

MICROBIAL BIOMASS PHOSPHORUS TURNOVER IN VARIABLE-CHARGE SOILS IN CHINA

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ABSTRACT

Turnover of microbial biomass phosphorus (Bp) in three acid red soils (one Arenic aquult and two Plinthic aquults) were investigated by ^{32}P -labeling microorganisms *in situ* and fumigation–extraction techniques. The turnover rates of Bp in the three red soils were 130, 190, and 217 days, respectively, that is 1.7 to 2.8 times per year. The contents of Bp were 12.2, 17.8, and 31.5 mg kg^{-1} , respectively, for the three soils. Microbial biomass turnover provided a dynamic source of available phosphorus (P) with the size of 2–3 times greater than the Bp. The annual fluxes of P through microbial biomass in these soils could amount to 91 to 146 kg ha^{-1} , which are 3–5 times greater than the amount of P annually removed by the harvested crops. The turnover rate of Bp was greater in the sandy soil than in the other two clayey soils. This was explained by a larger amount of

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Bp and lower mineralization in the clayey soils, as compared with the sandy soil. Apparently, the turnover of Bp plays a very important role in the supply of plant-available P and ecological cycling of P in the subtropical red soil region.

Key Words: Microbial biomass; Phosphorus availability; Red soils; Turnover rate

INTRODUCTION

Red soils are widespread in Southern China and other tropical and subtropical regions in the world. Because of strong adsorption of P by variable-charge minerals such as iron (Fe) and aluminum (Al) oxides, utilization of soil and fertilizer P in the red soils is generally $< 5-10\%$.^[1] Field trials revealed that plant utilization of P could be significantly enhanced by application of organic matter.^[2] In addition to chemical interactions such as release of organic acids from decomposition of applied organic matter, the input of organic matter stimulates the growth of microorganisms. Laboratory culture studies indicated that utilization of specifically adsorbed P by microorganisms could be 2–3 times greater than most crops observed in the field.^[3] Therefore, an enhanced growth of microorganisms may increase the transformation of specifically-adsorbed P to more readily available P pools such as microbial biomass P (Bp) and low molecular weight organic P.

Microbial biomass, defined as the living part of soil organic matter excluding plant roots and soil animals larger than $5 \times 10^3 \mu\text{m}^3$, has been considered to play an important role in the cycling and bioavailability of nutrients in agroecosystem.^[4,5] Microorganisms have the capability to utilize specifically adsorbed P in variable-charge soils, and incorporate soil P into their cells.^[3] Simultaneously, they release their own P in the forms of inorganic phosphate or low molecular weight organic P into soil through metabolic processes.^[3] Plant-availability of P in variable-charge soils may benefit from the turnover of Bp since both Bp and low molecular weight organic P are potentially available to plants.^[6,7] However, few literatures are available to provide this information.

Studies on the turnover of microbial biomass carbon (C) and nitrogen (N) in soils provided useful information regarding the quality of organic matter and cycling and availability of N in soils.^[8-12] The turnover of Bp in other types of soils was reported to be much faster than that of microbial biomass carbon (C) or nitrogen (N).^[13] Therefore, turnover of microbial biomass may contribute to the supply of soil available P to plants.^[14-16] The microbial mediation of P is particularly important for variable charge soils such as red soils, because of high

P adsorption capacity.^[17] However, minimal work has been performed to investigate the significance of Bp turnover in the plant-availability of P in the variable charge soils.

Turnover of microbial biomass C, N, and P in soil can be determined by labeling the microorganisms *in situ* using ¹⁴C, ¹⁵N, or ³²P and then measuring the decay of ¹⁴C, ¹⁵N, or ³²P that has been incorporated into microbial biomass and the change in microbial biomass C, N, and P.^[9,13] The decay of microbial ¹⁴C, ¹⁵N, or ³²P has been observed to follow the first order reaction,^[10,12,13] and the turnover rate can be calculated based on the rate constant obtained by fitting the data to the first-order equation. However, previous studies on microbial biomass P turnover did not take the synthetic ³²P from microbial metabolites into account, and this negligence may affect the accuracy and reliability of the Bp turnover estimation.

The objectives of this study were to measure turnover rate of microbial biomass P in three red soils with different textures using a modified ³²P labeling procedure and to examine major factors affecting the turnover rate.

