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PHOSPHORUS EFFECTS ON N_2O AND NO EMISSIONS FROM *ACACIA MANGIUM* SOIL

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Abstract: An incubation experiment was conducted to examine the effects of phosphorus (P) application on N_2O and NO emissions from soils of an *Acacia mangium* plantation in Indonesia. The soils were incubated for 30 d with and without adding P ($Ca(H_2PO_4)_2$; 2 mg P $g \cdot soil^{-1}$) after adjusting water-filled pore space (WFPS) to 75% or 100%. N_2O/NO ratio in both 75% and 100% WFPS in control soils were higher than 1, suggesting that N_2O and NO were emitted mainly from denitrifying bacteria. P addition increased N_2O emission under both WFPS conditions and NO emission under 75% WFPS, which was attributed to two reasons. Firstly, the stimulation of O_2 consumption by general heterotrophic activity, which was suggested by significant increase in CO_2 production in P treated soils, promoted developing stronger anaerobic conditions required for denitrification. Secondly, P addition could also have relieved the P shortage for denitrifying bacteria, producing N_2O and NO. Our results suggest the application of P fertilizer has a potential to stimulate N_2O and NO emissions from *Acacia mangium* plantation at least when soils are under relatively wet conditions. The results also suggest that P limitation in tropical soils might be suppressing the emissions of N_2O and NO, because it limits the activity of nitrification and/or denitrification.
Key Words: Nitrous oxide, Nitric oxide, Phosphorus, *Acacia mangium* plantation, Incubation experiment, Denitrification

1. INTRODUCTION

Acacia mangium, a legume, is one of the major fast growing species used in plantation forestry in the tropics. Due to its rapid growth, high tolerance to poor soils, and suitable quality for pulp, *Acacia mangium* plays an important role to sustain commercial supply of pulp (Duguma et al., 1994; Garay et al., 2004). Besides, afforestation by fast growing tree species including *Acacia mangium* is a typical mitigation option in the forestry sectors for global warming (IPCC, 2007). However, leguminous trees as *Acacia mangium* have been demonstrated to be the non-negligible emission source of N_2O and NO (Erickson et al., 2001; Dick et al., 2006; Arai et al., 2008; Konda et al., 2008) because of its high N-fixing ability. Arai et al. (2008) suggested the importance of N_2O emissions from leguminous tree stands will increase over the next several decades due to the increase of the area.

It is well known that nitrous oxide (N_2O) is an important greenhouse gas following CO_2 and CH_4 (IPCC, 2007), and nitric oxide (NO) contributes to the production of tropospheric ozone, acid rain, or photochemical smog (Crutzen, 1983). Soils are major sources for the production of N_2O and NO, which are byproducts or intermediate products of microbial nitrification and denitrification (Bremer et al., 1997; Davidson et al., 2000; Wrage et al., 2001).

It has been suggested that many tropical forests are impoverished in P relative to most temperate forests (Vitousek et al., 1993; Cleveland et at., 2002) and P fertilizers are commonly applied in plantation forestry in the tropics. Although P fertilization could change the N_2O and NO emissions by influencing the soil microbial activities, effects of P fertilization to N_2O and NO emissions were rarely reported.

Here we investigated the effects of release from P limitation by P fertilization on the production of N_2O and NO. For simplicity, we conducted incubation experiment and excluded the effects of vegetation, which could uptake nitrogen (N). Soils from an *Acacia mangium* plantation on Sumatra were used for incubation experiment.

2. MATERIAL AND METHODS

In September 2007, we selected a 1-yr old *Acacia mangium* plantation site in South Sumatra Province, Indonesia (3°47.394' E, 103°55.236' S). The climate of the area is humid tropical rain forest with an annual precipitation of 2000~3000 mm and a mean annual temperature of 27.3°C (Hardjono et al., 2005). This site is the second generation of the plantation activity. Although 85 g of P fertilizer per tree were added to each planting hall before planting in 1996, no fertilizer was applied subsequently. Soil samples at 0-5 cm depth were randomly collected from 10 points in a 1.5 ha plantation site. The soil samples were well-mixed and air-dried after sieved through a 2 mm sieve. Table 1 shows the soil pH (H_2O) in a 1:2.5 air-dried soil to distilled water ratio, the total carbon (C) and N (CN corder; JM1000CN, J SCIENCE, Japan), the available P extracted by Bray-1 method (Kuo, 1996), the particle density (Blake and Hartge, 1998), and the particle size distribution (Day et al., 1965) of the soil sample.

