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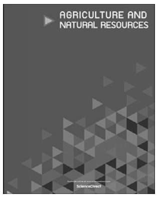

Original Article

Effects of phosphorus addition on nitrogen cycle and fluxes of N_2O and CH_4 in tropical tree plantation soils in Thailand

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ABSTRACT

An incubation experiment was conducted to test the effects of phosphorus (P) addition on nitrous oxide (N_2O) emissions and methane (CH_4) uptakes, using tropical tree plantation soils in Thailand. Soil samples were taken from five forest stands—*Acacia auriculiformis*, *Acacia mangium*, *Eucalyptus camaldulensis*, *Hopea odorata*, and *Xylia xylocarpa*—and incubated at 80% water holding capacity. P addition stimulated N_2O emissions only in *Xylia xylocarpa* soils. Since P addition tended to increase net ammonification rates in *Xylia xylocarpa* soils, the stimulated N_2O emissions were suggested to be due to the stimulated nitrogen (N) cycle by P addition and the higher N supply for nitrification and denitrification. In other soils, P addition had no effects on N_2O emissions or soil N properties, except that P addition tended to increase the soil microbial biomass N in *Acacia auriculiformis* soils. No effects of P addition were observed on CH_4 uptakes in any soil. It is suggested that P addition on N_2O and CH_4 fluxes at the study site were not significant, at least under laboratory conditions.

Introduction

Since the primary source of phosphorus (P) for terrestrial ecosystems is rock weathering, P has been considered to be the ultimate limiting soil nutrient in terrestrial ecosystems, and ecosystems with old soils can become depleted in P (Walker and Syers, 1976). Thus, highly-weathered lowland tropical forest soils have been considered to have low concentrations of biologically available P (Vitousek and Farrington, 1997; Elser et al., 2007; Vitousek et al., 2010), and it is widely believed that P limits the ecosystem process in tropical forests. Several studies have reported that P availability also limits soil microbial activities in tropical soils (Cleveland et al., 2002; Ilstedt et al., 2003, 2006; Cleveland and Townsend, 2006; Mori et al., 2010; Liu et al., 2012). Thus, changes in P availability may change soil microbial activities, metabolisms and community composition, which could be associated with altered nitrous oxide (N_2O) (Mori et al., 2013a, 2013b, 2014) and methane (CH_4) (Mori et al., 2013c) exchange between terrestrial ecosystems and the atmosphere (Mori et al., 2013d).

Soil of tropical forests is an important source of N_2O, which is a by-product or intermediate product of microbial nitrification and denitrification, respectively (Wrage et al., 2001). Tropical forest soils have also the potential to function as substantial sinks of CH_4 (Potter et al., 1996). CH_4 fluxes are the net result of the simultaneous microbial oxidation of CH_4 by methanotrophs in predominantly aerobic soil zones and microbial production of CH_4 by methanogenesis in predominantly anaerobic soil zones (Le Mer and Roger, 2001).

Recently several studies have reported that P addition reduced N_2O emissions through stimulated plant N uptake (Mori et al., 2013d; Baral et al., 2014; Zhang et al., 2014). Mori et al. (2014) experimentally confirmed this suggestion by showing that P addition reduced N_2O emissions from an *Acacia mangium* plantation, but did not if plant roots were excluded by the trenching method. The authors' understanding is still lacking on the effects of P

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addition on microbial activity, without plant interaction, and accompanying N_2O emissions. Hall and Matson (1999) suggested that a P shortage in tropical soils limits microbial N immobilization, resulting in an increase in N resources for nitrification or denitrification or both. Their report implied that P addition alleviates a P shortage and increases microbial N immobilization, resulting in a reduction in N resources for nitrification or denitrification or both and a reduction in N_2O emissions. However, previous papers have reported completely opposite results, with higher N_2O emission rates in P-added soils than in the non-added control (Mori et al., 2010, 2013a). Alleviation of P-limitation on nitrifying or denitrifying bacteria or both, stimulated whole N cycling in the soil, and improved reductive conditions with stimulated denitrifying activity being the suggested mechanism. However only one soil was used (soil taken from an *Acacia mangium* plantation in South Sumatra, Indonesia), and there are still very few reports testing the effects of P addition on N_2O emissions.