MATERIALS AND METHODS

Soils

Three red soils were sampled from 0–20 cm depth in Longyou county, Zhejiang province. Soil No. 1 was a light texture soil derived from red sandstone, and the other two (soil No. 2 and No. 3) were clayey soils developed on the quaternary red earths. Soil classification and some related properties of the soils are shown in Table 1. Soil organic matter was determined by a dichromate oxidation method.^[18] Soil microbial biomass C was measured by a chloroform fumigation–extraction and automated-analyzer method.^[19] Soil microbial biomass P was determined by the chloroform fumigation and 0.025 N HCl–0.03 N NH₄F extraction method.^[20] Soil pH was measured using a Beckman 120 pH meter (Beckman, Inc., CA) at a soil:water ratio of 1:1. Total P and extractable P were measured by the HClO₄–H₂SO₄ digestion method and 0.025 N HCl–0.03 N NH₄F extraction method (Bray1 P), respectively. Phosphorus concentration in the digest or extract was determined by the ascorbic acid reduction colorimetric method.^[21]

Determination of Microbial Biomass Phosphorus Turnover

Incubation Study

The ³²P-labeled NaH₂PO₄ solution that contained glucose, NH₄NO₃, and KH₂PO₄ was added to moist soil samples at a rate which supplied

Table 1. Basic Properties of the Soils

Soil No.	Soil Types	Vegetation and Cultivation History	Organic Matter (g kg ⁻¹)	Total P (g kg ⁻¹)	Extr. P (mg kg ⁻¹)	pH (H ₂ O)	MBC ^a (mg kg ⁻¹)	Clay (g kg ⁻¹)
1	Arenic aquult	Vegetable crops—5 yr	8.79	0.31	1.80	4.8	89.4	155
2	Plinthic aquult	Citrus—6 yr	9.05	0.24	43.8	6.0	185.2	342
3	Plinthic aquult	Tea bushes—40 yr	59.13	0.44	4.11	5.8	390.6	387

^a MBC—microbial biomass carbon.

1000 mg C, 100 mg N, and 30 mg P per kg of soil (oven-dry basis). The added ^{32}P -specific activity was 1.11 kBq g^{-1} soil. The moisture content of the treated and the control soil samples was adjusted to 70% of water holding capacity. The substrate-amended and the control (without any amendment) soil samples were then incubated for 80 days at 25°C . The experiment is a randomized complete block with three replications. Total microbial biomass P in both the control and treated soils and microbial biomass ^{32}P ($\text{Bp-}^{32}\text{P}$) in the treated soils were measured in triplicate on sub-samples collected at intervals of 2, 10, 20, 40, 60, and 80 days, respectively, after incubation. The incubation study was repeated and the statistical analyses were performed across the repeating and the replications.

Analytical Methods

Microbial biomass P (Bp) was determined by the fumigation–extraction method as described by Wu et al.^[22] The specific activity of ^{32}P in the $0.025 \text{ N HCl} - 0.03 \text{ N NH}_4\text{F}$ extract was measured using a liquid scintillation counter with automatic correction of ^{32}P natural decay (Wallace Winspectry-1414, Finland). The microbial biomass P- ^{32}P ($\text{Bp-}^{32}\text{P}$) was calculated using the following formula:

$$\text{Bp-}^{32}\text{P} = \frac{\text{Ep-}^{32}\text{P}}{\text{KEP}} \quad (1)$$

$$\text{Ep-}^{32}\text{P} = \frac{(\text{AF} - \text{AN})/r \times v/x}{\text{Ws} \times \text{SAs}} \quad (2)$$

where $\text{Ep-}^{32}\text{P}$ is the flush of ^{32}P due to fumigation against the unfumigated sample; KEP is the conversion factor used to calculate Bp from the P flush; AF and AN are the radioactive activities of ^{32}P (dpm) in the aliquot of extracts of the fumigated and unfumigated sample, respectively; r is the recovery of P added to the soil within 24 h; v is the volume of extract (mL); x is the volume of the extract taken for counting the activity of ^{32}P ; Ws is the weight of the soil (oven-dry basis); and $(\text{SA})_s$ is the specific activity of the added ^{32}P -labeled substrate solution.

