantage of biofertilizer application yet. A drastic change on the use of biofertilizers over N-fertilizer and manure is surely will not be accepted by the farmers at the area. A gradual change by combining biofertilizer application with common practices of N-fertilizer and manure usage is attempted. No report has been published regarding the effect of nitrogen fixing biofertilizer application combined with common practices of N-fertilizer and manure usage in agriculture, especially at tropical regions such as Indonesia, on the emission of N_2O. This study was aimed to examine on field N_2O emission in response to the application of nitrogen fixing biofertilizer combined with common practices of urea and fresh cattle manure usage at maize-growing field in Gunung Kidul, Indonesia

MATERIALS AND METHODS

Field Site and Experiment Design

The research was conducted on agricultural land located in Beji Village, Gunung Kidul, Yogyakarta, Indonesia (110° 40' 48.52" E, 7° 50' 34.20" S, 240

---

*Address correspondence to this author at the Dept. of Agricultural Microbiology, Fac. of Agriculture, Universitas Gadjah Mada, JL. Flora, Bulaksumur, Yogyakarta 55281, Indonesia; Tel: +62274523065; Fax: +62274563062; E-mail: irfan_prijambada@mail.ugm.ac.id

ISSN: 1814-8085 / E-ISSN: 1927-5129/14

amsl) during dry season from April to August 2011. The temperature was fluctuated from 25.4 to 27 °C, and the average humidity was 80 %. Numbers of days with precipitation for April and May were 10 and 8, with total precipitation of 154 and 168 mm, respectively. No precipitation occured on June to August. The soil at the experimental site has a clay texture which content of 8 % sand, 15 % silt and 77 % clay. The soil pH (in a 1:2.5 soil to water), soil moisture, organic C, total N, and C/N ratio were 6.8, 68 %, 0.9 mg.g^{-1}, 0.1 %, and 15.2 respectively. The experimental site was maize (*Zea mays*. L.) planted field. The experiment was arranged in a randomized block design with a plot size of 4 m x 6 m with three replications. Phosphorus and potassium were added as base fertilizers in the form of superphosphate (SP36) and potassium chloride (KCl) at a rate of 112.5 kg.ha^{-1} and 95 kg.ha^{-1}, respectively. The treatments were application of fresh cattle manure (M), fresh cattle manures added with nitrogen fixing biofertilizer (MB), urea (U), urea added with nitrogen fixing biofertilizer (UB). Plots without addition of nitrogenous fertilizer were used as a control (C). *Azotobacter sp.* was added as N_2 fixing biofertilizer in the amount of 1 kg.ha^{-1} (bacterial density 10^7-10^9 cfu.gr^{-1}). Fresh cattle manure and urea were given at 12, 30, and 48 DAP. Urea was given at a rate of 43.5, 29, and 15 kg.ha^{-1}, respectively, while fresh cattle manure was given at a rate of 6000, 4000, and 2000 kg.ha^{-1}, respectively. Total N content of fresh cattle manure and urea were adjusted and applied at an equal amount on each plots (40.83 kg.ha^{-1}).

Measurements

All samples for measurements were collected at 24, 42, 60, and 72 DAP. For NH_4^+-N, NO_3^--N and organic C analysis, soil samples were collected from the plots at a depth of 0–30 cm and sieved through 2 mm sieve. Soil NH_4^+-N was extracted by 1*M* KCl and was determined following the procedure of Anderson and Ingram [8], while NO_3^--N was determined colorimetrically after extraction by 0.5 *M* K_2SO_4 [9]. Soil organic C content was determined by wet oxidation with acid dichromate [10].

Air samples for N_2O analysis were taken manually using a closed 0.4 m x 0.15 m x 0.15 m polyvinyl chloride (PVC) chambers. Three closed chambers were used per plot per treatment. Gas samples were taken between 8.00 to 10.00 a.m. with regular intervals (0, 20, 30, and 40 min) after inserting the chambers into the soil at a depth of 0.05 m. At each time-point, gas samples (10 mL) were collected from the chamber headspace and then sealed with butyl rubber stoppers immediately. Nitrous oxide concentrations in the samples were determined using a Shimadzu Gas Chromatograph GC 2014 Greenhouse model, equipped with an electron capture detector (ECD) and a Porapack Q column at 70°C, using N_2 as the carrier gas at a flow of 26 mL.min^{-1}, injector temperature of 250°C and detector temperature of 325°C. The N_2O fluxes (μg.N.m^{-2}.min^{-1}) were calculated from the increase of N_2O concentration inside the chamber per unit time using the following equation [11].

$$F(N\text{-}N_2O) = d[N_2O]/dt \times k \times h \times (273/T)$$

where: $d[N_2O]/dt$= change in concentration per unit time (ppb.min^{-1}), k = constant for conversion from volume to weigh of N_2O = 1.250, h= height of chamber (m), and T= air temperature inside the chamber (°K).

