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Effect of phosphate rock, lime and cellulose on soil microbial biomass in acidic forest soil and its significance in carbon cycling

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Abstract Phosphate rock (PR), limestone, coal combustion by-product (CCBP) high in Ca and high organic manures are potential amendments for increasing agricultural production in the acidic soils of the Appalachian region. The objective of this study was to examine effects of PR, CCBP and cellulose addition on soil microbial biomass in an acidic soil based on the measurement of soil microbial biomass P (P_{mic}) and on the mineralization of organic matter. Application of PR alone or in combination with CCBP increased P_{mic}. The P_{mic} was far less when the soil received PR in combination with limestone than with PR application alone or PR in combination with CCBP. Either CCBP or limestone application alone considerably decreased P_{mic} in the soil due to reduced P solubility. Cellulose addition alone did not increase P_{mic}, but P_{mic} was significantly increased when the soil was amended with cellulose in combination with PR. The decomposition of added cellulose was very slow in the soil without PR amendment. However, mineralization of both native organic matter and added cellulose was enhanced by PR application. Mineralization of organic matter was less when the soil was amended with PR in combination with high rates of CCBP (> 2.5%) because PR dissolution varied inversely with amount of CCBP addition. Overall, CCBP had no detrimental effect on soil microbial biomass at low application rates, although, like limestone, CCBP at a high rate may decrease P_{mic} in P-deficient soils through its influence on increased soil pH and decreased P bioavailability in the soil. Application of PR to an acidic soil considerably enhanced the microbial activity, thereby promoting the cycling of carbon and other nutrients.

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Introduction

Soil microbial biomass plays a key role in maintaining soil fertility because its activity is the primary driving force for cycling elements such as carbon and nitrogen (Smith and Paul 1991). Soil microorganisms are reservoirs of potentially available plant nutrients (Tate and Salcedo 1988). Microbial biomass P has been suggested as a significant source of P to plants (Kouno et al. 1994).

The size and activity of the soil microbial biomass are considerably affected by the immediate energy supply and nutrient availability (Nielsen and Eiland 1980; Ocio et al. 1991; Wu 1992; Amador and Jones 1993; Reeve et al. 1993; Bauhus and Khanna 1994). The special role of P in controlling carbon cycling through its effect on soil microbial biomass was evident from soil sequence studies (Tate and Salcedo 1988). Phosphorus content of parent material affected the contents of organic matter, nitrogen and sulfur in soil (Walker and Syers 1976). By controlling N immobilization, P availability to organisms could ultimately control the organic matter content of soil (Walker and Syers 1976). A conceptual model proposed by McGill and Cole (1981) provides a rational framework for understanding C, N, P and S interrelationships over both pedological and biological time scales, with P playing the central role. Further research is needed to elucidate the effect of soil P status on cycling of C, N, P and S in a terrestrial ecosystem through soil microbial biomass.

Phosphate rock (PR) is by far the most cost-effective P source for acid soils in agriculture. The dissolution of PR in acid soil could be decreased considerably by addition of limestone and coal combustion by-product (CCBP) (He et al. 1996). However, the effect of PR, especially when applied in combination with liming materials such as limestone, CCBP and cellulose, on soil microbial biomass is

not well understood. Such an understanding is required because the input of soil amendments such as CCBP and PR may affect soil quality and raise environmental concerns (Carlson and Adriano 1993).

In the present study, the effect of PR, CCBP, lime and cellulose, applied alone or in various combinations, on soil microbial biomass in acid forest soil was investigated based on the measurement of P_{mic}. The relationship between P_{mic} and soil-labile P as affected by liming materials, PR and cellulose was examined. The influence of P-availability to microbial biomass on the mineralization of both native and added organic matter in soil was evaluated in relation to C and nutrient cycling.