Calculation of Microbial Biomass Phosphorus Turnover Rate

The turnover rate is defined as the time required for synthesizing or decomposing the amount of Bp equivalent to the original ‘stand crop’ at steady state. For a given cohort of Bp, e.g., ^{32}P -labeled Bp, the decay can be described

by the first-order reaction:

$$Y_t = Y_0 e^{-kt} \quad (3)$$

where Y_0 is the initial amount of Bp in the cohort at time $t = 0$, Y_t is the remaining amount after time t and k is the decay rate constant of the cohort. For this system, the turnover rate (T) can be expressed as the formula:

$$T = 1/k \quad (4)$$

These equations are theoretically true for a cohort of biomass, which, by definition, is not replenished as the organisms die. However, if Eq. (3) is applied to the labeled Bp present at the beginning and end of time t , an erroneous component of k occurred. This is because the labeled Bp pool is being replenished by the decomposition of labeled metabolites during time t . Let b represent this erroneous value and then the true decay rate constant k is calculated after correction of the measured value for b . If the amount of labeled Bp that is synthesized during t is known, we can deduct it from the quantity of labeled Bp present at time t , and obtain k according to Eq. (3) and calculate T . The labeled Bp synthesized during t can be calculated using the data obtained from the experiment.

RESULTS

Establishing Starting Time for Measuring Turnover Rate of Microbial Biomass Phosphorus

Addition of substrates (glucose and other nutrients such as N and P) enhanced the growth of soil microorganisms including microbial biomass P (Bp) in the first few days. Accordingly, Bp was increased by 80.0%, 67.4%, and 93.0%, respectively, for the three soils, as compared with the controls and reached its maximum values (21.9, 29.8, and 60.8 mg kg⁻¹, respectively, for soil No. 1, No. 2, and No. 3) at day two (Table 2). However, from day 2 to day 10 the Bp dropped down by 26.5%, 18.5%, and 30.3%, respectively, for the three soils due to depletion of the energy source (Figs. 1–3). From day 10 of the incubation, the decline in Bp became smaller, and the Bp decreased every 10 days only by 4.8%, 3.2%, and 2.2%, respectively, from day 10 to day 80 (Figs. 1–3). This indicates that the degradation of the Bp was more rapid than its growth rate at this stage and the effect of substrates addition on Bp was not significant after 10 days of the incubation.

The change of Bp-³²P was similar to the Bp during the whole period of the incubation. The Bp-³²P rapidly reached its maximum value (12.5, 15.8, and

Table 2. Dynamics of Total Microbial Biomass P (Bp) During the Incubation

Soil No.	Treatment	Total Bp (mg P kg ⁻¹) at Various Incubation Times (Days)					
		2	10	20	40	60	80
1	Unamended	12.2	11.7	11.2	10.6	9.9	9.0
	Amended	21.9	16.1	15.5	14.2	12.5	10.7
2	Unamended	17.8	17.2	16.6	15.8	15.5	14.3
	Amended	29.8	24.3	23.5	22.4	21.2	18.8
3	Unamended	31.5	31.2	30.8	30.0	28.7	27.0
	Amended	60.8	42.4	41.6	40.3	38.4	35.9

32.7 mg kg⁻¹, respectively, for soil No. 1, No. 2, and No. 3) in the first two days after incubation but decreased by 37.6%, 28.5%, and 34.9% from day 2 to day 10, respectively, for the three soils (Tables 3 and 4). From the day 10 of the incubation, the decline in ³²P-Bp was small, and the ³²P-Bp decreased every 10 days only by 9.7%, 7.5%, and 6.5%, respectively, for the three soils during the period of day 10 to day 80 (Fig. 4). This suggested that the turnover of ³²P-Bp attained a relatively steady state after 10 days of the incubation. In addition, the degradation of ³²P-Bp was observed to follow the first-order reaction for all the