On the harvesting time, five randomized selected plants samples in each plot were determined total plant biomass for measuring the harvest index and the grain yield.

Statistical Analysis

All statistical analysis were performed using StatView for Windows (SAS Institute, Cary, NC, USA) and were based on $P < 0.01$. Treatment effects were assessed using analysis of variance (ANOVA) and differences assessed by Duncan Multiple Range Test (DMRT) method. A multiple linear regression-procedure was used to establish the relationship between the observed soil properties and N_2O emissions.

RESULTS

Nitrous Oxide Fluxes

Different treatment significantly affected the N_2O fluxes. The N_2O emission from all treatments were higher than that of control from the 24 until 72 DAP ($P < 0.01$). These results indicated that the addition of nitrogenous fertilizers to agricultural soil significantly promoted the N_2O emission. All treatments stimulated N_2O emissions in the range of 1.5-10.5 μg.m^{-2}.min^{-1}. Their emissions were 12-40 times higher than the control plot (Figure **1**). Maximum N_2O emission was observed at 24 DAP ($P < 0.01$). The N_2O emissions decreased at subsequent observations (42, 60, and 72 DAP). The N_2O emission from plots receiving nitrogen fixing biofertilizer were lower compared to that without nitrogen fixing biofertilizer ($P < 0.01$). The average of

N_2O emission in the field of MB and UB treatment were 4.6 $\mu g.m^{-2}.min^{-1}$ and 4.0 $\mu g.m^{-2}.min^{-1}$, respectively, whereas the N_2O emission in the field of M and U treatments were 5.6 $\mu g.m^{-2}.min^{-1}$ and 4.9 $\mu g.m^{-2}.min^{-1}$, respectively.

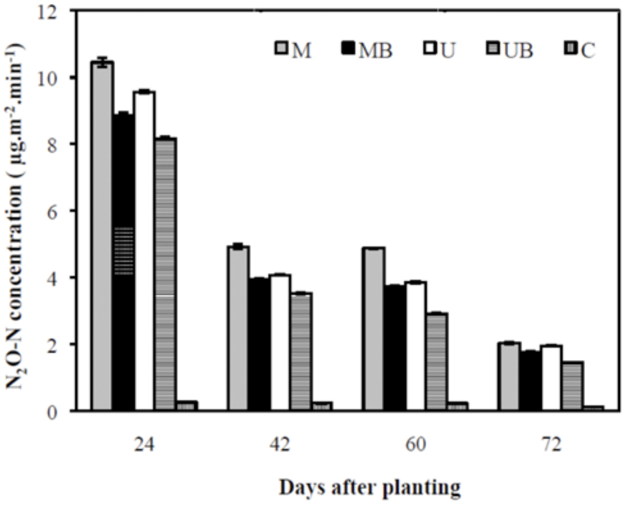

Figure 1: Nitrous oxide emission in the maize field after receiving treatments at 24, 42, 60, and 72 days after planting (DAP). Vertical bars indicate ± standard deviation of means (n=3).

Soil Properties

To further understand the factors which affect the reduction of N_2O emission in response to the application of nitrogen fixing biofertilizer, soil properties such as soil NO_3^--N, NH_4^+-N and organic C were further observed. The concentration of soil NO_3^--N, NH_4^+-N and organic C at 24 DAP were significantly higher than those observed at 42, 60, and 72 DAP (Figures **2**, **3** and **4**). The highest NO_3^--N concentration was found in soil of MB treatment, followed by M, U, and UB treatments. Those NO_3^- concentrations were 24.8-39.4 $mg.kg^{-1}$ soil, 22.3-38.3 $mg.kg^{-1}$ soil, 23.1-31.0 $mg.kg^{-1}$ soil, and 22.9-30.1 $mg.kg^{-1}$ soil, respectively.