It has been shown that P addition increased (Aerts and Toet, 1997), decreased (Conrad et al., 2000), or had no effects (Lund et al., 2009; Keller et al., 2005) on CH4 fluxes. It was also reported that P application lowered CH_4 fluxes indirectly by stimulating root water uptake (Zhang et al., 2011). Mori et al. (2013b) reported that P addition had no effects on CH_4 fluxes in an *Acacia mangium* plantation, but significantly increased CH_4 uptake when there were no plant interactions. Thus, there are several reports examining the effects of P addition on CH_4 fluxes, but few studies have reported the effects in tropical forest soils, especially tropical tree plantation soils.

The current study conducted another incubation experiment using soils taken from five different tropical, monoculture, tree plantations. The effects of P addition on N_2O and CH_4 fluxes were examined.

Materials and methods

Soil sampling

Soil samples were collected at plantation sites inside the Sakaerat Environment Research Station (SERS) in Nakhon Ratchasima, Thailand (14°30′N, 101°55′E). The climate of the region is classified as tropical savannah (Yamashita et al., 2010). The mean annual temperature was 25.5 °C and annual precipitation was 1407 mm for 2000–2008 (Yamashita et al., 2011). The main soil type is Acrisols (Yamashita et al., 2011). Five forest plantation stands were chosen—*Acacia auriculiformis* (Leguminosae), *Acacia mangium* (Leguminosae), *Eucalyptus camaldulensis* (Myrtaceae), *Hopea odorata* (Dipterocarpaceae), and *Xylia xylocarpa* (Leguminosae). At the beginning of the experiment, each stand was aged 9 yr. Soil samples (0–5 cm depth) were collected from six randomly selected points in each forest stand using 100 mL soil cores. Litter layers were removed before soil sampling. After collection, each soil sample was sieved through a 2 mm sieve. The general physicochemical characteristics of the 0–5 cm soil samples are shown in Table 1. The particle size distribution was determined using the pipette method (Gee and Bauder, 1986). The pH (H_2O) was determined for 1:2.5 water suspensions using a glass electrode (Horiba; Kyoto, Japan). The total C and total N contents were determined using an NC analyzer (JM 1000CN; J-Science Lab Co. Ltd.; Kyoto Japan). The available P contents were determined using the Bray-1 method (Kuo, 1996).

Incubation

Fresh soil samples of 30 g were placed in 223 mL wide-mouth jars for gas sampling, and 5 g samples were placed in 50 mL bottles for chemical analyses (each forest stand had six replications). For each analysis, two subsamples were prepared—one for P addition and the other for the non-P-added control. P was added as KH_2PO_4 solution (100 μg P/g soil, dissolved in distilled water). Non-P-added controls were prepared without P addition in the same manner. After the soil water condition was adjusted to 80% water holding capacity, the samples were incubated at 25 °C in the dark for 48 h. The wide-mouth jars were closed with butyl rubber stoppers equipped with sampling ports, and gas samples were taken at 0 h and 48 h after the closure of the stoppers. Gas concentrations were analyzed using a gas chromatograph (GC-14B; Shimadzu; Kyoto, Japan) equipped with an electron capture detector for N_2O and a flame ionization detector for CH_4. The gas fluxes were calculated from the differences between the gas concentrations at 0 h and 48 h.

Inorganic N and dissolved N were extracted by shaking 5 g of fresh soil with 25 mL of 0.5 M K_2SO_4 for 30 min. The NH_4 and NO_3^- contents were determined using a flow-injection analyzer (AQLA-700-NO; Aqualab; Tokyo, Japan). The dissolved N concentration was analyzed using a total organic carbon analyzer with a total organic nitrogen measurement unit (TOC-V_E/TNM-1; Shimadzu; Kyoto, Japan). The soil microbial biomass N was determined using a chloroform fumigation extraction method (Jenkinson et al., 2004). Fresh soil samples of 5 g were exposed to $CHCl_3$ vapor for 24 h in a vacuum desiccator at 25 °C. After the residual $CHCl_3$ had been removed, the fumigated soils were shaken with 50 mL of 0.5 M K_2SO_4 extractant for 30 min and the dissolved N was extracted. The soil microbial biomass element contents were calculated from the differences of the dissolved N contents between the fumigated and unfumigated samples using a conversion factor of 0.45 (Jenkinson et al., 2004). The soil pH (H_2O) was measured at the end of the incubation period.

Statistical analysis

Statistical analyses were performed using the Excel software (version 2013; Microsoft Corp; Redmond, WA, USA). The level of

Table 1
Physicochemical properties of soils at different plantation sites (data from Mori et al., 2016).