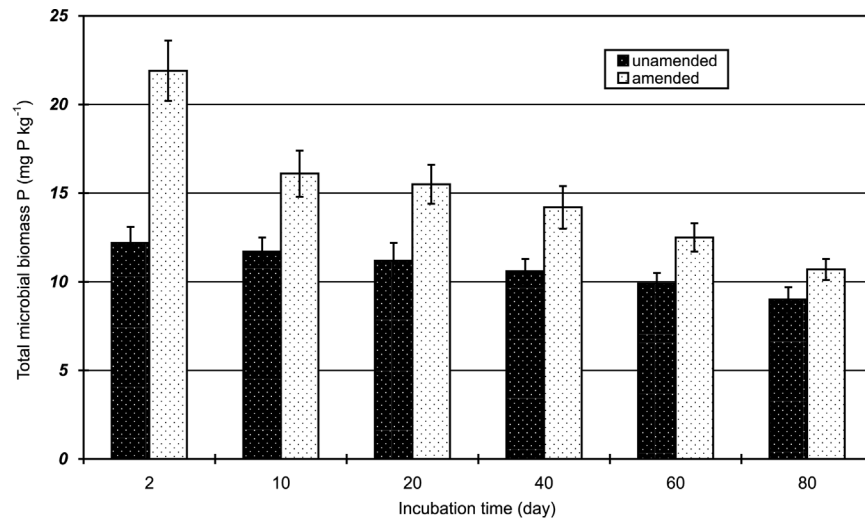


Figure 1. Dynamic change of microbial biomass P for soil No. 1 with and without substrate amendment (error bar indicates standard error).

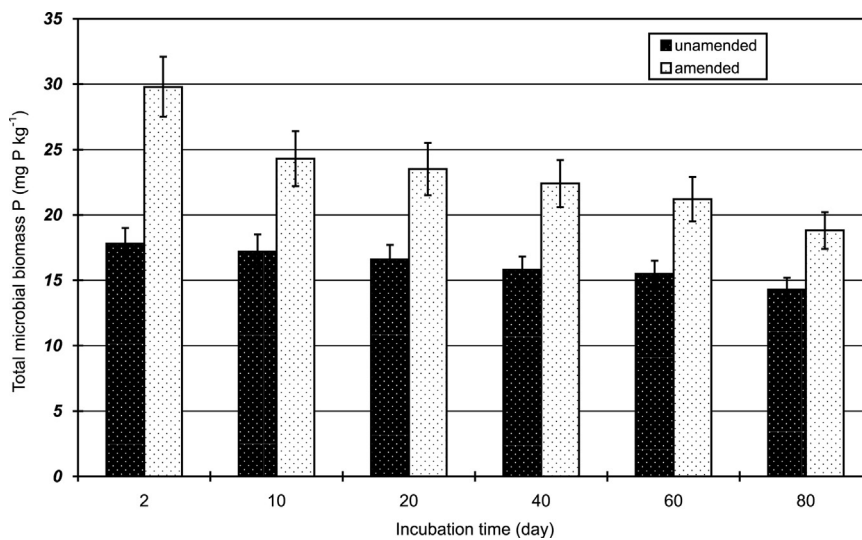


Figure 2. Dynamic change of microbial biomass P for soil No. 2 with and without substrate amendment (error bar indicates standard error).

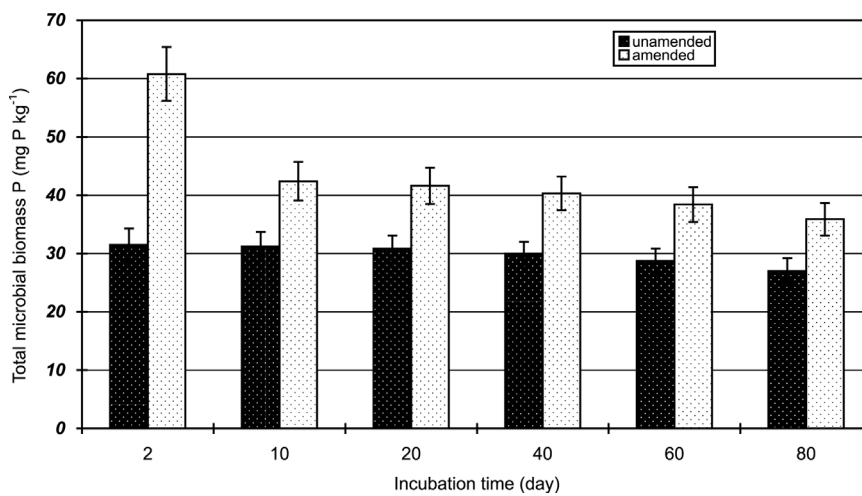


Figure 3. Dynamic change of microbial biomass P for soil No. 3 with and without substrate amendment (error bar indicates standard error).