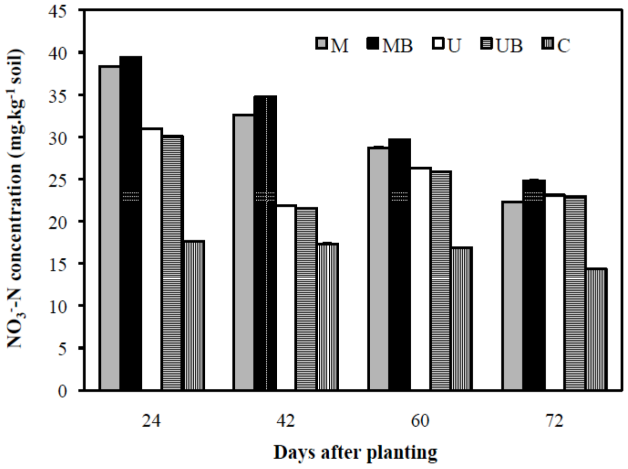

Figure 2: Soil NO_3^--N concentration in the maize field after receiving treatments at 24, 42, 60, and 72 days after planting. Vertitical bars indicate ± standard deviation of means (n=3).

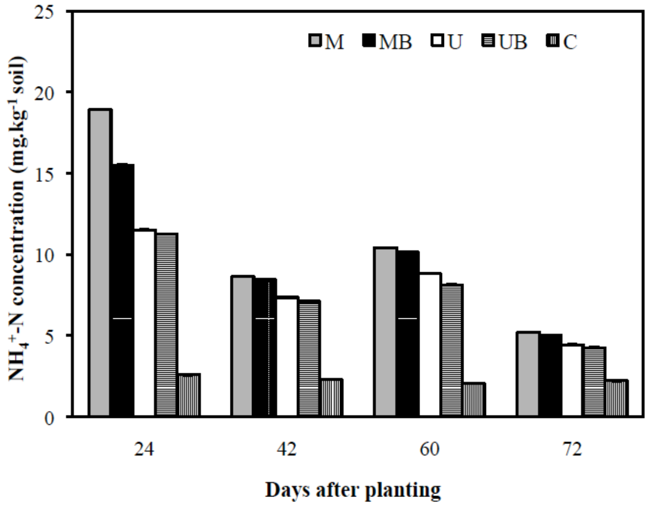

Figure 3: Soil NH_4^+- N concentration in the maize field after receiving treatments at 24, 42, 60, and 72 days after planting. Vertitical bars indicate ± standard deviation of means (n=3).

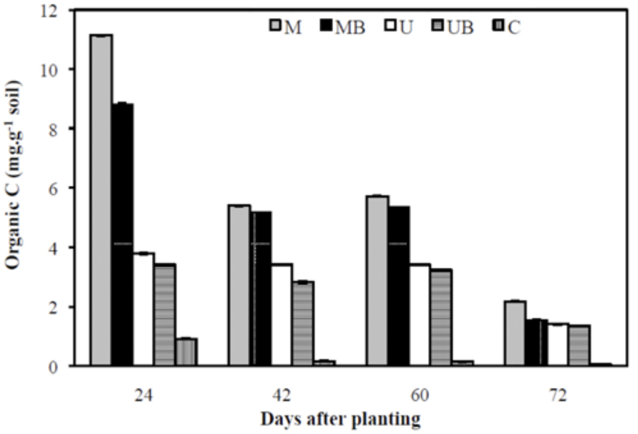

Figure 4: Soil organic C concentration in the maize field after receiving treatments at 24, 42, 60, and 72 days after planting. Vertical bars indicate ± standard deviation of means (n=3).

The highest NH_4^+-N and organic C concentrations were observed fromsoil of M, followed by MB, U, and UB treatments. Soil NH_4^+-N of M, MB, U, and UB were 5.2-18.9 $mg.kg^{-1}$ soil, 5.0-15.5 $mg.kg^{-1}$ soil, 4.4-11.5 $mg.kg^{-1}$ soil and 4.3-11.2 $mg.kg^{-1}$ soil, respectively. Soil organic C of M, MB, U, and UB were 2.2-11.1 $mg.g^{-1}$ soil, 1.5-8.8 $mg.g^{-1}$ soil, 1.4-3.8 $mg.g^{-1}$ soil, and 1.4-3.4 $mg.g^{-1}$ soil, respectively.

