

Acacia mangium植林地土壌ならびにImperata cylindrica
草原土壌への窒素添加がリンの存在形態ならびに酸性ホス
ファターゼ活性に及ぼす影響

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Effects of nitrogen application to phosphorous forms and acid phosphatase activity (APA) in soils of *Acacia mangium* plantation and *Imperata cylindrica* grassland

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Nitrogen (N)-rich high quality litter of leguminous tree may enhance the phosphorus (P) availability in a soil via the enhanced activity of microorganisms. We conducted sequential P extraction and incubation experiments with N and carbon (C) application using soils from an *Acacia mangium* plantation and an *Imperata cylindrica* grassland. We extracted inorganic P (Pi) and organic P (Po) with water on anion exchange resin (resin-P), NaHCO₃ (NaHCO₃-P), NaOH (NaOH-P), and HCl (HCl-P) in this sequence, and total P. Available P (resin-P + NaHCO₃-Pi) and organic P accounted for 4%–6% and 35%–45% of total P, respectively, in both soils. The differences in P fractions between the soils of different vegetations were unclear, and each fraction was significantly affected by the clay content of the soil. In an *in vitro* incubation experiment, acid phosphatase activity (APA) was higher in acacia soils than in grassland soils and further increased in acacia soil with N application. This suggests the soils in N fixing trees like acacia have higher APA due to continuous input of N rich litter and might have higher potential to acquire P even under P-limited soil condition in the tropics.

Key words : Organic phosphorus, Phosphorus sequential extraction, Acid phosphatase activity, *Acacia mangium* plantation, Fast-growing tree plantation, *Imperata cylindrica* grassland

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マメ科樹種植林における窒素(N)濃度の高いリターの投入は, 土壌中の微生物活性を高め, 植物によるPの利用性を促進する可能性がある。本研究では *Acacia mangium* 植林地とその前植生である *Imperata cylindrica* 草原の土壌において, 陰イオン交換樹脂 (resin-P), NaHCO₃ (NaHCO₃-P), NaOH (NaOH-P), HCl (HCl-P) を用いた逐次抽出法による無機態P (Pi) および有機態P (Po) の形態別分析とNおよびCの添加培養試験を実施した。両土壌における全Pに占める有効態P (resin-P + NaHCO₃-Pi) の割合は4-6%である一方, 有機態Pの割合は35-45%であった。アカシア土壌と草原土壌のP形態および全Pには有意な植生間差がほとんど認められず, 各土壌の粘土含量の違いがリンの存在様式をより強く規定していた。全培養期間を通じ, N無添加区におけるアカシア土壌の酸性ホスファターゼ活性 (APA) は草地土壌より高く, N添加によってアカシア土壌のAPAはさらに増加した。このことから, アカシアなど窒素固定樹種下の土壌はN濃度の高いリターの継続的な投入によって高いAPAを獲得しており, リン制限のかかった熱帯土壌においてより高いリン獲得ポテンシャルを備えている可能性が示唆された。

キーワード: 有機態リン, リンの形態別分析, 酸性ホスファターゼ活性, アカシアマングウム, 熱帯早生樹植林, チガヤ草原

1. Introduction

Phosphorus (P) is usually considered to be the primary nutrient limiting element to affect plant productivity in humid tropical soils (Christina and Coleman, 1999; Garcia-Montiel *et al.*, 2000). In highly weathered tropical soils, oxides and hydroxides of Fe and Al absorb P strongly, reducing P availability of plants significantly. Thus, enhancement of P availability will contribute to sustainable agriculture and forestry in the tropics.

Two major P dynamics are well known: one is a geochemical cycling involving P sorption and release on mineral soil along with the weathering of parent materials. The

other is a biological cycling which is the uptake and release by plants and microbes. These organisms synthesize organic P (Po) and recycling these Po through the mineralization after its death (Olander and Vitousek, 2004).

Many researchers (Garcia-Montiel *et al.*, 2000; Oberson *et al.*, 2001; Pearson and Vitousek, 2002) have used the sequential P extraction method developed by Hedley and Stewart (1982) and Tiessen and Moir (1993) to clarify existing form of P in soil, and shown that NaOH-extractable Po accumulates (Miller *et al.*, 2001; Kolawole *et al.*, 2003). In tropical soils, this Po is essential as a source of available P (Phiri *et al.*, 2001; Reddy *et al.*, 2001). However, the mechanisms of Po turnover in relation to available P have

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not yet been well understood.