Table 3. Dynamics of ^{32}P -Activity of Microbial Biomass P and $\text{HCl-NH}_4\text{F}$ -Extractable P of Unfumigated Soils During the Incubation

Soil No.	^{32}P -Activity of Microbial Biomass P (dpm μg^{-1} P)								^{32}P -Activity of $\text{HCl-NH}_4\text{F}$ -Extractable P (dpm μg^{-1} P)									
	Day 2	Day 10	Day 20	Day 40	Day 60	Day 80	Day 2	Day 10	Day 20	Day 40	Day 60	Day 80	Day 2	Day 10	Day 20	Day 40	Day 60	Day 80
1	917.9	810.5	781.2	632.3	470.6	300.4	517.8	485.4	500.3	516.9	520.3	525.1	360.4	296.3	300.6	317.9	320.2	325.4
2	662.8	590.4	510.6	445.2	370.3	289.4	360.4	296.3	300.6	317.9	320.2	325.4	290.5	224.8	230.6	248.9	263.5	273.4
3	480.1	425.2	400.3	352.2	300.7	243.3	290.5	224.8	230.6	248.9	263.5	273.4	290.5	224.8	230.6	248.9	263.5	273.4

Table 4. Measured Microbial Biomass ^{32}P , Metabolite-Synthesized Microbial Biomass ^{32}P , Apparent and Actual Rate Constants

Soil No.	Measured Microbial Biomass ^{32}P (mg P kg^{-1})						Microbial Biomass ^{32}P Synthesized from the Metabolites (mg P kg^{-1})						Rate Constant of Microbial Biomass ^{32}P Degradation ($\times 10^{-3}/\text{Day}$)	
	Day 2	Day 10	Day 20	Day 40	Day 60	Day 80	10-20	10-40	10-60	10-80	b (Apparent)	k (Actual)		
1	12.5 \pm 1.0	7.8 \pm 0.6	7.0 \pm 0.4	5.6 \pm 0.4	4.1 \pm 0.3	2.5 \pm 0.2	0.14	0.34	0.50	0.60	15.8	19.5		
2	15.8 \pm 1.3	11.3 \pm 1.2	10.6 \pm 1.1	8.9 \pm 0.6	7.3 \pm 0.6	5.4 \pm 0.3	0.07	0.24	0.37	0.49	10.4	11.7		
3	32.7 \pm 2.8	21.3 \pm 2.2	19.1 \pm 1.5	16.5 \pm 1.3	14.2 \pm 1.1	11.6 \pm 0.8	0.15	0.34	0.49	0.64	8.4	9.2		

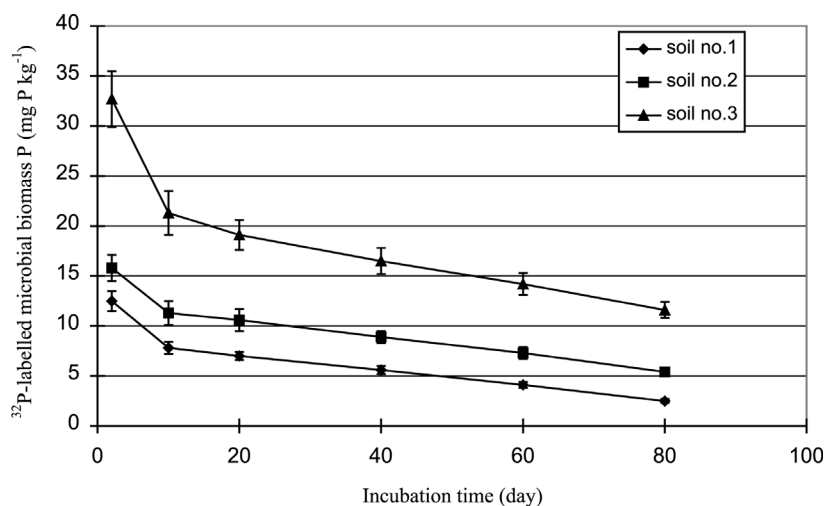


Figure 4. Dynamic change of ³²P-labeled microbial biomass P (error bar indicates standard error).

three soils (Fig. 5). Therefore, the 10th day after incubation was chosen as starting time for measuring turnover rate of Bp.