Relationship between Soil Properties with Nitrous Oxide Emission

A significant two-ways interactions (fertilizer usage and sampling time) was observed ($P < 0.01$) between soil NO_3^--N, NH_4^+-N, organic C contents and N_2O

emission (Table **1**). Those relationship may caused the highest rate of N_2O emissions observed in M treatment at 24 DAP where the concentration of NO_3^--N, NH_4^+-N and organic C were also high.

Table 1: Coefficient of Determination (r^2) for the Relationship between N_2O Emission and Soil Properties Including NO_3^--N, NH_4^+-N and Organic C

No	Relationship	N_2O
1.	NO_3^--N	0.85**
2.	NH_4^+-N	0.91**
3.	Organic C	0.83**

Value that followed by double asterisk symbol are significantly different ($P < 0.01$).

Grain Yield and Harvest Indext of Maize Plant

All treatments had significant ($P < 0.01$) effects on grain yield, and harvest index of maize plant (Table **2**). The higher grain yield, and harvest index were observed from data of MB, followed by UB, U, and M treatments. Maize grain yield of MB, UB, U, and M treatments were 4.06 $t.ha^{-1}$, 3.40 $t.ha^{-1}$, 2.23 $t.ha^{-1}$, and 1.31 $t.ha^{-1}$, respectively. Harvest index of maize plant of MB, UB, U, and M treatments were 0.46, 0.43, 0.40, and 0.34, respectively.

Table 2: The Grain Yield and Harvest Index of Maize Plant Receiving Different Treatment

Treatment	Grain yield ($t.ha^{-1}$)	Harvest index
M	1.31 ± 0.08^a	0.34 ± 0.016^a
MB	4.06 ± 0.03^b	0.46 ± 0.004^b
U	2.23 ± 0.09^c	0.40 ± 0.006^c
UB	3.40 ± 0.06^d	0.43 ± 0.006^d

Value with different superscript in the same column are significantly different ($P < 0.01$).

DISCUSSIONS

Differences in fertilizer usage during the growing period of maize significantly affected the N_2O emission. Soil N_2O emissions were varied with the highest rates of emissions occured on M treatment at 24 DAP (10.5 $\mu g.m^{-2}.min^{-1}$). The values in present study were higher compared to the result reported by Dambreville *et al.* [12] who observed the emission in the maize field receiving pig manure. The higher emission in this research may be caused by the higher clay content of soil, temperature and rainfall. Higher clay content of soil caused anaerob condition and induced for N_2O emission [13-15]. Temperature affects directly the activity of the nitrifying and denitrifying bacteria and the ratio N_2O/N_2, this ratio increase when the temperature increase [16]. Moreover, temperature controls biological oxygen consumption and this may also affect the emission of N_2O. In addition, many studies have reported the positive correlation of N_2O emission and rainfall [17-20]. However, that emission was lower compared to the results reported by Jumadi *et al.* [4] and Zhang *et al*. [21] who observed an increase of N_2O emission in maize plots experiments after receiving urea and amonium sulfat plus poultry manure.

The higher N_2O emission from soil receiving fresh cattle manure compared to the one receiving urea may be caused by the availability of organic C in fresh cattle manures which was stimulating denitrification process [22, 23]. The results were in accordance with the results of Morley and Baggs [24] who reported that N_2O production correlates positively with soil organic C. The lower N_2O emission from soil receiving nitrogen fixing biofertilizer compared to the one with no biofertilizer may be caused by the ability of *Azotobacter sp.* to reduce N_2O concentrations by denitrification process to produce N_2 gas [25]. Because of the high rates of N_2O emissions occured at the field receiving M treatment, further effort to mitigate N_2O emission from fresh cattle manure will be needed. Mahimairaja *et al*. [26] and Yamulki [27] reported an appropriate technique for composting of cattle manure was by adding straw or woodchips before application in the field which may reduce the N_2O emissions up to 30–35%.