Materials and methods

An infertile acid Lily loam (Typic Hapludult sand 51%, silt 38% and clay 11% from West Virginia) was used in the investigation. This soil was under natural vegetation of deciduous and coniferous trees, dominated by oak (*Quercus* L.), loblolly pine (*Pinus taedal.*), broad-leaf maple (*Acer amplum* Rehd.) and Pacific serviceberry (*Amelanchier florida* Linda). Soil was collected from the depth of 0–50 cm after removal of litter and surface organic materials. The soil sample was mixed, passed through a 2.0-mm sieve and stored below 4 °C prior to use. A subsample was air-dried for chemical analysis. Properties of the soil were: organic C 1.26%; pH (H_20), 4.5; pH (0.01 *M* $CaCl_2$), 3.9; 3.0 mg Bray-1 extractable P kg^{-1} soil and 0.4 mg Olsen extractable P kg^{-1} soil by the Olsen-P procedure (Olsen and Sommers 1982). A highly carbonate-substituted phosphate rock (PR) with 132 g P and 338 g Ca kg^{-1} from Texasgulf, Inc., Raleigh, North Carolina, was passed through a 150-μm sieve prior to use. The CCBP used (CCBP-#22 from the Beckley ARS collection) was produced by an in situ forced oxidation limestone-based scrubber. The CCBP contained up to 90% $CaSO_4$, < 500 mg total P kg^{-1} and nondetectable amounts of either Bray 1- or Olsen P-extractable P. An unburned, ground, dolomitic limestone (manufactured by the James River Limestone Company, Virginia)[1] was passed through a 150-μm sieve before use. The lime contained 46% calcium carbonate and 40% magnesium carbonate (95% calcium carbonate equivalent). High-purity cellulose (Sigmacell, type 100) was used as the model organic material in this study.

Incubation experiment

Portions of fresh soil were weighed and mixed with amendments for the treatments as follows: (1) CCBP at application rates of 0, 0.5, 1.0, 1.5, 2.5 and 5.0% of the soil mixture (by weight); (2) treatment (1) plus NCPR (North Carolina phosphate rock) at 397.2 mg P kg^{-1} soil; (3) treatment (2) plus dolomitic limestone (3.41 g kg^{-1}, which brought the soil pH to around 6.0 according to a limestone-soil pH relationship curve previously determined with this limestone); and (4) treatment (2) plus cellulose (10 g kg^{-1} of the total mixture), N (in form of NH_4NO_3) was added to adjust C/N ratio at 15:1 for the cellulose-treated soils. Controls (without amendments) were prepared for each of the treatments; and for each treatment, soil without PR was included. The total weight of each soil-amendment mixture was 1.0 kg (oven-dry basis). The moisture content of the mixtures was adjusted to 45% water-holding capacity, and the mixtures were each placed in a 3-l plastic container (12.5 × 12.5 × 19.2 cm) and incubated at 23 °C. Containers were covered with Parafilm to prevent moisture loss but to allow air exchange during incubation. At intervals of 0.1 (sampled immediately after being prepared), 10, 20, 30, 45 and 60 days after mixing, subsamples (5.0 g soil, oven-dried basis) of soil from each of three replicates were taken for the measurements of soil microbial biomass P and soil labile P extracted by the Bray-1 and Olsen-P procedures. Soil pH and organic C of the soils were determined at the end of incubation.

Soil microbial biomass P was determined by the $CHCl_3$-fumigation-0.5 *M* $NaHCO_3$ extraction procedure (Brookes et al. 1982). Triplicate subsamples of moist, incubated soil (5.0 g, oven-dry basis) were taken from each treatment and fumigated for 24 h in a vacuum desiccator with $CHCl_3$. After removal of $CHCl_3$ by evacuation, both fumigated and nonfumigated soils were extracted on a mechanical shaker (200 rpm) for 0.5 h with 0.5 *M* $NaHCO_3$ at a soil to solution ratio (mass/volume) of 1:4. After the suspension was filtered through Whatman No. 42 filter paper, P concentration in the supernatant was determined by the phosphomolybdate, colorimetric method (Olsen and Sommers 1982). Soil microbial biomass P (P_{mic}) was calculated as: $P_{mic} = E_P / (K_P \times r_P)$, where E_p is the difference between P extracted from the fumigated and nonfumigated soil, K_P is a conversion factor for P flush from fumigation to soil microbial biomass P with a value of 0.45 and r_P is the recovery ratio of added phosphate (which was previously determined with nonfumigated soil under the same conditions as the extraction of microbial biomass P) for correction of sorption of P released from microbial biomass after the soil being fumigated. P_{mic} is expressed as mg P kg^{-1}.

