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Original Article

Contrastive effects of inorganic phosphorus addition on soil microbial respiration and microbial biomass in tropical monoculture tree plantation soils in Thailand

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ABSTRACT

An incubation experiment was conducted to test the effects of inorganic phosphorus (P) addition on soil microbial activities in tropical monoculture tree plantation soils. The soils taken from an experimental tree plantation site in Nakhon Ratchasima, Thailand were incubated for 48 h with and without adding 100 μg of P (KH_2PO_4) per gram soil after adjusting the water holding capacity to 80%. During the incubation period, the microbial biomass carbon (MBC) contents determined using the chloroform fumigation extraction method decreased and P addition stimulated the decreased rate significantly. On the other hand, the P addition increased the dissolved organic carbon (DOC) contents and CO_2 emissions. The study suggested that P addition had changed soil microbial activities, possibly including a soil microbial community change. Furthermore, the study showed that the stimulated soil respiration by P addition is not necessarily accompanied by increased MBC. The assessment of the effects of P limitation on soil microbial activities should measure at least the effects of P addition on both soil respiration and MBC, possibly combined with soil microbial community analyses.

Introduction

Clarifying limiting nutrient in ecosystems has been one of the most important questions in the field of ecosystem ecology. It is believed that phosphorus (P) availability limits tropical forest ecosystems, including primary production (Elser et al., 2007; Vitousek and Farrington, 1997) and soil microbial activities (Cleveland et al., 2002; Mori et al., 2013) because most tropical forests are established on highly-weathered soils, where most of the remaining P is occluded on iron and aluminum oxides (Miller et al., 2001). Whether P availability limits soil microbial activities or not has been tested by observing the effects of P addition on soil microbial respiration or soil microbial biomass growth. Many researchers conducted incubation experiments and reported that P addition stimulated soil microbial respiration, suggesting the microbial activities in the soils used for their studies were limited by P availability (Cleveland et al., 2002; Ilstedt et al., 2003; Mori et al., 2010). On the other hand some researchers have reported that P addition increased the amount of microbial biomass carbon (MBC) and stated that the microbial activities in the soil were limited by P availability (Turner and Wright, 2014).

However the responses of soil respiration and microbial biomass are not necessarily linked to each other. For example, the addition of insufficient nutrients like P would improve respiratory efficiency, because nutrient shortages drive microbes to require more energy to maintain their activities and cause a lower efficiency of respiration (López-Urrutia and Morán, 2007; Schimel and Weintraub, 2003;

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Table 1
Physico-chemical properties of the top 5 cm of soil at each of the five experimental sites.

Site[a]	pH (H_2O)	Total C (mg C per g soil)	Total N (mg N per g soil)	Available P[b] (μg P per g soil)	Clay (%)	Silt (%)	Sand (%)
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*; HO, *Hopea odorata*; XX, *Xylia xylocarpa*.
[b] Available P was extracted using the Bray-1 method (Kuo, 1996).

Sinsabaugh et al., 2013). P addition, therefore, may rather reduce soil microbial respiration (energy required to maintain the activities of soil microbes) even though microbial activities are limited by P availability. P addition may also shift the soil microbial community from being oligotrophic-like to copiotrophic-like, resulting in an uncoupled response of soil respiration and microbial biomass to P addition. However few studies have shown the effects of P addition on the soil microbial biomass and respiration simultaneously. The current study tested the effects of P addition on soil microbial respiration and soil MBC simultaneously by conducting an incubation experiment using tropical tree plantation soils.