Microorganisms are responsible for soil P_o transformations through the mineralization of P from organic sources and synthesis and release of P_o (Oberson *et al.*, 1997, 2001; Colvan *et al.*, 2001; Kwabiah *et al.*, 2003; Santruckova *et al.*, 2004). P uptake by microorganisms (bio-P) also forms a labile P_o pool that acts as a sink and a source of plant-available P (Chen *et al.*, 2000; Oehl *et al.*, 2001). Indeed, Ayaga *et al.*, (2006) reported that bio-P was a suitable indicator of P availability. Thus, microorganisms play a key role in biological P cycling, particularly in P_o transformation directly related to P availability.

Acacia is one of the most important leguminous (i.e., N-fixing) tree species for industrial plantations because of its fast growth and tolerance of acidic and nutrient-poor environments. Large-scale plantations of *Acacia mangium* (hereafter called "acacia") are currently expanding in Southeast Asia, especially in Indonesia, Malaysia, Vietnam and Thailand. Because its vigorous growth even on degraded *Imperata cylindrica* grassland (hereafter called "grassland") appeared after timber exploitation of natural forests in 1970s–1980s, acacia should contribute to soil amelioration (Evans, 1992) and also to fulfill the increasing wood demand. N-fixing acacia supplied more N to soil via the mineralization of N-rich litter than did non-leguminous tree species (Rao and Reddy, 2002). With regard to the effect of N-fixing plants on soil P, Oberson *et al.*, (1999, 2001) suggested that high-quality litter grass legume may increase microbial activity, accumulation of P_o , and its availability, and biological turnover of P. In an incubation experiment, Kouno *et al.*, (1999) found that bio-P increased with increasing N application. This implied that P availability may be closely linked to N resources. However, little information is available on the effects of N abundance in leguminous tree plantations on soil P dynamics in the tropics.

In this study we compared the P existing forms in soil between acacia plantation and *Imperata* grassland which is precedent vegetation before acacia plantation establishment, to reveal the effect of N-fixing acacia to the status of soil P. And also effect of N application to soil biological activity in relation to P_o transformation was examined using an *in vitro* incubation.

2. Materials and methods

2.1 Soils used

The samples were collected in 8-year old acacia stands and *Imperata* grasslands which are still present in some patches in inland South Sumatra province, Indonesia (lat. $3^{\circ}30'4^{\circ}05'S$, long. $103^{\circ}50'104^{\circ}10'E$). The acacia plantation was established in 1993, and the logging interval is 6 to 8 years. The geology of the study area consists of sandy clay shale and quartzitic sandstone, and the soil is categorized as an Acrisol (FAO, 1979). The climate is classified as Aw by the Koppen classification (FAO, 1979). Annual rainfall ranges from 2,200 to 3,000 mm and occurs mainly from October to June. The mean annual temperature is $27.3^{\circ}C$ (Hardjono *et al.*, 2005). The soils in the plantation are char-

acterized by low pH, low base saturation, and high Al saturation (Yamashita *et al.*, 2008). The effective cation exchange capacity in the surface soil is around $6\text{ cmol}_e\text{ kg}^{-1}$, and the total carbon content is around 30 gC kg^{-1} . The clay content ranges from 22% in the surface to $>55\%$ at 150 cm. The chemical properties of the surface soils are notably different: the pH and base saturation averaged 5.5 and 65% in the grassland and 4.4 and 35% in the acacia plantations (Yamashita *et al.*, 2008).

2.2 Soil sampling

To compare the P forms between the soil under the acacia and grasslands, we collected soil samples at 12 locations, 7 in 8-year-old acacia stands and 5 in grassland, from March to September 2003. A $20\text{ m} \times 20\text{ m}$ plot was set up in each of the 12 locations and, soil samples were collected at 0–5 cm and 5–10 cm using three 100-mL sampling cylinders at six different sampling points randomly arranged. We mixed the samples from the same depth from the six sampling points in each plot. Thus, we prepared 2 composite layer samples for each of the 12 locations. The samples were air-dried, sieved (2 mm) after removing plant debris contained, and then used for analysis.