Calculating Turnover Rate of ³²P-Labeled Biomass Phosphorus in Soil

The apparent rate constant (*b*) for the degradation of Bp-³²P was obtained by fitting a first-order regression equation to the data of residual Bp-³²P measured at different times (Table 4). The *b* value was calculated according to the following formula:

$$b = -(\ln Y_{t_2} - \ln Y_{t_1}) / (t_2 - t_1) \quad (5)$$

where Y_{t_1} and Y_{t_2} are the Bp-³²P measured at time t_1 and t_2 , respectively. Assuming that the *b* value is constant from day 10 to day 80, the error caused by fitting *b* to the data was minimized by measuring the Bp and Bp-³²P at 20, 40, 60, and 80 days, respectively, after the incubation. The *b* value thus obtained was more accurate than that calculated from the Bp-³²P data measured from a single period (day 10 to day 80). In the same way, the actual rate constant of Bp-³²P degradation (*k*) in the soils was obtained by measuring the activity of Bp-³²P (Table 3) and then correcting them for the Bp-³²P activity synthesized from metabolites (Table 4).

As stated above, after 10 days of incubation, the turnover rate of Bp declined and quickly reached a relatively steady state. However, the decline in

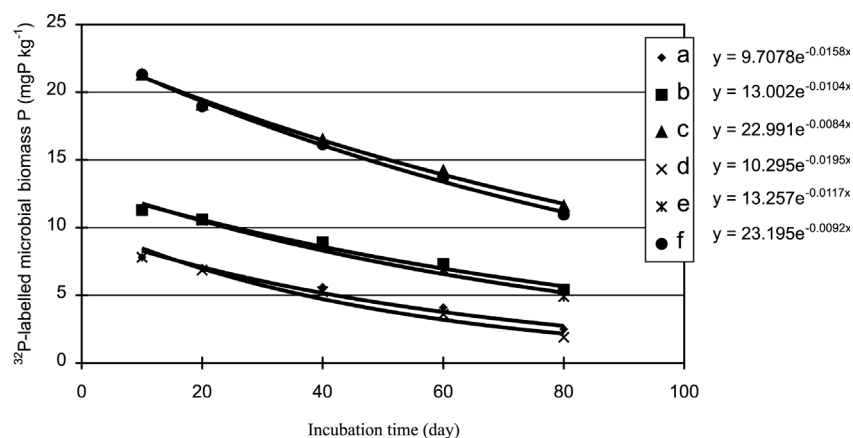


Figure 5. Decline of ^{32}P -labelled microbial biomass P and its first-order regression equation: a, b, and c are the apparent decline fitting curves and d, e, and f are the actual decline fitting curves of microbial ^{32}P for soil No. 1, 2, and 3, respectively.

total Bp during the 10–80 days of incubation was faster in substrate-amended soils than the unamended control soils (Table 5). This suggests that during this period, the turnover of Bp was still faster in the substrate-amended soils than the unamended soils. Therefore, the k value obtained from the amended samples might not represent the rate constant of Bp in the unamended soils, and needs to be further corrected for substrate effect. Given that the faster decline of total Bp in the substrate-amended soils mainly resulted from the enhanced turnover of Bp due to the residual effects of substrate incorporation, the turnover rate constant (k_u) of Bp in the unamended soil can be calculated from the following formula: $\Delta P = e^{-kt} - e^{-k_u t}$, where ΔP is the difference in the proportion of the total Bp

Table 5. The Corrected Turnover Rate Constants and Turnover Rates of Microbial Biomass P in the Unamended Soils

Soil No.	Percent Decrease in Bp (10–80 days) (%)		Rate Constants of Bp Turnover in the Unamended Soils (k_u) ($\times 10^{-3}/\text{day}$)	Turnover Rates Measured in Lab (days)	Turnover Rates Under Field Conditions (days)
	Unamended	Amended			
1	23.1	33.5	14.6	68	130
2	16.9	22.6	9.96	100	190
3	13.5	15.3	8.72	115	217

that declined between 10 and 80 days of the incubation between the substrate-amended soil and the unamended control, k and k_u are the turnover rate constants of Bp in the substrate-amended and the unamended soils, respectively, and t is the time of incubation in days starting at day 10.

