Phosphorus addition reduced microbial respiration during the decomposition of *Acacia mangium* litter in South Sumatra, Indonesia

Taiki Mori^{1*}, Shigehiro Ishizuka², Ryota Konda¹, Agus Wicaksono³, Joko Heriyanto³, Arisman Hardjono³ and Seiichi Ohta⁴

¹ Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

² Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

³ PT. Musi Hutan Persada, Muara Enim 31172, South Sumatra, Indonesia

⁴ Japan International Forestry Promotion and Cooperation Center, Koraku 1-7-12, Bunkyo-ku, Tokyo 112-0004, Japan

* Corresponding author: taikimori7@gmail.com

ABSTRACT Assessing the effects of nutrient addition on organic matter decomposition in tropical tree plantations is essentially important because of the increasing importance of the afforestation along with the increases in the intensified management practices and fertilizer use. We tested the effects of phosphorus (P) addition on microbial respiration during the decomposition of *Acacia mangium* litters and leaves by using an incubation experiment. Following hypotheses were examined: (i) P addition reduces microbial respiration during the litter decomposition if labile carbon (C) and nitrogen (N) are little; while (ii) the effect is not clear in the fresh leaf decomposition due to the large amount of labile C and N. P addition significantly reduced the rates of microbial respiration during the litter decomposition, with the following possible mechanisms; (i) decrease in C or N investment in phosphatase due to the reduced P requirement makes microbes decompose fewer litters to get C or N, (ii) P addition improved microbial respiratory efficiency and reduced energy required to maintain microbial activity. Meanwhile in high-quality leaf, such decrease in microbial respiration rates during the decomposition by P addition did not occur. We suggested that P fertilization in fast-growing tree plantations in tropics may reduce organic matter decomposition. Since the present study is a short-term incubation study, longer studies or field experiments are required to fully understand the effects of P addition on the organic matter decomposition in P-limited tropical forests.

Key words: *Acacia mangium*, decomposition, nitrogen mining, phosphorus, tropics

INTRODUCTION

Increase in anthropogenic nutrient loading to ecosystems (ex. Galloway et al. 2008) has motivated researchers to study effects of nutrient addition to ecosystems including organic matter decomposition (Knorr et al. 2005). In tropical Asia, tree plantations of fast-growing species such as *Acacia mangium* are expanding (FAO et al. 2006) for industrial pulp and timber production, C sequestration (Zhang et al. 2012), and greening the land (Wang et al. 2010). Due to the increasing importance of the afforestation along with intensified management practices and fertilizer use (Six et al. 2002), assessing the effects of nutrient addition on organic matter decomposition in tropical tree plantations is essentially important.

According to the traditional theory, organic matter decomposition is controlled by limiting nutrients as nitrogen (N) or phosphorus (P); hence the addition of the limiting nutrients stimulates the decomposition (Mack et al. 2004,

Vitousek & Howarth 1991). Nevertheless many studies have reported that N addition had negative effects on organic matter decomposition (Janssens et al. 2010, Ramirez et al. 2012, Treseder 2008) especially at the later stage of decomposition. This phenomenon could be explained well by microbial N mining theory (Moorhead & Sinsabaugh 2006) as follows; some microbes use labile C to decompose recalcitrant organic matter in order to get N, and N addition makes microbes no longer mine recalcitrant organic matter to get N. In contrast, P addition has been thought to stimulate organic matter decomposition (ex. Cleveland et al. 2002; Ilstedt et al. 2003; Mori et al. 2013a, 2013b), and P mining does not occur. This is partly because of McGill and Cole (1981)'s conceptual model that pointed out N mineralization is coupled to respiration of C due to the bounding of C and N together in the organic matter, while P is mineralized by phosphatase independently of C because most phosphorus in organic matter is bound in phosphate esters. Thus this model suggests that P acquisition is not accompanied

by recalcitrant C mineralization as N acquisition is, thereby P addition would not suppress the decomposition (Craine et al. 2007).