Organic carbon content of the incubated soils was measured with a Carbon-Hydrogen-Nitrogen Instrument (LECO, CHN-600) according to the procedure provided by LECO Inc. The reliability of the procedure was ascertained by the fact that the recovery of standard samples (soil with known content of organic C) by the procedure was >99.5%. The incorporation of limestone and CCBP into the soil for the treatment prior to incubation was not found to affect the determination of organic C in the incubated soils. Soil pH was measured with a glass electrode at a soil to solution ratio (mass/volume) of 1:1.

Results and discussion

Effect of PR, CCBP, limestone and cellulose on soil microbial biomass

Soil microbial biomass P (P_{mic}) in the control (without any amendment) slightly increased during incubation (Fig. 1), probably reflecting the effect of increased incubation temperature, compared to field temperatures. For the CCBP-amended soil, P_{mic} decreased over time, mostly in the first 20 days of incubation. Terminal P_{mic} was inversely related to CCBP rates when the CCBP was applied alone (Table 1). This result was consistent with a previous report that the activity of phosphatase and dehydrogenase was considerably reduced by CCBP application (McCarty et al. 1994). Limestone addition alone decreased P_{mic} from 3.6 to 1.8 mg P kg^{-1} in the soil by the end of the experiment (Fig. 3, Table 1). Decreases in P_{mic} due to liming are well documented (Haynes and Swift 1988; Urbasek and Chalupsky 1992). A marked increase in soil pH due to liming is usually considered to be the principal mechanism responsible for the reduced soil microbial biomass (Haynes and Swift 1988; McCarty et al. 1994). The CCBP used contained 4.7% CaO and increased soil pH similar to liming materials and, thus, may be a key factor responsible for the decreased microbial biomass or enzyme activity in the acid soil (McCarty et al. 1994).

[1] Mention of particular companies or commercial products does not imply recommendations or endorsement by the Virginia Polytechnic Institute and State University, Blacksburg, Virginia, or the U.S. Department of Agriculture over other companies or products not mentioned.

Table 1 Selected soil chemical properties and microbial biomass after 60 days' incubation

Sample No.	Treatment[a]	pH (0.01 *M* $CaCl_2$)	Bray 1 (mg P kg^{-1})	Olsen-P (mg P kg^{-1})	P_{mic} (mg P kg^{-1})
1	Control	4.0	2.3 ± 0	0.5 ± 0	3.6 ± 0.3
2	CCBP 0.5%	4.1	1.2 ± 0.3	0.5 ± 0	2.5 ± 0.2
3	CCBP 1.0%	4.2	0.6 ± 0	0.4 ± 0	0.7 ± 0
4	CCBP 1.5%	4.2	0.6 ± 0	0.4 ± 0	0.5 ± 0
5	CCBP 2.5%	4.3	0.4 ± 0	0.4 ± 0	0.4 ± 0
6	CCBP 5.0%	4.7	0.3 ± 0.1	0.4 ± 0	0.2 ± 0
7	PR alone	4.3	27.3 ± 0.1	23.4 ± 0.2	10.3 ± 0.4
8	PR+CCBP 0.5%	4.3	9.0 ± 0.2	7.2 ± 0.5	10.7 ± 0.5
9	PR+CCBP 1.0%	4.3	6.7 ± 0.1	6.7 ± 0.1	11.4 ± 0.5
10	PR+CCBP 1.5%	4.4	6.3 ± 0.2	6.4 ± 0.1	11.9 ± 0.6
11	PR+CCBP 2.5%	4.5	5.6 ± 0.1	5.8 ± 0.1	11.5 ± 1.0
12	PR+CCBP 5.0%	4.9	4.2 ± 0.1	3.7 ± 0.1	11.3 ± 0.5
13	L alone	5.6	1.1 ± 0	0.5 ± 0	1.8 ± 0.2
14	PR+L+CCBP 0.0%	5.7	3.4 ± 0	2.8 ± 0	7.9 ± 0
15	PR+L+CCBP 0.5%	5.7	2.5 ± 0	1.1 ± 0	7.2 ± 0.2
16	PR+L+CCBP 1.0%	5.8	2.4 ± 0	1.0 ± 0	6.3 ± 0.3
17	PR+L+CCBP 1.5%	5.9	2.3 ± 0	1.0 ± 0	4.7 ± 0
18	PR+L+CCBP 2.5%	6.1	2.2 ± 0	1.0 ± 0	3.9 ± 0.5
19	PR+L+CCBP 5.0%	6.4	2.1 ± 0	0.9 ± 0.1	2.7 ± 0
20	C alone	3.9	3.1 ± 0	0.9 ± 0	3.6 ± 0.3
21	PR+C+CCBP 0.0%	4.3	23.0 ± 0.1	19.0 ± 0.2	12.4 ± 0.1
22	PR+C+CCBP 0.5%	4.4	7.5 ± 0.1	5.9 ± 0.1	12.8 ± 0.3
23	PR+C+CCBP 1.0%	4.5	5.6 ± 0	5.1 ± 0.1	13.5 ± 1.0
24	PR+C+CCBP 1.5%	4.5	4.8 ± 0	3.5 ± 0.1	14.2 ± 0.3
25	PR+C+CCBP 2.5%	4.7	4.1 ± 0	2.7 ± 0.1	12.8 ± 0.5
26	PR+C+CCBP 5.0%	5.1	3.7 ± 0	0.9 ± 0.1	12.4 ± 0