For the soil incubation experiment, in September 2003, field-moist soils at a depth of 0–5 cm were collected from two plots each of acacia (Aca-1 and Aca-2) and grassland (Grass-1 and Grass-2) which were randomly selected from the 12 locations. The soils were passed through a 2 mm-mesh sieve after plant debris contained in the soils, and refrigerated without drying until use.

2.3 Sequential P extraction and analysis of general soil properties

P was extracted sequentially from 0.5-g soil samples (Hedley and Stewart 1982; modified by Tiessen and Moir, 1993) using the solutions in the order of water (resin-P), 0.5 M NaHCO_3 ($\text{NaHCO}_3\text{-P}$), 0.1M NaOH (NaOH-P), and 1M HCl (HCl-P). After the extraction, the residual soil was oxidized with H_2O_2 and extracted using H_2SO_4 solution (residue-P). The organic P in a part of the solutions extracted by NaHCO_3 and NaOH containing both inorganic P ($\text{NaHCO}_3\text{-P}_i$ and NaOH-P_i) and organic P ($\text{NaHCO}_3\text{-P}_o$ and NaOH-P_o) were digested to P_i using acidified ammonium persulfate. All P_i concentration in each solution was colorimetrically measured (Murphy and Riley, 1962). Total P was defined as sum of resin-P, $\text{NaHCO}_3\text{-P}_i$, $\text{NaHCO}_3\text{-P}_o$, NaOH-P_i , NaOH-P_o , HCl-P and residue-P. Total available P_i (ava- P_i) was defined as the sum of resin-P and $\text{NaHCO}_3\text{-P}_i$. Total P_o was calculated by subtracting other P_i fractions from total P.

Total C and total N in pulverized samples ($<0.2\text{ mm}$) were determined with an NC analyzer (Sumigraph NC-900). After digestion with H_2O_2 and shaking with sodium hexametaphosphate dispersant, the particle size distribution was measured by the pipette method (Gee and Bauder 1986).

2.4 Incubation experiment

To clarify any change in soil biological activity in relation to P_o transformation, we conducted an incubation experiment. Different levels of $(\text{NH}_4)_2\text{SO}_4$ with a constant level of

powdered cellulose were used to represent N and C input via litterfall in an acacia plantation.

One set of 16 samples comprised 4 treatments of 4 soils. Each soil sample was adjusted to 60% of its water-holding capacity, and 50.0 g was placed in a 200-mL polypropylene bottle sealed with polyethylene film. Four treatments were prepared: (1) unamended control, (2) (NH₄)₂SO₄ at 202 mg N kg⁻¹ (1N), (3) 404 mg N kg⁻¹ (2N), and (4) 808 mg N kg⁻¹ (3N). Treatments 2, 3, and 4 also received powdered cellulose equal to 12.1 g C·kg⁻¹ soil, which was based on the 9.7 t·ha⁻¹·year⁻¹ annual input of acacia litterfall (Bernhard-Reversat, 1993). This C application was based on the assumption that all litterfall (with a C concentration of 50%) was decomposed and was deposit in the 0–5 cm soil layer (1.25 Mg m⁻³ bulk density). The C/N ratios in these applications were equal to 59.9 (1N), 30.0 (2N), and 15.0 (3N). The C/N ratio of fresh acacia litter is 30.1 (Bernhard-Reversat, 1993).

All bottles were incubated in the dark at 25°C for a month with occasional addition of distilled water to maintain soil moisture. At day 0 (0d, when no cellulose was applied), 1 week (1w), 2 weeks (2w), and 4 weeks (4w), we extracted P, determined microbial biomass C and P, and measured acid phosphatase activity (APA).

Microbial biomass C (bio-C) was determined by the fumigation-extraction (FE) method of Vance *et al.*, (1987). Organic C in the extracted solution was determined with a total organic carbon analyzer (Shimadzu TOC-V_{CHS}). Bio-C was calculated as the difference between C extracted from samples fumigated with CHCl₃ and C extracted from unfumigated samples multiplied by a k_c factor of 2.15 (Sakamoto and Inubushi 1997). Microbial biomass P (bio-P) was determined by the FE method of Wu *et al.*, (2000). P in extracts was measured colorimetrically (Murphy and Riley 1962). Bio-P was calculated as the difference between P in fumigated samples and P in unfumigated samples multiplied by a k_p factor of 0.4 (Wu *et al.*, 2000). APA was determined by the method of Tabatabai (1982).