Materials and methods

Site description

The study site was located in plantations within the Sakaerat Environment Research Station in Nakhon Ratchasima, Thailand (14°30′N, 101°55′E). The station was established by the Thai government in 1967 and was declared a United Nations Educational, Scientific, and Cultural Organization biosphere reserve in 1977 (Yamashita et al., 2010). The climate of the region is classified as tropical savannah (Yamashita et al., 2010). The mean annual temperature and annual precipitation were 25.5 °C and 1407 mm, respectively, from 2000 to 2008 (Yamashita et al., 2011). The main soil type is Acrisols (Yamashita et al., 2011). Five forest stands were chosen: *Acacia auriculiformis* (AA, Leguminosae), *Acacia mangium* (AM, Leguminosae), *Xylia xylocarpa* (XX, Leguminosae), *Eucalyptus camaldulensis* (EC, Myrtaceae) and *Hopea odorata* (HO, Dipterocarpaceae). Each stand was aged 9 yr at the beginning of the experiment. The general physico-chemical characteristics of the top 5 cm of soil are shown in Table 1. 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 after being finely ground (JM 1000CN; J-Science Lab Co. Ltd.; Kyoto, Japan). Available P values (Bray-1 P, Table 1) in the present study were relatively low compared to other highly-weathered soils (see the review of Johnson et al., 2003).

Soil sampling and incubation

In August 2014, soil samples at depths of 0–5 cm were randomly collected from 6 points in each forest stand using 100 mL soil cores. The litter layer was removed before the soil was sampled. Each soil sample was sieved through a 2 mm sieve. Samples of 30 g fresh soil were placed in 223 mL wide-mouth jars for gas sampling, and 5 g samples were placed in 50 mL bottles for dissolved organic C (DOC) and 5 g in 50 mL glass bottle for soil microbial biomass carbon (MBC) analysis (six replicates per forest stand).

Two subsamples were prepared for each analysis—one for P addition and the other for the control. P was added as KH_2PO_4 solution (100 μg P per gram dry soil, dissolved in distilled water). Controls were prepared without P addition in the same manner. 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 inserting the stoppers. The CO_2 concentration was analyzed using a gas chromatograph (GC-14B; Shimadzu; Kyoto, Japan) equipped with a thermal conductivity detector, using He as the carrier gas. The column, injector and detector temperatures were kept at 60 °C, 60 °C and 100 °C, respectively. The CO_2 emission rates were calculated from the differences between gas concentration at 0 h and 48 h.

Dissolved organic C (DOC) was extracted at the beginning and the end of the incubation by shaking 5 g soil with 50 mL extractant

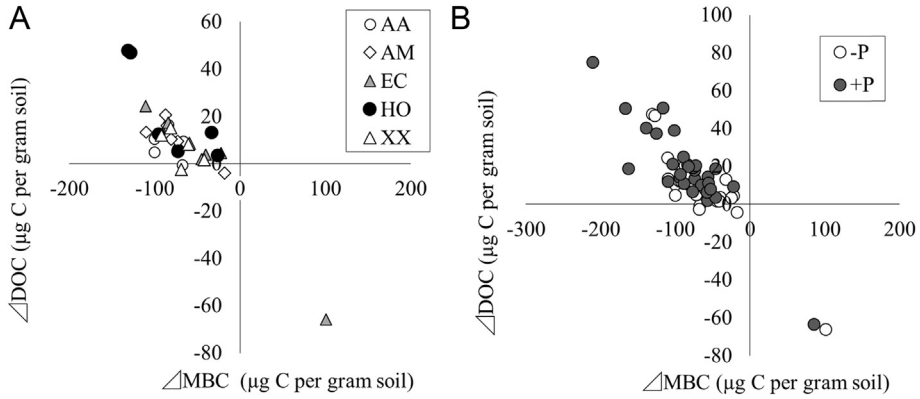

Fig. 1. Relationship between ΔMBC (differences between microbial biomass carbon (MBC) before and after the incubation) and ΔDOC (differences between dissolved organic carbon (DOC) before and after the incubation) in: (A) control soils; (B) P-added soils. AA, *Acacia auriculiformis*; AM, *Acacia mangium*; EC, *Eucalyptus camaldulensis*; HO, *Hopea odorata*; XX, *Xylia xylocarpa*.

for 30 min. The supernatants were filtered and refrigerated until analysis. The DOC was analyzed using a total organic carbon analyzer (TOC-V_E; Shimadzu; Kyoto, Japan). The soil microbial biomass C (MBC) was determined using a chloroform fumigation extraction method (Vance and Jenkinson, 1987) at the beginning and the end of incubation. Samples of 5 g fresh soil were exposed to $CHCl_3$ vapor for 24 h in a vacuum desiccator at 25 °C. After 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 soluble C was extracted. Equivalent portions of unfumigated soils were also extracted. Soluble C was determined using the total organic carbon analyzer. The MBC was calculated from the differences between the fumigated and unfumigated samples using a conversion factor of 0.45 (Jenkinson et al., 2004). Soil pH (H_2O) was measured at the end of the incubation period.