[a] *CCBP* coal combustion by-product, *PR* North Carolina phosphate rock (397 mg P kg^{-1}), *L* limestone 3.41 g kg^{-1}, *C* cellulose (10 g kg^{-1})

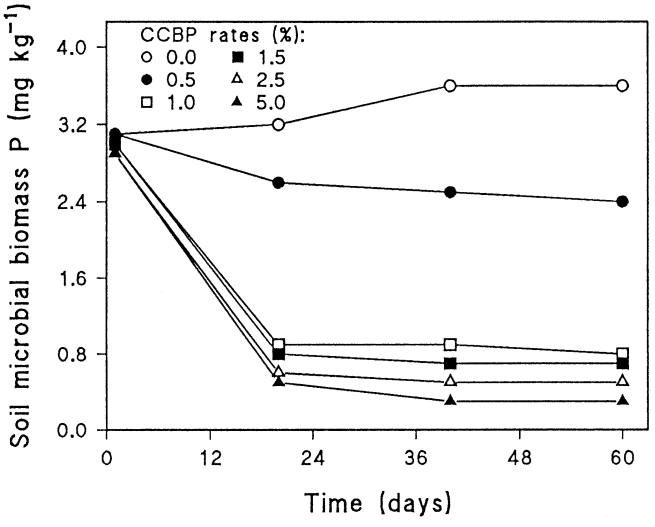

Fig. 1 Effect of CCBP application alone on soil microbial biomass P in an acid forest soil

However, the CCBP effect on soil microbial biomass was distinctly different from that of limestone addition. When soil pH changed from 4.0 to 5.6 (ΔpH = 1.6) by applying limestone, P_{mic} decreased by 1.8 mg P kg^{-1}. When the soil pH changed from 4.0 to 4.7 (ΔpH = 0.7) by applying 5% CCBP, P_{mic} decreased by 3.3 mg P kg^{-1} (Table 1). Hence, it appears that the pH change was not the only factor responsible for the reduced P_{mic} observed from CCBP application. The results from this study showed that CCBP application significantly decreased the labile P content in the soil, and the decline of P_{mic} followed closely the decrease in labile P with increasing CCBP application rates (Table 1). Limestone addition raised soil pH to a greater extent but decreased labile P to lesser extent than the 1–5% CCBP treatments, and subsequently the decease in P_{mic} was less than that applied with 5% CCBP.