Site[a]	pH (H_2O)	Total C[b] (mg C/g soil)	Total N[b] (mg N/g soil)	Available P[c] (μg P/g soil)	Clay[d] (%)	Silt[d] (%)	Sand[d] (%)
AA	4.9	21.6	2.1	12.1	7.3	21.3	71.5
AM	5.1	12.0	1.4	7.0	3.8	16.0	80.2
EC	5.3	13.5	1.4	12.2	6.4	15.6	78.0
HO	5.0	10.7	1.5	9.9	5.1	17.3	77.5
XX	5.2	11.7	1.4	10.9	3.1	16.5	80.4

[a] AA, *Acacia auriculiformis*; AM, *Acacia mangium*; EC, *Eucalyptus camaldulensis*; and HO, *Hopea odorata*; XX, *Xylia xylocarpa*.
[b] Average of three replications.
[c] Average of six replications.
[d] Average of two replications.

significance was examined using a paired *t*-test assuming normality. The correlation coefficient was obtained by single simple regression analysis. One of the six *Acacia auriculiformis* samples and three of the six *Eucalyptus camaldulensis* samples showed unnaturally high N_2O emissions and so these were excluded from further analyses.

Results and discussion

P addition significantly ($p < 0.05$) increased N_2O emissions from *Xylia xylocarpa* soils, from 1.0 ± 0.16 ng N/g soil to 1.4 ± 0.22 ng N/g soil (Fig. 1A). In other soils, no differences were observed between the P-added soils and non-added controls. P addition had no effects on the CH_4 uptake in all soil samples (Fig. 1B). Both the NH_4^+ and NO_3^- contents did not change by P addition, except for *Xylia xylocarpa* with a tendency for higher NH_4^+ contents in P-added soils ($p < 0.1$, Table 2). In *Xylia xylocarpa* soils, net ammonification rates tended to be higher in P-added soils ($p < 0.1$), but net nitrification rates showed no differences. In *Acacia auriculiformis*, *Acacia mangium*, *Eucalyptus camaldulensis*, and *Hopea odorata*, no differences were observed in the NH_4^+ and NO_3^- contents, net ammonification rates, and net nitrification rates between the control and P-added soils (Table 2). P addition had no effects on the dissolved N and microbial biomass N, except that P addition tended to increase the microbial biomass N contents in *Acacia auriculiformis* soils ($p < 0.1$, Table 3). P addition significantly reduced the soil pH in *Acacia mangium* and *Hopea odorata* soils, but the decrease was very small (Table 3). In *Xylia xylocarpa* soils, N_2O emissions were negatively correlated with net ammonification rates, and the slope was lower in P-added soils (Fig. 2).

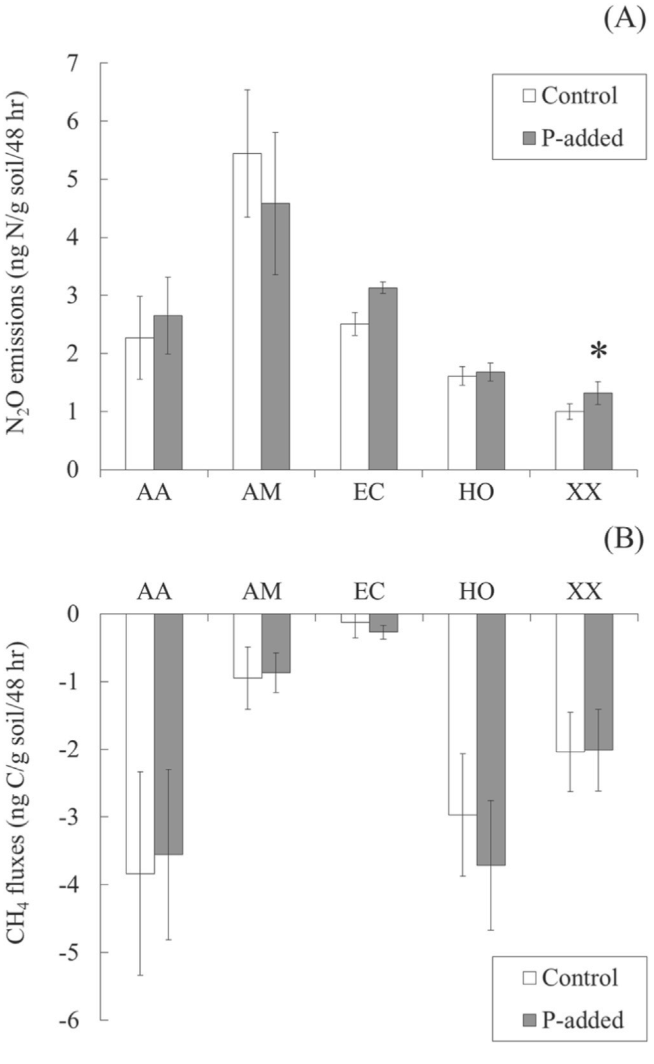

Fig. 1. Effects in different plantations of P addition on: (A) N_2O emissions; (B) CH_4 fluxes. *Above a column indicates significant differences between P-added soil and non-added control determined by paired *t*-test ($p < 0.05$). Error bars show mean ± SE. AA, *Acacia auriculiformis*. AM, *Acacia mangium*. EC, *Eucalyptus camaldulensis*. HO, *Hopea odorata*. XX, *Xylia xylocarpa*.