Air-dried soil sample equivalent to 30 g in oven-dry weight was placed in a 223 ml wide mouth jar for gas emission analysis, and 5 g in a 50 ml plastic bottle for inorganic N analysis. Triplicate jars and bottles were prepared for each treatment.

Table 1. Selected physico-chemical properties of the soil

pH (H_2O)	Total C (mg C g^{-1})	Total N (mg N g^{-1})	Available P (mg P kg^{-1})	Clay (%)	Silt (%)	Sand (%)	Texture	Particle density
4.74	37.3	3.0	5.87	48.9	16.6	34.5	Clay	2.49

After the 48 h pre-incubation at 40% WFPS, a solution of $Ca(H_2PO_4)_2$ equivalent to 2 mg P g·soil^{-1} dissolved in distilled water was applied and the soil water content was adjusted to 75% or 100% WFPS because N_2O emission was reported to be much larger in relatively wet condition in our study site (Arai et al., 2008). Bulk density for WFPS calculation was determined to 1.01 by re-compacting soil sample in the glass jar for incubation. We also prepared the control without P application in the same manner. The samples were incubated at 25°C in the dark. The water content was maintained as constant during the incubation by adding distilled water occasionally. Since P addition significantly decreased soil pH from 4.74 to 3.95 ($P < 0.05$, t-test, n=3), we made an experiment to assure whether this change of pH alters N_2O and NO emissions or not. We concluded that the change in N_2O and NO emission rates of this soil due to decreases in soil pH is negligible, because N_2O and NO emissions from soil samples adjusted to 3.96 in pH by adding H_2SO_4 did not significantly differ from those of control ($P > 0.05$, t-test, n=3; unpublished data).

N_2O, NO, and CO_2 emission rates were measured 8 times during the incubation period, i.e., 0-, 0.5-, 1-, 3-, 8-, 15-, 22-, and 30-d after the beginning of the incubation. Fifteen ml of gas samples were collected at 0, 20, and 40 min after closing the jar with a butyl rubber stopper equipped with a sampling port and an air bag to regulate the inside pressure to atmospheric level. The N_2O concentration in the gas sample was analyzed using a gas chromatograph (GC-14B, SHIMADZU, Kyoto, Japan) equipped with an electron capture detector. The NO concentration was analyzed with a NO-NO_2-NO_x Analyzer (Model 42i, Nippon Thermo Co. Ltd., Kyoto, Japan). The CO_2 concentration was analyzed with a gas chromatograph (GC-14B, SHIMADZU, Kyoto, Japan) equipped with a thermal conductivity detector. We calculated fluxes using a linear regression slope, using data at 0, 20, and 40 min, because the increase in gas concentration linearly appeared.

Inorganic N was analyzed at 0- and 8-d after the beginning of the incubation. Both NH_4^+ and NO_3^- were extracted by shaking 5 g soil with 50 ml 2M KCl for 1 hour. The supernatants were filtered and refrigerated until analysis. NH_4^+ was determined by indophenol blue absorptiometry and NO_3^- by naphthyl ethylenediamine method using a flow injection (AQLA-700-NO, AQUA LAB, Japan).

Since the emission rates of N_2O and NO after 8-d remained at very low level, we will not include the results of N_2O and NO at 15-, 22-, and 30-d for further calculation. The cumulative emissions of N_2O and NO until 8-d were estimated using the linear trapezoidal method. Net ammonification rate and net nitrification rate were calculated from the concentration of NH_4^+ and NO_3^- at 0-d and 8-d as follows.

Net ammonification rate:
(μg N g·soil^{-1} day^{-1}) = (NH_4^+ at 8-d - NH_4^+ at 0-d) / 8.