The reasons for the greatly decreased soil labile P and P_{mic} from CCBP application are not fully understood. In acid soil, P adsorbed on soil mineral surface is the major source of labile P pool. At high Ca^{2+} concentrations, multilayer sorption of Ca^{2+} could occur on the soil surface, where the sorbed P is covered by the Ca^{2+} sorption layers. The sorbed Ca^{2+} could block the release of sorbed P, thereby making it less extractable by the Bray-1 or Olsen-P reagents, and also possibly less available to soil microorganisms. Phosphorus deficiency probably limits the growth of soil microbial biomass because the labile P content in the soil was very low (2.98 mg P kg^{-1} by the Bray-1 and 0.42 mg P kg^{-1} by the Olsen-P procedure). CCBP application might exacerbate soil P deficiency through such a Ca^{2+} blockage mechanism, and thus decrease P_{mic} in the amended soil.

The results from the PR treatment supported the hypothesis that decreased P availability from CCBP might limit the growth of soil microbial biomass. PR application alone markedly increased P_{mic} in the soil (Fig. 2). With increasing incubation time, P_{mic} steadily increased up to the end of incubation (60 days) (Fig. 2), in contrast to the

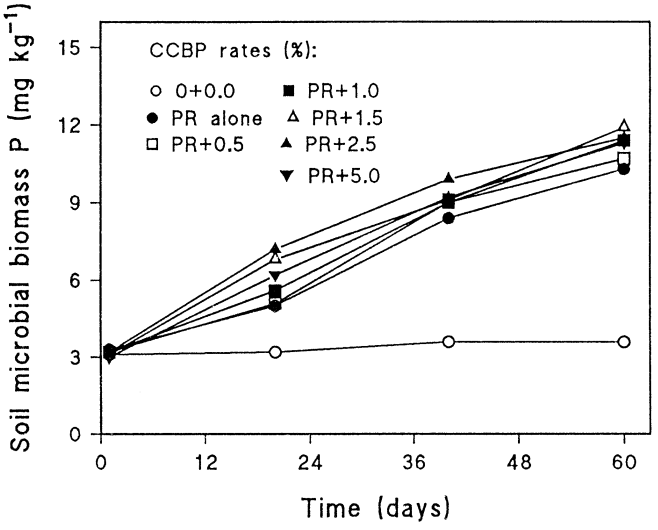

Fig. 2 Effect of CCBP in combination with PR (397.2 mg P kg^{-1}) on soil microbial biomass P in an acid forest soil

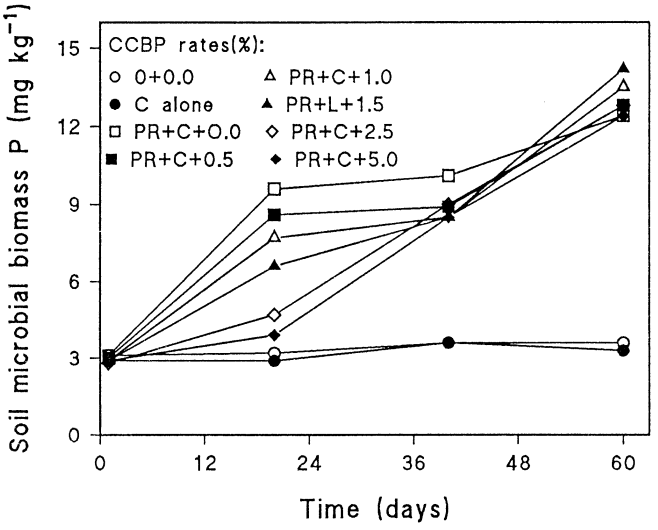

Fig. 4 Effect of CCBP in combination with cellulose (10 g kg^{-1}) and PR (397.2 mg P kg^{-1}) on soil microbial biomass P in an acid forest soil