In this experimental field, eventhough all plots were given fresh cattle manure and urea with the same N content over periods of growing maize plant, the soil mineral N contents (NO_3^--N and NH_4^+-N) in soil receiving them were extremely different. The soil NH_4^+-N of M > MB > U > UB may be caused by the existence of a higher number of microorganisms in the fresh cattle manures which were able to convert organic N in the fresh cattle manure into soil NH_4^+-N [5]. In addition *Azotobacter sp.* possess an active transport system which can take up N-NH_4^+ [28], resulted to the condition in which N-NH_4^+ of M > MB and U > UB.

Under aerobic condition existing in the maize growing field, NH_4^+-N would be converted into NO_3^--N by nitrifying microorganisms as shown by the rise of soil NO_3^--N content at treatment plots compared control plots. The addition of nitrogen fixing bacteria to manure induced highest rate of nitrification activity compared to other treatments and resulted to NO_3^--N concentrations of MB treatment > M > U > UB. On other hand, the treatment of nitrogen fixing bacteria addition to urea may caused the nitrogen fixing bacteria to become a competitor for one group of nitrifying microorganism, namely heterotrophic nitrifying microorganism to gain organic carbon in which was low in the treatment of urea addition [29, 30]. It was resulted in lowering NO_3^--N concentration of UB treatment. The NO_3^--N (highest at MB treatment) furthermore caused inhibition of nitrogenase which then promoted denitrification process by *Azotobacter sp.* [31] as a few of strain *Azotobacter sp*. has been reported to have both abilities on N_2 fixation and denitrification [32].

Biofertilizer addition with manure (MB treatment) and urea (UB treatment) increased maize grain yield, and harvest index significantly ($P < 0.01$) than M and U treatment, while simultaneously reducing soil N_2O emissions during their growing significantly ($P < 0.01$). These conditions may be partly explained by reducing of soil nutrient N losses through N_2O emissions, furthermore maize plant can take up efficiently and convert available nutrient N in soil to biomass and grain yield. The increase of grain yield, and harvest index also indicate a cumulative effect of successive biofertilizer applications in maize plant crop. As reported by Vessey *et al*. [33], biofertilizer not only contribute to N fixation, but they also involved in the biological control of plant pathogens, solubilization of nutrients and phytohormone synthesis. Moreover they can also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential [34]. Eventhough soil N_2O emissions of MB treatment was higher than UB treatment, maize grain yield, and harvest index of MB treatment was higher 20 % and 7 % as compared to UB treatment. These situations may be caused by rich nutrient composition inside fresh cattle manure that support for growing *Azotobacter* (Biofertilizer). On the contrary, urea less supportive of *Azotobacter* growth [35].

CONCLUSIONS

It may be concluded that application of nitrogenous fertilizer significantly stimulated N_2O emission. The application of fresh cattle manure may produce higher N_2O emission. Addition of nitrogen fixing biofertilizer in combination with nitrogenous fertilizer could reduce the emission and simultaneusly increased maize grain yield, and harvest index ($P < 0.01$). The application of nitrogen fixing biofertilizer can mitigate the problem of N_2O emission.

ACKNOWLEDGEMENT

We acknowledge financial support from the I-MHERE Project Sub Activity 3.1.1-II of Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. (Contract no: UGM/BI/1263/I/05/04).

APPENDIX

N_2O EMISSION AND SOIL PROPERTIES

Class Level Information

Class	Level	Values
Fertilizers	5	M0, M1, M2, M3, M4
Dates of sampling	4	W1. W2. W3. W4
Replications	3	1, 2, 3
Numbers of Observations Read	60	
Numbers of Observations Used	60	

1. Dependent Variable: N_2O

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	21	579.7964177	27.6093532	306117	<.0001
Error	38	0.0034273	0.0000902		
Corrected Total	59	579.7998450			

R-Square	Coeff Var	Root MSE	N_2O Mean		
0.999994	0.246868	0.009497	3.846983		

Source	DF	Anova SS	Mean Squares	F value	Pr>F
Replications	2	0.0005380	0.0002690	2.98	0.0626
Fertilizers	4	213.4973566	53.3743391	591785	<.0001
Dates of sampling	3	292.8998018	97.6332673	1082503	<.0001
Fertilizers *Dates of sampling	12	73.3987213	6.1165601	67817.0	<.0001

2. Dependent Variable: NH_4^+-N

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	21	895.7974971	42.6570237	1938337	<.0001
Error	38	0.0008363	0.0000220		
Corrected Total	59	895.7983333			
R-Square	Coeff Var	Root MSE	NH_4^+-N Mean		
0.999999	0.056920	0.004691	8.241667		