2.5 Statistical analyses

To compare the mean values of each P fraction and the

ratios of each P fraction to total P between acacia and grassland at each depth, a one-way ANOVA and a test of equal proportions were performed respectively. The relationships between clay content and each P fraction were analyzed by Pearson’s correlation coefficient. SPSS 10.0 J (SPSS Inc., Chicago, USA) was used for all statistical analyses.

3. Results

3.1 P fractions in acacia and grassland soils

At 0–5 cm depth, ava-P_i accounted for only 5.2% of total P in acacia soil and 5.4% in grassland soil, whereas total P_o accounted for 39.1% and 39.3% of total P, respectively (Table 1). NaOH-P_o was higher than NaHCO₃-P_o in both soils for 0–5 and 5–10-cm depth. P fractions strongly adsorbed into soil minerals (i.e. NaOH-P, HCl-P and residue-P) accounted more than 80% of total P in both soils, although HCl-P_i was less than 1% of total P.

These seven P fractions and the ratios of P fractions to total P did not differ significantly between the soils (Table 1). Meanwhile, the ratios of some P fractions to total P were significantly correlated with clay contents (Fig. 1). Residue-P and NaOH-P_i were positively correlated with clay contents, whereas total P_o and ava-P_i were negatively correlated with clay contents.

3.2 Changes in biological properties and P fractions during incubation

After 4 weeks, bio-C in Aca-1 increased from 593 to 1,088 μg C g⁻¹ soil by 3N application (Table 2). After 2 weeks, bio-C in Aca-1 was greater in 3N (1,880 μg C g⁻¹ soil) than in the control (426 μg C g⁻¹ soil). In contrast, bio-P showed no clear change during incubation. As a result, bio-C/P showed the same tendency as bio-C and increased with time and N application; for example, bio-C/P in Aca-1 and Grass-1 increased from 80.6 and 139 to 155 and 209, respectively, in 3N after 4 weeks.

APA was higher in the control acacia soils (Aca-1 and Aca-2) than in the control grassland soils (Grass-1 and Grass-2) during the entire incubation period (Fig. 2). This was the only distinct difference found between acacia and grassland soils among the parameters obtained in the incu-

Table 1 P fractions (μg P g⁻¹) and the ratio of each P fraction to total P (% , in parentheses) in acacia and grassland soils.

Vegetation type	Depth (cm)	Resin	NaHCO ₃		NaOH		HCl	Residue	Ava-P _i ^c	Total P _o ^d	Total P
		P	P _i ^a	P _o ^b	P _i	P _o	P _i	P			
Acacia plantation	0-5	3.3 (3.1)	2.3 (2.2)	9.5 (8.9)	12.8 (12.0)	32.1 (30.2)	0.47 (0.4)	46.0 (43.2)	5.6 (5.2)	41.6 (39.1)	106.4 (100)
	5-10	2.0 (2.2)	2.09 (2.3)	8.7 (9.4)	11.1 (11.9)	24.7 (26.7)	0.38 (0.4)	43.7 (47.1)	4.1 (4.5)	33.4 (36.0)	92.7 (100)
Grassland	0-5	2.9 (2.9)	2.4 (2.5)	8.7 (8.9)	9.3 (9.5)	29.9 (30.4)	0.66 (0.7)	44.3 (45.2)	5.3 (5.4)	38.6 (39.3)	98.1 (100)
	5-10	2.4 (2.6)	2.3 (2.5)	8.6 (9.4)	8.0 (8.7)	31.6 (34.3)	0.64 (0.7)	38.6 (41.9)	4.6 (5.0)	40.2 (43.7)	92.1 (100)

^aInorganic P. ^bOrganic P. ^cresin-P + NaHCO₃-P_i. ^dTotal P – (Resin-P + NaHCO₃-P_i + NaOH-P_i + HCl-P_i + Residue-P)