Statistical analysis

The level of significance was examined by a repeated two-way ANOVA (tree species and P addition, P addition was treated as a repeated item) or a paired t test after confirming the normality of each dataset using the Kolmogorov–Smirnov test. Since some data were not normally distributed, the Wilcoxon signed-ranks test was used to compare between controlled and P-added measurements. Spearman's correlations were used to assess the relationship among parameters. Each statistical analysis was performed using the Excel software package (version 2013; Microsoft Corp.; Redmond, WA, USA) with statistical add-in software (Excel statistics version 2015 and its updates; Social Survey Research Information Co., Ltd.; Tokyo, Japan).

Results

During the incubation experiment, most of the soil showed a decrease in the MBC content; most of the soil showed negative values for ΔMBC (differences between MBC before and after the incubation, see Fig. 1A). The decrease in the MBC contents was significantly (coefficient of determination (R^2) = 0.78, $p < 0.001$, Spearman's correlations, analyzing all five tree species) correlated with an increase in DOC (Fig. 1A). P addition stimulated a decrease in MBC and an increase in DOC (Fig. 1B); ΔMBC decreased significantly ($p < 0.05$, Wilcoxon signed-ranks test) by P addition and ΔDOC (differences between DOC before and after the incubation) increased significantly ($p < 0.001$, Wilcoxon signed-ranks test) by P addition.

P addition significantly increased CO_2 emissions without interaction with tree species (Fig. 2A). On the other hand, P addition and tree species influenced MBC (Fig. 2B) and DOC (Fig. 2C) with significant ($p < 0.01$ and $p < 0.001$, respectively) interactions. Simple main effect analysis suggested that P addition reduced the MBC contents in the HO stand ($p < 0.05$, Fig. 2B). The DOC contents increased significantly (simple main effect analysis, $p < 0.05$, Fig. 2C) by P addition in the EC and HO stands. Although P addition significantly ($p < 0.05$) reduced the pH(H_2O) values, the decreases were very small (Table 2).

Discussion

Since additional C was not applied in the study, microbial activity may have been limited by C availability, which may have caused the decrease in MBC during incubation. However, the initial DOC contents had no co-relationship with changes in MBC (ΔMBC) during the incubation experiment (coefficient of determination (R^2) = 0.037, $p = 0.76$, Spearman's correlations), implying that other factors may have caused the decrease. Birge et al. (2015) observed a decrease in the soil microbial biomass during 42 d incubation despite C addition. They suggested that sample processing and the controlled incubation setting may have changed the variation and quantity of microhabitats in the soil. The decrease in MBC was