Source	DF	Anova SS	Mean Squares	F value	Pr>F
Replications	2	0.0003397	0.0001699	7.72	0.0015
Fertilizers	4	242.0684673	60.5171168	2749901	<.0001
Dates of sampling	3	531.9816357	177.3272119	8057758	<.0001
Fertilizers *Dates of sampling	12	121.7470543	10.1455879	461016	<.0001

3. Dependent Variable: NO_3^--N

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	21	2625.609771	125.029037	1314408	<.0001
Error	38	0.003615	0.000095		
Corrected Total	59	2625.613386			
R-Square	Coeff Var	Root MSE	NO_3^--N Mean		
0.999999	0.037206	0.009753	26.21373		

Source	DF	Anova SS	Mean Squares	F value	Pr>F
Replications	2	0.000246	0.000123	1.29	0.2862
Fertilizers	4	1510.473411	377.618353	3969835	<.0001
Dates of sampling	3	825.615954	275.205318	2893185	<.0001
Fertilizers *Dates of sampling	12	289.520159	24.126680	253640	<.0001

4. Dependent Variable: Organic C

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	21	471.7535250	22.4644536	660038	<.0001
Error	38	0.0012933	0.0000340		
Corrected Total	59	471.7548183			
R-Square	Coeff Var	Root MSE	Organic C Mean		
0.999997	0.168021	0.005834	3.472167		

Source	DF	Anova SS	Mean Squares	F value	Pr>F
Replications	2	0.0001733	0.0000867	2.55	0.0917
Fertilizers	4	248.5367267	62.1341817	1825592	<.0001
Dates of sampling	3	139.2603383	46.4201128	1363890	<.0001
Fertilizers *Dates of sampling	12	83.9562867	6.9963572	205563	<.0001

THE CORR PROCEDURE

5. Variables: N_2O NH_4^+N NO_3^-N Organic C

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
N_2O	60	3.84698	3.13482	230.81900	0.11600	10.47600
NH_4^+N	60	8.24167	3.89654	494.50000	2.70700	18.93900
NO_3^-N	60	26.21373	6.67098	1573	14.39300	39.42200
Organic C	60	3.47217	2.82769	208.33000	0.05000	11.14000

Pearson Correlation Coefficients, N = 60

Prob > |r| under H0: Rho=0

	N_2O	NH_4^+N	NO_3^-N	Organic C
N_2O	1.00000	0.91089 <.0001	0.84518 <.0001	0.83151 <.0001
NH_4^+N	0.91089 <.0001	1.00000	0.86678 <.0001	0.92810 <.0001
NO_3^-N	0.84518 <.0001	0.86678 <.0001	1.00000	0.90679 <.0001
Organic C	0.83151 <.0001	0.92810 <.0001	0.90679 <.0001	1.00000

GRAIN YIELD AND HARVEST INDEX

Class Level Information

Class	Level	Values
Fertilizers	4	M1, M2, M3, M4
Replications	5	1, 2, 3, 4, 5
Numbers of Observations Read	20	
Numbers of Observations Used	20	

1. Dependent Variable: Grain Yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	22.22605500	3.17515071	4214.80	<.0001
Error	12	0.00904000	0.00075333		
Corrected Total	19	22.23509500			
R-Square	Coeff Var	Root MSE	Grain Yield Mean		
0.999593	0.999706	0.027447	2.745500		

Source	DF	Anova SS	Mean Square s	F value	Pr>F
Replications	4	0.00212000	0.00053000	0.70	0.6045
Fertilizers	3	22.22393500	7.40797833	9833.60	<.0001

2. Dependent Variable: Harvest Index

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	4.06330500	0.58047214	198.17	<.0001
Error	12	0.03515000	0.00292917		
Corrected Total	19	4.09845500			
R-Square	Coeff Var	Root MSE	Harvest index Mean		
0.991424	1.326027	0.054122	4.081500		

Source	DF	Anova SS	Mean Squares	F value	Pr>F
Replications	4	0.01113000	0.00278250	0.95	0.4689
Fertilizers	3	4.05217500	1.35072500	461.13	<.0001

REFERENCES

[1] Intergovernmental Panel on Climate Change (IPCC). Climate Change 2007: The Physical Science Basis. The Fourth Assessment Report. Cambridge: University Press 2007; pp. 996.