However, since microbes need C and N for synthesizing phosphatase, microbes may decompose organic matter (including N mining) for getting C or N to synthesize phosphatase if labile C and N are insufficient. Thus, since P acquisition is accompanied by organic matter decomposition by indirect way, P addition may also reduce organic matter decomposition as N addition does. On the other hand, P addition may stimulate microbial activity and respiration in the condition with enough labile C and N as observed in previous studies where labile C was added together with P (Cleveland et al. 2002, Ilstedt et al. 2003). Nutrient addition may also change microbial community from more-oligotrophic one to more-copiotrophic one (Ramirez et al. 2012), with increasing labile fraction decomposition and reducing recalcitrant fraction decomposition (Fierer et al. 2007). Since nutrient shortage makes microbes require more energy to maintain their activities (Sinsabaugh et al. 2013), increase in respiratory efficiency after nutrient addition may also occur, which may reduce the rates of organic matter decomposition.

Thus P addition may not always stimulate organic matter decomposition, rather may reduce. However effects of P addition on organic matter decomposition were rarely tested in P-limited tropical forests. Here we tested the following hypotheses using litter and fresh leaf samples taken from an *Acacia mangium* plantation in South Sumatra, Indonesia: (i) P addition reduces microbial respiration during the litter decomposition if labile C and N are little; while (ii) the effect are not clear in the fresh leaf decomposition due to the large amount of labile C and N. For testing the two contrasting hypotheses, we did not provide additional C and N.

MATERIALS AND METHODS

Sampling

Sampling site is located at an *Acacia mangium* plantation in South Sumatra Province, Indonesia (3°47' S, 103°55' E). This region is humid tropical rain forest with an annual precipitation of 2750 mm and a mean annual temperature of 27.3 °C (Hardjono et al. 2005). The period from April to September is relatively dry season and October to May is wetter season, although there is no clear distinction. The soils in the area are Acrisols with Tertiary sedimentary rock as the parent material. In September 2007, we took litter layer samples from six different places in three acacia

stands. In these acacia stands, the first rotation (8 years duration) had been harvested in July 2007. We collected every fraction of Oi (L) layer, Oe (F) layer, and Oa (H) layer. Only few Oi (L) layer was observed in the litter layer, partly because the sampling was conducted two months after the harvesting. Details of the study area are reported by Mori et al. (2013b). At the beginning of February 2008, acacia seedlings were planted in the stands. In March 2009, leaves were collected from 24 trees in the three 1-yr old acacia stands (in total 72 trees). Three pieces of fresh leaves were taken from each tree (in total 216 pieces). Samples were mixed and smashed (<2.5 mm) after air-dried. The sample was kept until the incubation study. We also took another litter layer in December 2011, for inoculating microbes at the start of the incubation. The litter layer for inoculation was kept at 4 °C before the start of the incubation. Table 1 shows the chemical properties of the used samples. Total C and N were determined by an NC analyzer after being finely ground (JM1000CN; J-Science Lab Co., Ltd., Kyoto, Japan). Total P (TP) content was colorimetrically determined by the molybdenum blue method after samples were finely ground and digested by H₂SO₄ and H₂O₂ (Kjeldahl method). Soluble C and N were extracted by 0.5 M K₂SO₄ (Rennert et al. 2007), and analyzed using a total organic carbon analyzer with a total organic nitrogen measurement unit (TOC-V_{CSH}/TNM-1, SHIMADZU, Kyoto, Japan). Higher amount of total P, soluble C and N, and lower CP and NP ratios indicate that leaf sample has higher amount of labile C, N, and P than litter sample (Table 1).