Net nitrification rate:
(μg N g·soil^{-1} day^{-1}) = (NO_3^- at 8-d - NO_3^- at 0-d) / 8.

The level of significance of every analysis was examined by ANOVA followed by Tukey's multiple comparison tests or an unpaired t-test after confirm the normality of each data by Kolmogorov-Smirnov test. Since gas emission data were log-normally distributed, we log-transformed these data before analyses. Each statistical analysis was performed using SPSS version 10.0 (SPSS Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

The results suggested that the P application to *Acacia mangium* plantation might increase the unwelcomed emissions of N_2O and NO in this site. P addition significantly increased N_2O emission rates at 0.5- and 1-d at 75% WFPS and 1-d at 100% WFPS (Figure 1ab). NO emission rates significantly increased with P addition at 75% WFPS at 0.5-, 1-, and 3-d (Figure 1c), while such increase was not observed at 100% WFPS except 1-d (Figure 1d). At both WFPS conditions, the cumulative N_2O emissions for 8 d significantly increased with P addition (Table 2). P addition also significantly increased the cumulative NO emission at 75% WFPS, but there was no significant difference at 100% WFPS (Table 2).

Some possible mechanisms are considered as follows. At first, the development of stronger anaerobic conditions due to promoting O_2 consumption by heterotrophic microbes stimulated by P addition increased denitrification. In fact, P addition stimulated CO_2 production and N ammonification rate at both WFPS conditions (Table 2 & 3). Some previous studies suggested that the decrease of O_2 due to microbial consumption generated more anaerobic condition favour for denitrification (Klemedtson et al., 1988; Nobre et al., 2001; Azam et al., 2002). Secondary, P addition stimulated nitrifying and/or denitrifying bacteria due to the relief for their P shortage. Since tropical soils are believed to be impoverished in P (Vitousek et al., 1993), denitrification also could be limited by P. It is possible that P addition lead to increase in denitrification activity, which resulted in higher N_2O and NO.

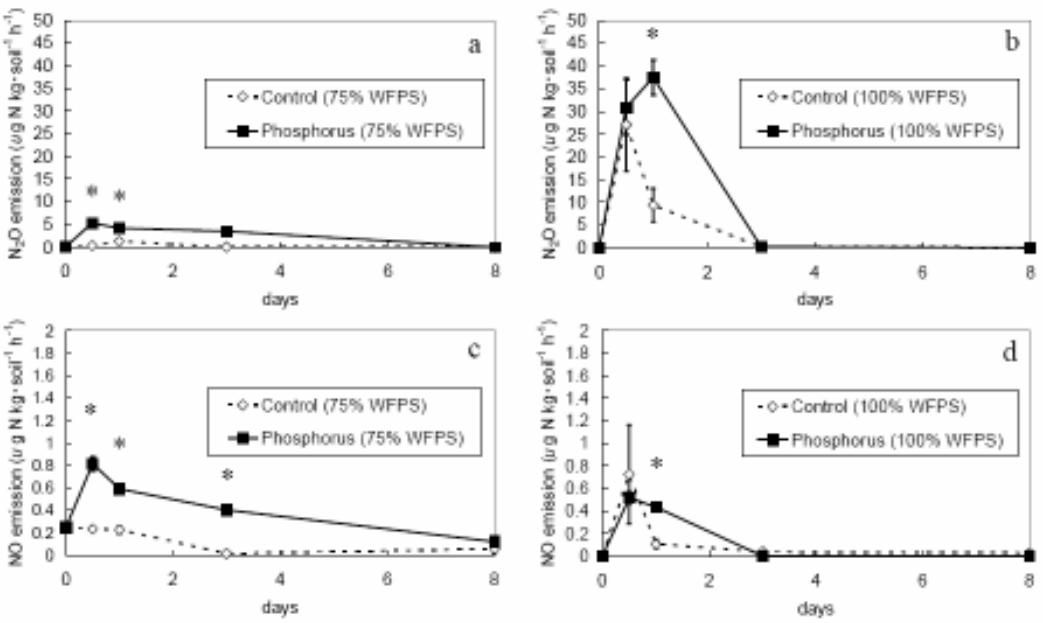

Figure 1. Effects of P addition on emissions of (a) N_2O under 75% WFPS, (b) N_2O under 100% WFPS, (c) NO under 75% WFPS, and (d) NO under 100% WFPS during 8 days. The error bars indicate the mean ± standard error of three replicates. * indicates the significant difference between control soil and P-added soil using an unpaired t-test ($P < 0.05$).