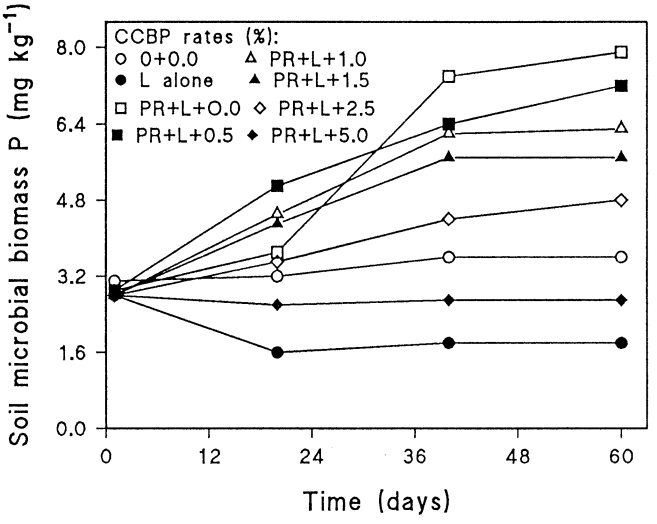

Fig. 3 Effect of CCBP in combination with limestone (3.42 g kg^{-1}) and PR (397.2 mg P kg^{-1}) on soil microbial biomass P in an acid forest soil

P_{mic} decrease by various rates of CCBP in the soil without PR. However, the P_{mic} content in the PR-amended soil increased slightly with increasing levels of CCBP application (Table 1). At day 60 of the incubation, P_{mic} in the soil amended with PR alone increased by 2.9 times compared to that in the control soil (without amendment), and P_{mic} in the soil amended with PR in combination with 5% CCBP increased by 57 times as compared to that in the soil amended with 5% CCBP alone (Table 1).

The effect of PR and CCBP application on P_{mic} was related to soil labile P status. PR application alone markedly increased soil-labile P (Table 1), and thus raised the P_{mic} in the soil. A positive relationship between P_{mic} and labile P was also reported by Chauhan et al. (1981) and Hedley et al. (1982). In the presence of limestone and PR, P_{mic} increased gradually with incubation time and reached a maximum in about 40 days (Fig. 3). P_{mic} content in the soil treated with limestone plus PR was significantly higher than that for limestone alone but considerably lower than that for PR alone (Table 1). For the treatment of PR plus limestone and CCBP, P_{mic} generally decreased with an increase in CCBP application rates, and at the application rate of 5% CCBP, P_{mic} was lower than that in the control. These results paralleled the decrease in PR dissolution (He et al. 1996) and soil labile P (Table 1) from the application of limestone and CCBP. For example, in the soil amended with limestone in combination with 5% CCBP, little dissolution of PR took place (He et al. 1996), and labile P was slightly lower than that in the control (Table 1). Consequently, P_{mic} in the soil amended with limestone and 5% CCBP was lower than that in the soil without any amendment (Table 1).

Application of cellulose alone did not increase P_{mic} (Fig. 4), which indicates that the energy source was not a limiting factor for the growth of microbial biomass in the soil. The application of cellulose in combination with PR increased P_{mic}. However, P_{mic} decreased slightly with increasing CCBP application rates in the soil amended with cellulose in combination with PR (Table 1), probably because the addition of cellulose increased the requirement for P by the soil microbial biomass and, in addition, CCBP decreased the soil-labile P in the soil (Table 1). For the treatment of PR plus cellulose, at low CCBP application rates (0.5–1.5%), the P_{mic} increased rapidly for the first 20 days, then increased slowly, whereas at higher application rates (2.5–5.0%), P_{mic} increased slowly for the first 20 days, then increased rapidly from 20 to 60 days (Fig. 4). This observation probably reflects the effect of P on soil microbial biomass because PR dissolution was more rapid at low CCBP application rates than at high application rates. Consequently, microbial biomass developed more rapidly in the former case than in the latter.

Table 2 Effect of phosphate rock and coal combustion by-product application on the decomposition of added cellulose in a acid forest soil