An increase in the N_2O emissions by P addition was in accordance with reports by Mori et al. (2010; 2013a), where P addition increased the N_2O emissions from an *Acacia mangium* plantation soil. The suggested mechanisms by Mori et al. (2010, 2013a) were: 1) P addition stimulated whole N cycling including N mineralization, providing more N for nitrifying or denitrifying processes or both; 2) P addition directly activated nitrifying or denitrifying bacteria or both by alleviating P limitation; and 3) P addition stimulated O_2 consumption by heterotrophic activities and created a more reductive condition, which was suitable for denitrifying bacteria and stimulated denitrification. The results from the present study suggested that P addition stimulated whole N cycles for two reasons. First, P addition tended to increase the net ammonification rates in *Xylia xylocarpa* soils (Table 2). Second, P addition reduced the slope of the regression line between N_2O emissions and net ammonification rates (Fig. 2). Since net ammonification is the sum of the NH_4^+ production (ammonification) and NH_4^+ consumption (NH_4^+ immobilization and nitrification), the negative correlation between N_2O emissions and net ammonification rates was most probably because of the more-active nitrification in the soils with lower net ammonification rates (larger NH_4^+ consumption by nitrification). A decrease in the slope by P addition indicates higher N_2O emissions in P-added soils at the same net ammonification rates, suggesting that the flow of N was accelerated (both NH_4^+ production and consumption were stimulated) by P addition. Thus, it can be suggested that the stimulated whole N cycles caused higher N_2O emissions in the present study. Although two-way ANOVA using all samples showed that P addition increased CO_2 emissions (Mori et al., 2016), a paired *t*-test using *Xylia xylocarpa* samples alone did not show significant differences between the control and P-added soils. In *Xylia xylocarpa* soils, two of six replicates showed a decrease in CO_2 emissions after P-addition, and these two soils showed the largest increase of N_2O after P addition. Thus we concluded that the promoted reductive condition was not the reason for the stimulated N_2O emissions by P addition in the present study.

P addition had no effects on N_2O emissions from *Acacia auriculiformis*, *Acacia mangium*, *Eucalyptus camaldulensis*, and *Hopea odorata* soils (Fig. 1A). Although insufficient data were available for a complete explanation, several mechanisms can be suggested. First, P addition may have improved the respiratory efficiency. Since nutrient shortage causes a lower efficiency of microbial respiration (López-Urrutia and Morán, 2007; Sinsabaugh et al., 2013), nutrient supply could increase respiratory efficiency conversely. P addition possibly improved nitrifying or denitrifying respiratory efficiency or both, reducing the emissions of N_2O, as a by-product and an intermediate of nitrifying and denitrifying respiration, and possibly offset the increased N_2O emissions deriving from the stimulated N cycling. Another suggested mechanism is that P addition stimulated mainly N immobilization, not nitrification or denitrification or both (Hall and Matson, 1999). Although P addition did not increase the microbial biomass N contents (Table 2), P addition may have also stimulated microbial lysis and transformed the microbial biomass N into organic N. In the present study, no effects of P addition on N_2O emissions were observed in *Acacia mangium* soils, while previous studies showed higher N_2O emissions from P-added

Table 2
Soil N contents for different plantation sites and treatments.