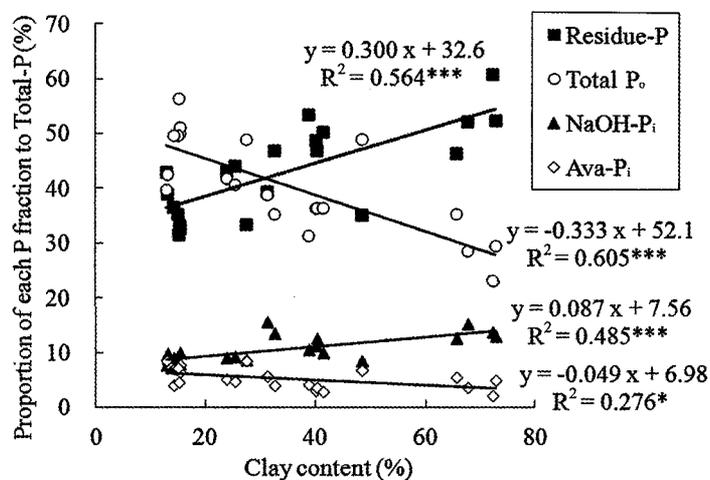


Fig. 1 Relationships between clay contents and ratios of residue-P, NaOH-P_i, ava-P_i and total P_o to total P (%).

*P < 0.05, **P < 0.01, ***P < 0.001

Table 2 Changes in biomass-C, biomass-P, and biomass-C/P during incubation after N application.

Site		Bio-C μg C g ⁻¹ soil (incubation period)				Bio-P μg P g ⁻¹ soil (incubation period)				Bio-C/P (incubation period)			
		0d	1w	2w	4w	0d	1w	2w	4w	0d	1w	2w	4w
Aca-1	C	593	505	426	-	7.4	5.7	5.2	6.1	80.6	88.9	82.5	-
	1N	274	1456	281		6.1	5.0	5.2		45.1	294	54.4	
	2N	529	1678	457		5.2	4.7	5.8		102	357	78.2	
	3N	614	1880	1088		7.9	5.6	7.0		78.1	338	155	
Aca-2	C	329	149	314	317	1.9	1.2	1.0	1.3	173	129	302	235
	1N	214	348	752		1.5	1.1	0.9		139	327	815	
	2N	159	298	762		1.5	1.3	1.2		106	238	619	
	3N	172	242	-		2.0	1.4	1.5		84.5	176	-	
Grass-1	C	479	251	249	106	3.4	3.4	3.2	3.7	139	74.3	77.5	28.5
	1N	578	610	688		4.5	3.3	4.2		128	183	164	
	2N	555	658	861		3.8	3.7	3.9		147	180	222	
	3N	528	782	831		4.0	4.1	4.0		133	193	209	
Grass-2	C	285	208	311	245	6.3	7.1	7.1	7.3	45.2	29.1	43.9	33.4
	1N	443	493	931		6.2	5.3	4.6		71.2	93.5	201	
	2N	489	605	1296		6.4	6.0	4.6		76.9	101	279	
	3N	537	609	1215		6.5	5.8	4.4		82.0	105	274	

-Not determined.

bation experiment. APA increased with N application after 1w, except in Grass-1, although these trend were not observed at 2w and 4w (Fig. 2).

Any distinct trends were not found in P fraction for all soils during incubation (Fig. 3 and Fig. 4). For ava-P_i, control soil of Aca-1 and Grass-1 decreased from 0d to 1w and almost constant from 1w to 4w (Fig. 3). For total P_o, control soil of Aca-1 slightly decreased from 0d to 1w and almost constant from 1w to 4w (Fig. 4). N application increased ava-P_i in Aca-1 after 2w and Grass-2 after 4w (Fig. 3), whereas any effects of N application on total P_o were not found for all soils (Fig. 4).

4. Discussion

4.1 Characteristics of P fractions in acacia and grassland soils

The ratios of P_o to total P in this study (around 35%–45%, Table 1) were within the range of values for soils from other tropical regions: 40% in grassland Oxisol in Colombia (Oberson *et al.*, 2001), 20% to 60% in forested volcanic soils in Hawaii (Miller *et al.*, 2001), and 65% in forested allophanic soils in New Zealand (Chen *et al.*, 2003).