[2] Hua X, Guangxi X, Cai Z, Tsuruta H. Nitrous oxide emissions from three rice fields in China. Nutr Cycl Agroecosyst 1997; 49: 23-8. http://dx.doi.org/10.1023/A:1009779514395

[3] Jones SK, Rees RM, Skiba UM, Ball BC. Influence of organic and mineral N fertilizer on N_2O fluxes from temperate grassland. Agric Ecosyst Environ 2007; 121: 74-83. http://dx.doi.org/10.1016/j.agee.2006.12.006

[4] Jumadi O, Hala Y, Muis A, Ali A, Palennari M, Yagi K, *et al*. Influences of chemical fertilizers and a nitrification inhibitor on greenhouse gas fluxes in a corn (*Zea mays* L.) field in Indonesia. Microb Environ 2008; 23: 29-34. http://dx.doi.org/10.1264/jsme2.23.29

[5] Hutchinson GL, Davidson EA. Processes for production and consumption of gaseous nitrogen oxides in soil. In: Harper LA, Mosier AR, Duxbury JM. Rolston DE, Eds. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. Madison: Spec Publishers 1993; pp. 79-93.

[6] Skiba U, Smith KA. The control of nitrous oxide emissions from agricultural and natural soils. Chemosphere Glob Change Sci 2000; 2: 379-386. http://dx.doi.org/10.1016/S1465-9972(00)00016-7

[7] Kennedy IR, Choudhury ATMA, Kecskes ML. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol Biochem 2004; 36:1229-1244. http://dx.doi.org/10.1016/j.soilbio.2004.04.006

[8] Anderson JM, Ingram JSI. Colorimetric determination of ammonium. In: Anderson JM, Ingram JSI, Ed. Tropical Soil Biology and Fertility, A Handbook of Methods. Wallingford UK: CAB International 1993; pp. 73-4.

[9] Cataldo DA, Haroon M, Schrader LE, Young V. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 1975; 6: 71-80. http://dx.doi.org/10.1080/00103627509366547

[10] American Public Health Association (APHA). Standard Methods for The Examination of Water and Wastewater. 18th ed. Washington: American Public Health Association 1985; pp. 54-8.

[11] Hutchinson GL, Mosier, AR. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci Soc Amer J 1981; 45: 311-16.
http://dx.doi.org/10.2136/sssaj1981.03615995004500020017x

[12] Dambreville C, Morvan T, Germon J. N_2O emission in maize-crops fertilized with pig slurry, matured pig manure or ammonium nitrate in Brittany. Agric Ecosystems Environ 2008; 123: 201-10.
http://dx.doi.org/10.1016/j.agee.2007.06.001

[13] Brentrup F, Kusters J, Lammel J, Kuhlmann H. Methods to estimate on-field nitrogen emissions from crop production as an input to LCA studies in the agricultural sector. Int J Life Cycl Assessment 2000; 5: 349-357.
http://dx.doi.org/10.1007/BF02978670

[14] Tan IYS, Duxbury JM, Melkonian JJ, Schindelbeck RR, Geohring LD, Hively WD, *et al*. Single-event nitrous oxide losses under maize production as affected by soil type, tillage, rotation, and fertilization. Soil Till Res 2009; 102: 19-26.
http://dx.doi.org/10.1016/j.still.2008.06.005

[15] Velthof G, Hoving I, Dolfing J, Smit A, Kuikman P, Oenema O. Method and timing of grassland renovation affects herbage yield, nitrate leaching, and nitrous oxide emission in intensively managed grasslands. Nutr Cycl Agroecosyst 2010; 86: 401-412.
http://dx.doi.org/10.1007/s10705-009-9302-7

[16] Keeney DR, Fillery IR, Marx GP. Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Sci Soc Amer J 1979; 43: 1124-1128.
http://dx.doi.org/10.2136/sssaj1979.03615995004300060012x

[17] Smith KA, Thomson PE, Clayton H, McTaggart IP, Conen F. Effects of temperature, water content and nitrogen fertilization on emissions of nitrous oxide by soils. Atmospheric Environ 1998; 32: 3301-3309.
http://dx.doi.org/10.1016/S1352-2310(97)00492-5