Incubation

Leaf or litter layer samples equivalent to 4 g were incubated for 75 days in 223 ml wide mouth jars in 4 replications. Five g litter sample was shaken with 30 ml distilled water for 18 h, and 0.2 ml of the supernatants was added to each sample as microbial inoculant. P (Ca(H₂PO₄)₂; 1 mg P g leaf·litter⁻¹) dissolved in distilled water was added. We confirmed that Ca(H₂PO₄)₂ addition does not change pH of fresh leaf sample significantly (Ca(H₂PO₄)₂ equivalent to 2 mg P g leaf⁻¹ changed fresh leaf pH from 5.3 to 5.2), by using another leaf samples (taken on December 2008). The fresh leaf pH was determined in a 1:2.5 leaf to distilled water ratio. We also prepared control samples without P addition in the same manner. Water content was adjusted to 70 % (approximately same condition as the litter layer in wetter season).

Table 1. Chemical properties of used samples.

	Total C mg C g sample ⁻¹	Total N mg N g sample ⁻¹	Total P μg P g sample ⁻¹	CN ratio	CP ratio	NP ratio	Soluble C mg C g sample ⁻¹	Soluble N mg N g sample ⁻¹
Litter layer	525	16.0	26	32.8	20222	616	3.1	0.28
Leaf	527	23.5	156	22.4	3374	150	33.9	0.79

Gas and chemical analysis

Carbon dioxide (CO₂) fluxes were measured 12 times during the incubation period; 0, 2, 8, 16, 24, 72, 120, 216, 312, 408, 600, 1080, and 1800 h after the beginning of the incubation. Fifteen ml of gas samples were collected at 0 and 30 min after closing the jars with a butyl rubber stoppers equipped with the sampling port. The CO₂ concentration was analyzed with gas chromatograph (GC-14B, SHIMADZU, Kyoto, Japan). The emission rates of these gases were calculated from the differences of gas concentration at 0 and 30 min. At the end of the incubation experiment, we determined soluble C and N, microbial C (MBC) and N (MBN). Soluble C and N were extracted by 0.5 M K₂SO₄ (Rennert *et al.* 2007), and analyzed using a total organic carbon analyzer with a total organic nitrogen measurement unit (TOC-V_E/TNM-1, SHIMADZU, Kyoto, Japan). MBC and MBN were determined by a chloroform fumigation extraction method (Vance *et al.* 1987). One g of leaf or litter samples were weighed into glass jars and exposed to CHCl₃ vapor for 24 h in a vacuum desiccator at room temperature. After residual CHCl₃ was removed, fumigated samples were shaken with 5 mL of 0.5 M K₂SO₄ for 30 min, and soluble C or N was extracted. Equivalent portions of unfumigated samples were also extracted. Microbial biomass element content was calculated as the difference between the fumigated and unfumigated samples using conversion factors of 0.45 (Jenkinson *et al.* 2004).

Calculation and data analysis

The level of significance was examined by a paired t-test after confirming the normality of each data by using Kolmogorov-Smirnov test. Some data sets not normally distributing were log-transformed prior to statistical analysis. For some data sets not following the normal or log-normal distribution, we adopted non-parametric Mann-Whitney's U test. Statistical analyses were performed by Excel 2010 with Statcel3.

RESULTS AND DISCUSSION

P addition clearly reduced the microbial respiration during the decomposition of litter layer samples (Fig. 1a), supporting our hypotheses. Cumulative CO₂ emissions also decreased from 109.1 ± 12.0 to 62.7 ± 2.7 (mg C g litter⁻¹) significantly, suggesting that P addition reduced the litter decomposition. Reduced soluble C and N contents (Table 2) by P addition in litter layer sample also suggested that the litter decomposition was suppressed by P addition. Although we did not determine the enzymatic activity, it is possible that P addition reduced decomposition conducted to get C or N for synthesizing phosphatase. Turner *et al.* (2014) reported that P addition reduced N-mineralizing enzyme, discussing that P addition reduced investment in N-rich phosphatase enzymes, reducing investment in N-acquiring enzymes. Another mechanism is also applicable; microbial respiration efficiency was improved by supplying P. Nutrient supply would increase respiratory efficiency because nutrient shortage makes microbes require more energy maintaining their activities and causes lower efficiency of respiration (Lopez-Urrutia & Moran 2007, Sinsabaugh *et al.*, 2013). Relieving P-limitation for decomposing-microbial activity might have improved respiratory efficiency, although we could not confirm this suggestion because we determined MBC and MBN only at the end of the experiment and they did not change by P addition (Table 1). Some previous studies reported P addition reduced soil respiration, although authors wrote the negative effect of P addition on CO₂ emissions was "a puzzle" (Ouyang *et al.* 2008, Changhui *et al.* 2014). Mechanisms suggested in the present study might explain the phenomenon. The present study was conducted under severely C and N limited condition without no additional C and N. In the field, there are continuous C and N input through root exudate, fresh litter supply, and rainfall, and these additional C and N might make the phenomena observed in the present study not clear. However we suggested that P addition reduces organic matter decomposition at least in a certain condition with severe C and N limitation.