Meanwhile, there were no clear differences in P fractions between the vegetations. P fractions might be controlled not only by the vegetations but also the mineralogical soil properties. In this study area, there was very large variation in

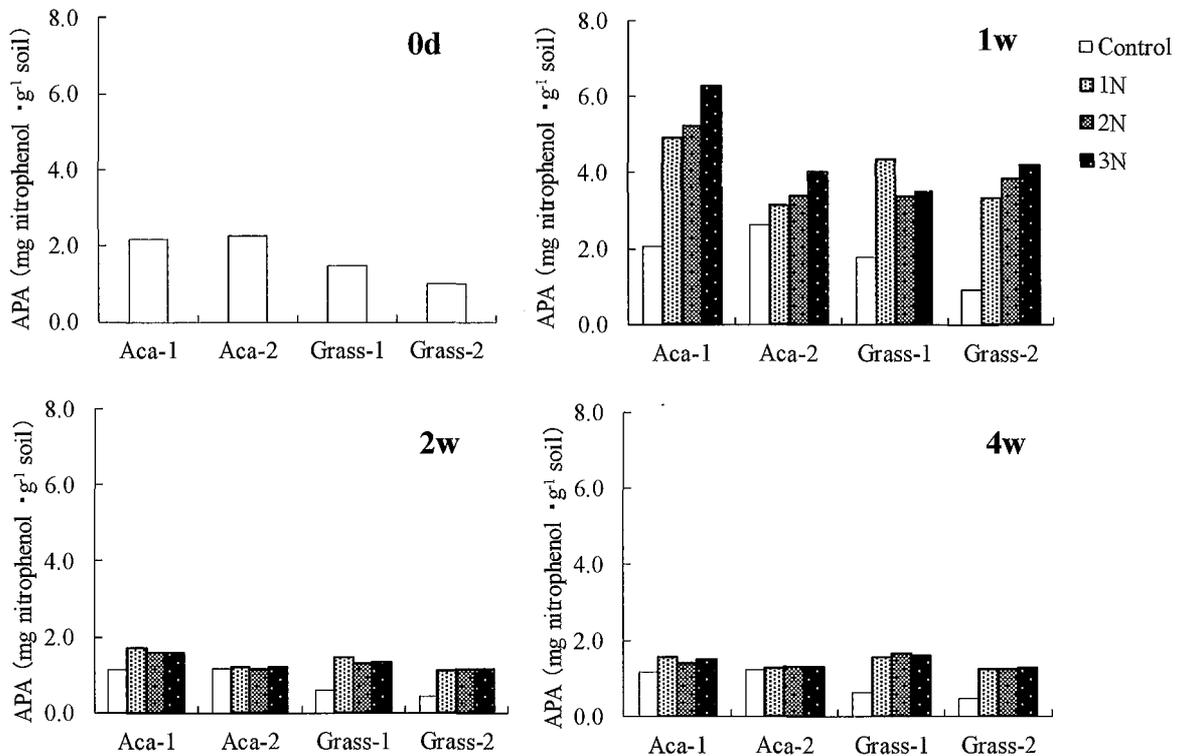


Fig. 2 Effect of N application on APA after 1 week, 2 week and 4 week's incubation.