Our incubation study suggested the application of P fertilizer might have a potential to stimulate N_2O and NO emissions from *Acacia mangium* plantation at least when soils are under relatively wet conditions. However our study was based on an incubation experiment conducted in the laboratory, where no vegetation existed.

Since plant growth is dependent on N availability (Aerts et al., 1995), vegetation is presumably the main competitor of nitrifying and denitrifying microbes for N. Although it has been recognized in various forest ecosystems that microorganisms are stronger competitors for inorganic N than plants, (Johanson, 1992; Kaye and Hart, 1997), some studies have shown plants to be the stronger competitor (Silvan et al., 2005). Hall and Matson (2003) reported the emissions of N_2O and NO were smaller in the N+P-fertilized plots compared to plots fertilized with N alone, and they attributed this phenomenon to the promotion of plant growth and N uptake accelerated by P addition. Therefore, also in *Acacia mangium* plantation, P addition might promote N uptake by vegetation and suppress the N_2O and NO emissions. Experiment is strongly requested to understand the effect of P application on the emissions of N_2O and NO in the field condition.

4. CONCLUSION

Our incubation study suggested the application of P fertilizer might have a potential to stimulate N_2O and NO emissions from *Acacia mangium* plantation at least when soils are under relatively wet conditions. We suggest that P fertilizer should be carefully used in *Acacia mangium* plantation. Further experiments in the field condition are strongly requested.

5. ACKNOWLEDGEMENT

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Table 2. Cumulative gas emissions

		N_2O *u*g N kg･soil^{-1} 8day^{-1}		NO *u*g N kg･soil^{-1} 8day^{-1}		CO_2 mg C kg･soil^{-1} 30day^{-1}	
Water content	Treatment	Avr.	SE	Avr.	SE	Avr.	SE
WFPS 75%	Control	52	19	25	1.9	829	38
	Phosphorus	498*	113	90*	3.0	1012*	36
WFPS 100 %	Control	622	52	19	5.3	1129	18
	Phosphorus	1512*	172	21	1.4	1376*	48

Notes: * indicates the significant difference between control soil and P-added soil using an unpaired t-test ($P < 0.05$).

Table 3. Soil inorganic N and rates of net ammonification and net nitrification.

WFPS	Treatment	NH_4^+				NO_3^-				Net ammonification rate		Net nitrification rate	
		μg N g^{-1} soil^{-1}				μg N g^{-1} soil^{-1}				μg N g^{-1} soil^{-1} day^{-1}		μg N g^{-1} soil^{-1} day^{-1}	
		0-day		8-day		0-day		8-day					
		Avr.	SE	Avr.	SE	Avr.	SE	Avr.	SE	Avr.	SE	Avr.	SE
75%	Control	94.3^a	1.50	170.9^b	3.65	2.77^a	0.03	2.40^a	0.10	9.7	0.16	-0.05	0.01
	Phosphorus	94.9^a	1.35	180.0^b	2.77	3.07^a	0.03	3.10^a	0.17	10.7*	0.26	0.00	0.02
100%	Control	96.5^a	1.03	163.8^b	1.25	2.80^a	0.00	2.13^a	0.37	8.4	0.09	-0.08	0.05
	Phosphorus	94.8^a	1.24	167.5^b	0.59	3.00^a	0.00	2.13^a	0.77	9.1*	0.10	-0.11	0.10

Notes: Figures with same letters within each parameter in the same WFPS condition indicate insignificant difference among them using ANOVA followed by Tukey's multiple comparison tests ($P < 0.05$). * indicates the significant difference between control soil and P-added soil using an unpaired t-test ($P < 0.05$).

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