On the other hand, such decrease in decomposition did not occur in leaf samples, rather P addition stimulated the

Fig. 1. Effects of P addition on the decomposition of (a) litter and (b) leaf samples. § indicates significantly higher CO₂ emissions in P-added sample ($P < 0.05$). * indicates significantly higher CO₂ emissions in control sample ($P < 0.05$). Error bars indicate standard errors of replications.

Table 2. Soluble C and N, microbial C and N at the end of the incubation experiment.

Sample	treatment	Soluble C ($\mu\text{g C g sample}^{-1}$)		Soluble N ($\mu\text{g N g sample}^{-1}$)		MBC ($\mu\text{g C g sample}^{-1}$)		MBN ($\mu\text{g N g sample}^{-1}$)	
		aver.	SE	aver.	SE	aver.	SE	aver.	SE
Litter layer	Control	585	27	149	6	570	112	106	28
	P-added	420	32	71	6	492	20	88	2
Leaf	Control	429	38	43	20	967	166	105	11
	P-added	378	22	68	49	765	10	92	18

MBC, microbial biomass C. MBN, microbial biomass N. ** $P < 0.01$, *** $P < 0.001$. SE, standard error.

microbial respiration at the beginning of the decomposition (Fig. 1b). Leaf samples with higher quality than litter layer samples (Table 1) probably provided enough labile C, N, and P for microbes, making microbes not mining P as the following two possible mechanisms; (i) microbes did not have to mine P because of enough labile P, (ii) microbes did not have to get C or N for synthesizing phosphatase from leaf decomposing. Instead, P addition stimulated microbial activities as most of the previous studies reported (Hobbie & Vitousek 2000, Cleveland et al. 2002, 2006). Phosphorus addition may have also changed microbial community from more-oligotrophic one to more-copiotrophic one (Ramirez et al. 2012). Since copiotrophs have less ability to decompose recalcitrant organic matter but decompose labile C more quickly (Fierer et al. 2007), the microbial community shift would have stimulated the decomposition of fresh leaf samples (Fig. 1b) with large amount of labile C (Table 1), while not the decomposition of litter samples (Fig. 1a). At 216 h after the start of the incubation, P-added samples showed lower CO₂ emissions (Fig. 1b), possibly because labile C was depleted quickly.

Thus we demonstrated P addition reduced microbial respiration during the decomposition of *Acacia mangium* litters (relatively recalcitrant organic matter), but not during the decomposition of fresh leaf samples (fresh organic matter). We suggested that P fertilization in fast-growing tree plantations in tropics may reduce organic matter decomposition. In addition, our result may partly explain the quick decomposition rate in tropical forests with low P availability; P shortage may stimulate litter decomposition at the later stage. Since the present study is a short-term incubation study, longer studies or field experiments are required to fully understand the effects of P addition on the organic matter decomposition in P-limited tropical forests, especially in fast-growing tree plantations with intensified management practices and fertilizer use. Furthermore, experiments with (i) more frequent microbial biomass analysis, (ii) microbial community analysis using PLFA analysis or 16-S RNA analysis, or (iii) measuring changes in enzyme activities are required to elucidate the precise mechanism.

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