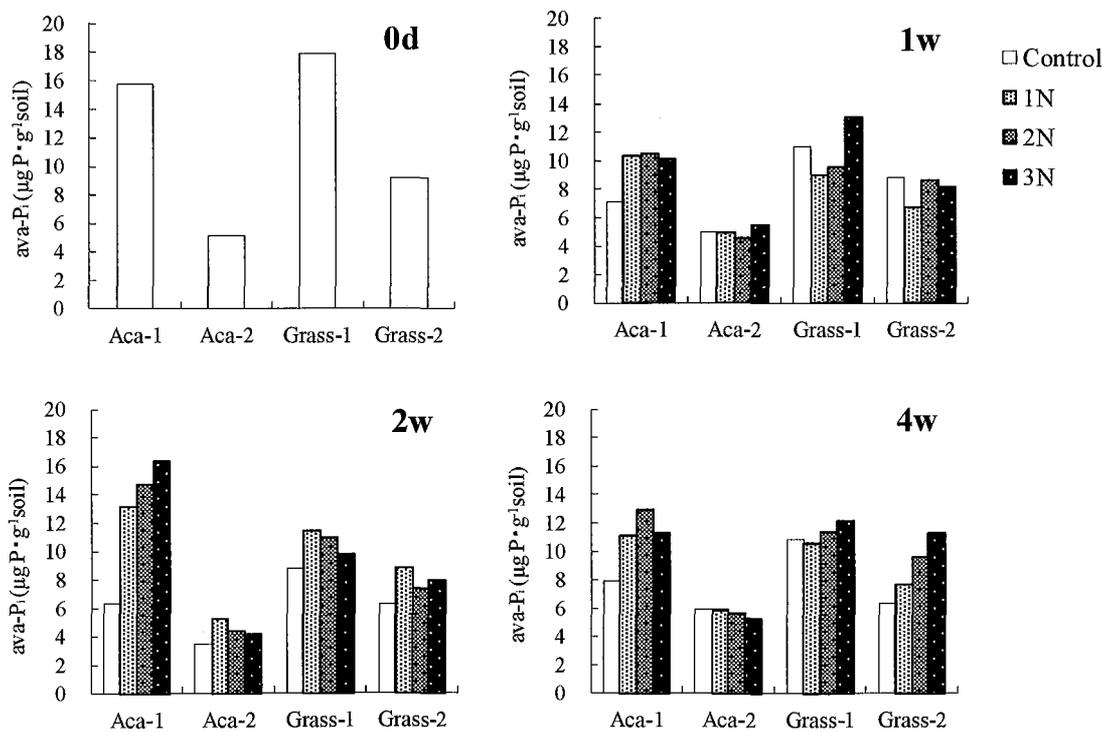


Fig. 3 Effect of N application on ava-P_i after 1 week, 2 week and 4 week's incubation.

clay contents (around 10%–70%, Fig. 1), and we assumed that soil mineralogy have a greater effect on some P fractions than vegetation. Tiessen *et al.*, 1984 show that active aluminum and iron in mineral soils, which were positively correlated with clay contents (Ohta *et al.*, 1993), could provide adsorption sites for NaOH-P. The positive correlation

between NaOH-P_i and clay contents in our study support this hypothesis. The adsorption of residue-P might also depend on the clay content (Fig. 1).

In these soils, more clayey soil had larger total P pool (data not shown). This may be because residue-P + NaOH-P_i accounted for about 50–60% of total P (Table 1) and