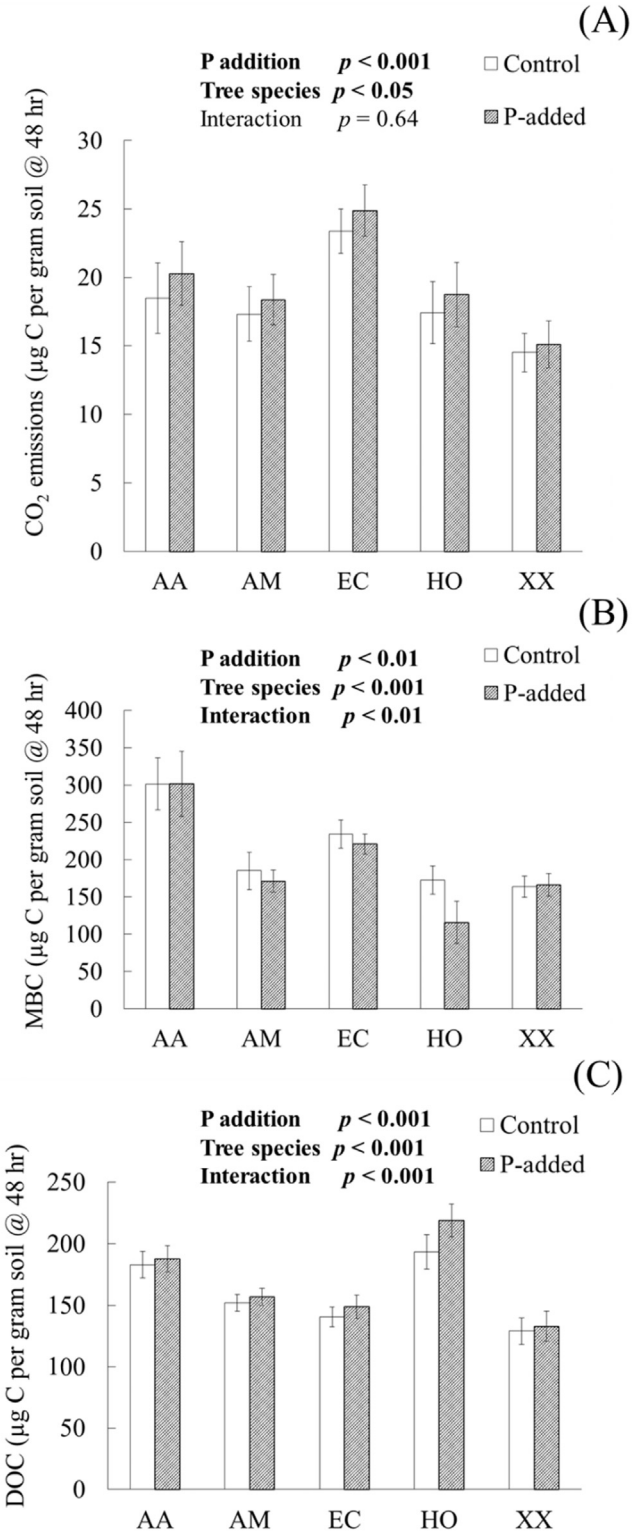

Fig. 2. Effects of P addition on: (A) CO_2 emissions; (B) microbial biomass carbon (MBC) contents; (C) dissolved organic carbon (DOC) contents. Significant levels of repeated two-way ANOVA are shown on the top of the figures. Values were measured at the end of the incubation experiment; Error bars show mean ± SE; AA, *Acacia auriculiformis*; AM, *Acacia mangium*; EC, *Eucalyptus camaldulensis*; HO, *Hopea odorata*; XX, *Xylia xylocarpa*.

Table 2
Changes in pH (H_2O) during the incubation.

Site[a]	Treatment	pH (H_2O) after
AA	Control	4.98
	P-added	4.96
AM	Control	4.79
	P-added	4.76
EC	Control	5.15
	P-added	5.11
HO	Control	4.91
	P-added	4.88
XX	Control	5.24
	P-added	5.27
		p values
Two-way repeated ANOVA	P-addition	0.03
	Tree species	0.02
	Interactions	0.98

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

correlated with an increase in the DOC contents (Fig. 1), probably because lysis of soil microbes provided DOC into the soils (Wu and Brookes, 2005).

P addition significantly stimulated a decrease in MBC, while significantly stimulating an increase in DOC and CO_2 emissions. This may have been because P addition accelerated soil microbial activities and the commencement of lysis. Another possible reason was that P addition changed the microbial community from being oligotrophic-like to copiotrophic-like, with quicker growth and higher respiration. Soil microbial community analysis using phospholipid fatty acid analysis or 16S rRNA analysis is necessary to clarify the whole mechanism. It could be that P addition changed soil microbial activities, possibly including a soil microbial community change. Further studies are necessary to fully understand the effects of P addition on soil microbial activities and the effects on other soil properties. The current study has shown that stimulated soil respiration by P addition is not necessarily accompanied by increased MBC. The effects of P addition on soil respiration and MBC showed completely opposite responses, with stimulated soil respiration and reduced MBC. The current study suggested that the attempt to clarify whether or not P availability limits soil microbial activities by measuring only one parameter (soil respiration or MBC) may be misguided. For accessing P-limitation on soil microbial activities, the effects should be measured of at least P addition on both soil respiration and MBC, possibly combined with soil microbial community analyses.