Treatments	Cellulose added (g C kg^{-1})	Organic C content (g C kg^{-1})		Reduction in organic C	
		Pre-incubation	Post-incubation	g C kg^{-1} lost (%)	Cellulose
Control	0	12.6 ± 0.1	12.4 ± 0.2	0.2 (1.6%) [a]	
+PR	0	12.6 ± 0.1	12.0 ± 0.0	0.6 (4.8%)	
–PR	4.5	17.0 ± 0.1	16.8 ± 0.2	0.2 (1.2%)	4.4 (0) [b]
+PR	4.5	17.0 ± 0.1	14.9 ± 0.1	2.1 (12.4%)	46.7 (33.3)
PR+CCBP 0.5%	4.5	16.9 ± 0.1	14.9 ± 0.1	2.0 (11.8%)	44.4 (31.1)
PR+CCBP 1.0%	4.5	16.8 ± 0.1	14.7 ± 0.1	2.1 (12.5%)	46.7 (33.3)
PR+CCBP 1.5%	4.5	16.8 ± 0.1	14.9 ± 0.3	1.9 (11.3%)	42.2 (28.9)
PR+CCBP 2.5%	4.5	16.7 ± 0.1	14.8 ± 0.1	1.9 (11.4%)	42.2 (28.9)
PR+CCBP 5.0%	4.5	16.3 ± 0.1	15.0 ± 0.2	1.3 (8.0%)	28.9 (22.2)

[a] Values inside parentheses are the percentages of total soil organic C lost during incubation
[b] Values inside parentheses are the percentages of cellulose lost after correction for soil organic C lost due to PR addition

PR effect on cellulose decomposition

P_{mic} is an estimate of the size of soil microbial biomass (Smith and Paul 1991). A large microbial biomass may not indicate high microbial activity due to possible variation in the metabolic state of microorganisms under different P supply conditions (Chauhan et al. 1981). To validate the effect of PR on soil microbial biomass, as measured in this study, organic C content in the soil of different treatments was determined at the end of the experiment to examine the effect of the PR addition on the mineralization of native and/or added organic C through microbial activities. The results from this study showed that after a 2-month period of incubation at 23 °C, only about 1.6% of native soil organic C was lost in the control soil, but about 4.8% was lost when PR was applied to the soil (Table 2). This relationship suggests that PR application increased soil-labile P, i.e., the availability of P to the soil microorganisms (Table 1), and thus promoted the mineralization of native soil organic matter. For the soil applied with cellulose alone, the decrease in organic C accounted for only 4.4% of the added cellulose C after the 2-month incubation period, whereas > 42.2% of the added organic C was decomposed, with the exception of the 5.0% CCBP treatment, when the cellulose was applied in combination with PR (Table 2). CCBP application at 5% decreased the decomposition rate of cellulose, probably because of lower labile P due to the decrease in PR dissolution.

Soil microorganisms play an important role in the cycling of C, N, P and S in the terrestrial ecosystem (McGill and Cole 1981). On the other hand, soil microorganisms rely primarily on the availability of C and P in soil for growth and performance (Amador and Jones 1993; Bauhus and Khanna 1994; Ocio et al. 1991). It has been commonly accepted that the energy supply is the key factor that controls the size and activity of soil microbial biomass in most cultivated soils and fertile natural soils (Lee 1994). However, in peat and forest soils where energy supply is not limiting, the availability of P limits the growth of soil microorganisms. Accordingly, application of fertilizer P has been reported to stimulate soil respiration rate in peat soils with low and intermediate total P (Amador and Jones 1993). Phosphorus fertilization was also found to enhance heterotrophic nitrification and $N_2(C_2H_2)$ fixation by *Ulex gallii* Planchon in acid forest soils and to increase subsequently plant biomass production (Bauhus and Khanna 1994; Toole et al. 1991). In cultivated soils, it has been observed that the contents of ATP, microbial P and phosphatase activity were positively related to the soil labile-P status (Nielsen and Eiland 1980; Hedley et al. 1982). In the present study, it was found that application of phosphate rock significantly increased soil microbial biomass P, and enhanced markedly the mineralization of both native and added organic C (Table 2). In the field site where the soil sample was collected, it was observed that raw organic matter accumulated mainly in the 0- to 5-cm surface layer. Soil organic C content decreased abruptly below 10 cm in the profile. Chemical analysis showed that from horizon A (0–10 cm) to horizon B (11–50 cm), organic C decreased from 84 to 4.5 g kg^{-1}, and total N decreased from 4.4 to 0.1 g kg^{-1}. These results suggest that P deficiency in this acidic forest soil may limit the growth of soil microbial biomass and possibly slow down the cycling of C, N and S in forest ecosystems.

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