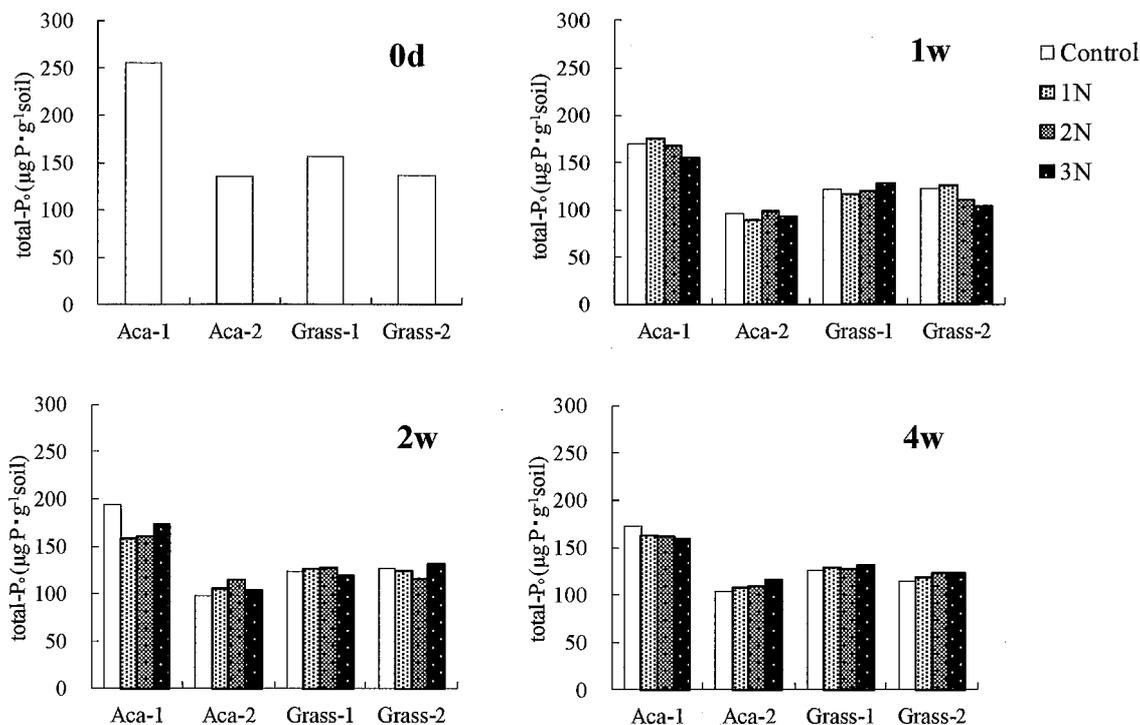


Fig. 4 Effect of N application on total P₀ after 1 week, 2 week and 4 week's incubation.

more clayey soil had larger residue-P + NaOH-P_i (Fig. 1). The ratios of ava-P_i or total P₀ to total P were higher in sandy soil than in clayey soil (Fig. 1) because total P pool was smaller in sandy soil; the amounts of ava-P_i or total P₀ were not correlated with clay contents. We speculate that sampling for clarifying temporal variation might be needed because these labile and biological fractions might change rapidly in accordance with changes of biotic and abiotic soil environment.

4.2 Changes in biological properties and P fractions during incubation

Higher APA in acacia soil throughout the incubation period (Fig. 2) suggests that N-rich litter promotes utilization of P₀ by microbes in soils. Microbes take up P by: (1) absorption of orthophosphate (PO₄³⁻) directly from the soil solution; (2) excreting organic acids, such as citric acid, which increase the desorption of insoluble P_i to soil; and (3) enhancing the activity of phosphatase, which hydrolyzes P₀ into available P_i (Olander and Vitousek, 2004). Higher APA in the acacia soils suggested the third mechanism worked in acacia soil relative to grasslands. Because the N application might stimulate the APA synthesis after 1 week incubation (Fig. 2), the relatively higher APA in acacia soil at 0d should be due to the continuous N-rich litter input. This result agrees with the finding that APA increased with long-term application of N fertilizer to Hawaiian rainforest soil (Treseder and Vitousek, 2001).

However, in our incubation experiment, N application did not increase bio-P (Table 2) unlike the enhanced APA after 1w (Fig. 2). In addition, N application did not change significantly the ava-P_i or total P₀ for all soils throughout the incubation, except ava-P_i in Aca-1 after 2w (Fig. 3 and Fig.

4). Although bio-C was likely to accumulate with increasing N application and time except Aca-2, bio-P did not similarly accumulate (Table 2). Rather, the simultaneous increase of bio-C/P and bio-C indicated an increase in microbial biomass by N application. The results were contrary to a number of reports that the application of substrates such as glucose and (NH₄)₂SO₄ to soil resulted in enhanced P transformation with microbial activities (e.g. Hedley *et al.*, 1982; Kouno *et al.*, 1999; Oehl *et al.*, 2001).

We speculate that P absorbed by microorganisms (bio-P) rapidly turned into the form of available P_i through death and lysis under the enlarged microbial population due to C and N application. In almost all soils (Aca-2, Grass-1 and Grass-2), the effects of enhanced APA by N application were not observed except the soil of Aca-1 in which an increase in ava-P_i by N application after 2w (Fig. 3) implied the effect of enhanced APA by N application after 1w (Fig. 2). Therefore, the contribution of enhanced APA to an increase in P availability measured by ava-P_i in the Acacia soil was not demonstrated clearly in this study. As a result, we did not find any parameters controlling labile and biological P fractions (Table 1) in these soils.

5. Conclusion

Sequential P extraction revealed that available inorganic P and total organic P accounted for 4%–6% and 35%–45% of total P in both soils. These results suggest that the organic P pool plays an important role in the P dynamics in this study area, as in other tropical regions. Although the establishment of the acacia plantation might have not changed the organic P pool, the incubation experiment indicates that the microorganisms have higher APA in the plantation, which

might be due to the continuous input of N rich litter. The soils in N fixing trees like acacia might have higher potential to acquire P even under P-limited soil condition in the tropics. Meanwhile, the effect of enhanced APA by N application on P-availability was not observed in our experiment. To reveal the direct relation between APA and supply of available P approach to detect the rapid cycling of labile P in soil might be required.

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