[18] Flynn HC, Smith J, Smith KA, Wright J, Smith P, Massheder J. Climate-and crop-responsive emission factors significantly alter estimates of current and future nitrous oxide emissions from fertilizer use. Global Change Biol 2005; 11: 1522-1536.
http://dx.doi.org/10.1111/j.1365-2486.2005.00998.x

[19] Zhang J, Han X. N_2O emission from the semi-arid ecosystem under mineral fertilizer (urea and superphosphate) and increased precipitation in northern China. Atmospheric Environ 2008; 42: 291-302.
http://dx.doi.org/10.1016/j.atmosenv.2007.09.036

[20] Rochette P, Worth DE, Lemke RL, McConkey BG, Pennock DJ, Wagner-Riddle C, *et al*. Estimation of N_2O emissions from agricultural soils in Canada. I. Development of a country-specific methodology. Can J Soil Sci 2008; 88: 641-654.
http://dx.doi.org/10.4141/CJSS07025

[21] Zhang YL, Liu J, Mu Y, Xu Z, Pei S, Lun X, *et al*. Nitrous oxide emissions from a maize field during two consecutive growing seasons in the North China. Plain J Environ Sci 2012; 24: 160-68.
http://dx.doi.org/10.1016/S1001-0742(10)60594-3

[22] Paul JW, Beauchamp EG, Zhang X. Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory. Can J Soil Sci 1993; 73: 539-553.
http://dx.doi.org/10.4141/cjss93-054

[23] Philippot L, Hallin S, Borjesson G, Baggs EM. Biochemical cycling in the rhizosphere having an impact on global change. Plant Soil 2009; 321: 61-81.
http://dx.doi.org/10.1007/s11104-008-9796-9

[24] Morley N, Baggs EM. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biol Biochem 2010; 42: 1864-1871.
http://dx.doi.org/10.1016/j.soilbio.2010.07.008

[25] Yamazaki Y, Yoshida N, Wada E, Matsuo S. N_2O reduction by *Azotobacter vinelandii* with emphasis on kinetic nitrogen isotope effects. Plant Cell Physiol 1986; 28: 263-271.

[26] Mahimairaja S, Bolan NS, Hedley MJ. Denitrification losses of N from fresh and composted manures. Soil Biol Biochem 1995; 27: 1223-1225.
http://dx.doi.org/10.1016/0038-0717(95)00042-D

[27] Yamulki S. Effect of straw addition on nitrous oxide and methane emissions from stored farmyard manures. Agric Ecosyst Environ 2006; 112: 140-145.
http://dx.doi.org/10.1016/j.agee.2005.08.013

[28] Gordon JK, Moore RA. Ammonium and methylammonium transport by the nitrogen-fixing bacterium *Azotobacter vinelandii*. J Bacteriol 1981; 148: 435-442.

[29] Wong T, Maier RJ. H_2-Dependent mixotrophic growth of N_2-fixing *Azotobacter vinelandii*. J Bacteriol 1985; 163: 528-533.

[30] Wrage N, Velthof GL, Van Beusichem ML, Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol Biochem 2001; 33: 1723-1732.
http://dx.doi.org/10.1016/S0038-0717(01)00096-7

[31] Sorger GJ. Regulation of nitrogen fixation in *Azotobacter vinelandii* OP: the role of nitrate reductase. J Bacteriol 1969; 98: 56-61.

[32] Zumft WG. Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 1997; 61: 233-616.

[33] Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 2003; 55: 571-586.
http://dx.doi.org/10.1023/A:1026037216893

[34] Kohler J, Caravaca F, Carrasco L, Roldan A. Contribution of *Pseudomonas mendocina* and *Glomus intraradices* to aggregates stabilisation and promotion of biological properties in rhizosphere soil of lettuce plants under field conditions. Soil Use Manage 2006; 22: 298-304.
http://dx.doi.org/10.1111/j.1475-2743.2006.00041.x

[35] Mikanova O, Novakova J, Hanzlikova A, Kubat J, Filip Z. Effect of long-term fertilization and anthropogonic load on biomass and the incidence of some groups of microorganisms. Plant Prod 1996; 42: 391-397.

Received on 30-12-2013 Accepted on 20-02-2014 Published on 05-03-2014

http://dx.doi.org/10.6000/1927-5129.2014.10.07