The afforestation of degraded lands along with intensified management practices and fertilizer use (Schulze et al., 2000; Six et al., 2002) has an increasingly important role. The current study may also suggest that P fertilization in tropical tree plantations may substantially change microbial activities. Since microbial activities play a key role in terrestrial nutrient dynamics through their own turnover and the mineralization of other organic nutrients (Arunachalam and Arunachalam, 2000; Paul and Clark, 1997), the changes in microbial activity caused by P addition would affect the soil nutrient (other than P) dynamics in tropical plantations.

Conflict of interest

Authors declare that they have no conflict of interest.

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References

Arunachalam, A., Arunachalam, K., 2000. Influence of gap size and soil properties on microbial biomass in a subtropical humid forest of north-east India. Plant Soil 223, 187–195.
Birge, H.E., Conant, R.T., Follett, R.F., Haddix, M.L., Morris, S.J., Snapp, S.S., Wallenstein, M.D., Paul, E.A., 2015. Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations. Soil Biol. Biochem. 81, 304–310.
Cleveland, C.C., Townsend, A.R., Schmidt, S.K., 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems 5, 0680–0691.
Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.
Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods, second ed. Soil Science Society of America, Madison, WI, pp. 383–411.
Ilstedt, U., Giesler, R., Nordgren, A., Malmer, A., 2003. Changes in soil chemical and microbial properties after a wildfire in a tropical rainforest in Sabah, Malaysia. Soil Biol. Biochem. 35, 1071–1078.
Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biol. Biochem. 36, 5–7.
Johnson, A.H., Frizano, J., Vann, D.R., 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. Oecologia 135, 487–499.
Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Analysis. Part 3, pp. 869–919. Chemical Methods. Soil Science Society of America, Madison, WI, USA.
López-Urrutia, Á., Morán, X.A.G., 2007. Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. Ecology 88, 817–822.
Miller, A.J., Schuur, E.A.G., Chadwick, O.A., 2001. Redox control of phosphorus pools in Hawaiian montane forest soils. Geoderma 102, 219–237.
Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J., Hardjono, A., 2013. Effects of phosphorus and nitrogen addition on heterotrophic respiration in an *Acacia mangium* plantation soil in South Sumatra, Indonesia. Tropics 22, 83–87.
Mori, T., Ohta, S., Konda, R., Ishizuka, S., Wicaksono, A., 2010. Phosphorus limitation on CO_2, N_2O, and NO emissions from a tropical humid forest soil of South Sumatra, Indonesia. In: Proceeding of International Conference of Environmental Engineering and Application (ICEEA), 2010, pp. 18–21.
Paul, E.A., Clark, F.E., 1997. Soil Microbiology and Biochemistry, second ed. Academic Press, New York, New York, USA.
Schimel, J., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35, 549–563.
Schulze, D.E., Wirth, C., Heimann, M., 2000. Climate change. Managing forests after Kyoto. Science 289, 2058–2059.
Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecol. Lett. 16, 930–939.
Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul, E.A., Paustian, K., 2002. Measuring and understanding carbon storage in afforested soils by physical fractionation. Soil Sci. Soc. Am. J. 66, 1981.
Turner, B.L., Wright, S.J., 2014. The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. Biogeochemistry 117, 115–130.
Vance, E.D., Jenkinson, D.S., 1987. Microbial biomass measurement in forest soils: the use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biol. Biochem. 19, 697–702.
Vitousek, P.M., Farrington, H., 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37, 63–75.
Wu, J., Brookes, P.C., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol. Biochem. 37, 507–515.
Yamashita, N., Ohta, S., Sase, H., Kievuttinon, B., Luangjame, J., Vsaratana, T., Grivait, H., 2011. Seasonal changes in multi-scale spatial structure of soil pH and related parameters along a tropical dry evergreen forest slope. Geoderma 165, 31–39.
Yamashita, N., Ohta, S., Sase, H., Luangjame, J., Visaratana, T., Kievuttinon, B., Garivait, H., Kanzaki, M., 2010. Seasonal and spatial variation of nitrogen dynamics in the litter and surface soil layers on a tropical dry evergreen forest slope. For. Ecol. Manag. 259, 1502–1512.