Site[a]	Treatment	NH_4^+ (µg N/g soil)		NO_3^- (µg N/g soil)		Net ammonification rate (µg N/g soil/d)		Net nitrification rate (µgvN/g soil/d)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
AA	Control	17.6	1.2	21.6	1.4	1.1	0.8	2.2	0.4
	P-added	16.6	1.1	21.3	1.8	0.6	1.1	2.1	0.6
AM	Control	15.9	0.8	17.6	2.6	0.3	0.5	1.9	0.4
	P-added	16.7	0.9	17.1	2.2	0.7	0.9	2.1	0.2
EC	Control	18.3	2.0	12.4	2.6	0.3	0.4	0.7	0.0
	P-added	17.9	2.3	12.7	2.6	0.1	0.7	0.8	0.1
HO	Control	16.9	2.1	12.8	2.1	1.8	0.3	0.5	0.2
	P-added	16.0	2.6	12.7	2.0	1.4	0.4	0.5	0.2
XX	Control	13.6	0.6	14.1	0.5	−0.6	0.5	1.5	0.3
	P-added	15.2[b]	1.0	14.1	0.5	0.2[b]	0.2	1.5	0.2

[a] AA, *Acacia auriculiformis*; AM, *Acacia mangium*; EC, *Eucalyptus camaldulensis*; HO, *Hopea odorata*; XX, *Xylia xylocarpa*.

[b] Indicates the tendency of differences between P-added soils and non-added controls ($p < 0.1$, paired *t*-test).

Table 3
Dissolved nitrogen, microbial biomass nitrogen, and soil pH for different plantation sites and treatments.

Site[a]	Treatment	DN[b] (µg N/g soil)		MBN[c] (µg N/g soil)		Soil pH	
		Aver.	SE	Aver.	SE	Aver.	SE
AA	Control	39.9	1.6	50.6	6.3	5.02	0.1
	P-added	39.4	1.7	56.1[e]	6.2	4.99	0.1
AM	Control	31.9	2.0	36.2	4.9	4.79	0.1
	P-added	31.7	1.2	31.2	3.2	4.76[d]	0.1
EC	Control	29.1	1.2	38.8	5.1	4.97	0.1
	P-added	29.9	1.0	37.3	5.6	4.93	0.1
HO	Control	36.0	1.7	34.3	2.9	4.91	0.1
	P-added	38.1	2.2	26.2	5.1	4.88[d]	0.1
XX	Control	27.4	1.5	34.9	1.5	5.29	0.2
	P-added	28.0	1.8	34.2	2.5	5.27	0.1

[a] AA, *Acacia auriculiformis*; AM, *Acacia mangium*; EC, *Eucalyptus camaldulensis*; and HO, *Hopea odorata*; XX, *Xylia xylocarpa*.

[b] DN, dissolved nitrogen.

[c] MBN, soil microbial biomass nitrogen.

[d] Indicates the significant differences between P-added soils and non-added controls ($p < 0.05$, paired *t*-test).

[e] Indicates the tendency of differences between P-added soils and non-added controls ($p < 0.1$, paired *t*-test).

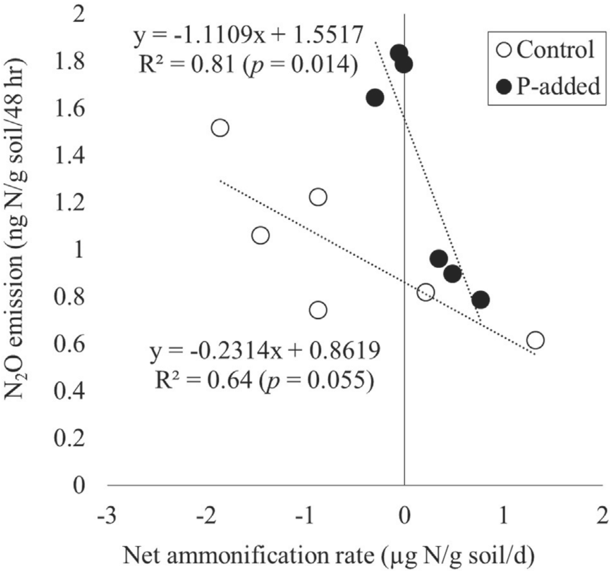

Fig. 2. Relationship between N_2O emissions and net ammonification rates in *Xylia xylocarpa* soils. In other soils, no significant relationships were observed. Dotted lines show equations. R^2 = coefficient of determination.

soil taken from an *Acacia mangium* plantation in South Sumatra, Indonesia (Mori et al., 2010, 2013a), suggesting that the effects of P addition on N_2O emissions are different even among soils with the same trees planted.

In the present study, P addition had no effects on CH_4 uptakes. In previous reports, P application directly stimulated methanogenesis activities (Archer, 1985) or methanotrophic activities (Zhang et al., 2011; Song et al., 2012), or inhibited methanogenesis activities (Conrad et al., 2000; Song et al., 2012). It was possible that P addition stimulated both methanogenesis and methanotrophic activities, with each offsetting the other. It is suggested that the effects of P addition on N_2O and CH_4 fluxes in the current study site were not significant, at least under laboratory conditions.

Conflict of interest

None declared.

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