Turnover Rates of Microbial Biomass Phosphorus

Table 5 shows the corrected turnover rate constants of microbial biomass P (Bp) and the corresponding turnover rates of the unamended soils (i.e., the native soil under laboratory conditions). The turnover rates of Bp were 68 days for the sandy soil (soil No. 1) and 100 and 115 days for the other two clayey soils (soil No. 2 and No. 3, respectively). However, the temperature (25°C) at which the turnover rates were obtained is higher than the average annual mean air temperature (17.5°C) in the fields where the soils were collected. Consequently, these turnover rates measured in the lab may be significantly different from those under field conditions. To correct the lab turnover rate for field conditions, the Q_{10} relationship, i.e., the Arrhenius equation was used in this study, provided that temperature is the dominant factor that influences the turnover rates.^[23] The Arrhenius equation is expressed as:

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (6)$$

where k_1 and k_2 are turnover rates corresponding to T_1 and T_2 . The Q_{10} is a factor resulting from a temperature difference of 10°C and can be obtained by determining k_1 and k_2 at T_1 and T_2 . A Q_{10} value of 2.344 was obtained from the data of Jenkinson et al.^[23] and used to calculate the turnover rates of microbial biomass P under field conditions. The obtained turnover rates of the Bp were 130, 190, and 217 days, respectively, for soil No. 1, No. 2, and No. 3 (Table 5). Microbial biomass P in these soils turned over 1.7 to 2.8 times in a year under field conditions. The sandy soil had a much greater Bp turnover rates than the two clayey soils.

DISCUSSION

The results from this study indicate that turnover rates of microbial biomass P (Bp) are related to soil texture. The soils with heavier texture had lower turnover rates, as compared with the light-texture soil (Tables 1 and 5). Measurements of the turnover rates of microbial biomass carbon and nitrogen using the same soils provided similar conclusions.^[11,12] These findings are consistent with previous observations by other workers.^[9,10,13] The lower microbial biomass turnover rate in a clayey soil is attributed to (1) greater

capacity to preserve or protect biomass; (2) closer interactions between microorganisms and products of their decay; and (3) a higher efficiency of utilization of added glucose and metabolic products by the soil biota.^[9] In addition, we observed that the clayey soil had a much greater microbial biomass C and P than the sandy soil at a very comparable organic carbon level (Table 1), and therefore, it took much longer to complete their turnover. The faster turnover of Bp in a sandy soil may serve as a good compensation to its smaller Bp pool for supply of available P to plants. The turnover rates of Bp in the three red soils were generally lower than those reported for other types of soil by Kouno et al.^[13] This may be due to a greater P adsorption capacity of the red soils than other types of soil, which decreases availability of P to the microorganisms.

The relationship between microorganisms and P availability in the red soils is tentatively described in Fig. 6. Microbial biomass serves as an immediate source or sink of P and as a driving force of P transformation and cycling.^[6] A close relationship between P availability and Bp has been observed in the red soils.^[24] Due to dominant variable-charge minerals, the red soils had a very high adsorption capacity for P.^[17,25] Microorganisms can enhance P availability by (1) providing organic acids (citric acid and oxalic acid, etc.) from decomposition of organic matter or metabolism,^[26] which can release adsorbed P by ligand exchange or chelating reaction;^[27] and (2) converting unavailable inorganic P into organic P including Bp and low molecular weight organic P,^[3] which are readily mineralized to provide available P for plant. Therefore, the size of Bp in red soils can be a useful measure of available P pool.

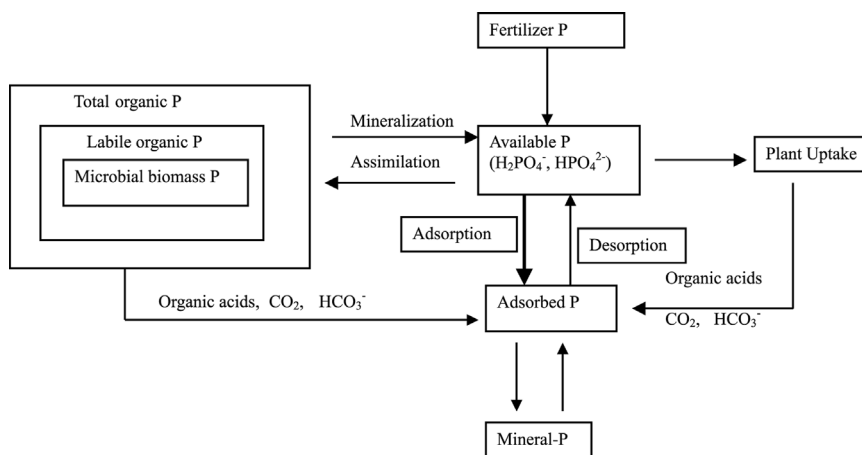


Figure 6. Soil P availability and microbial biomass P turnover relationships.

On the other hand, the turnover of Bp serves as a source of P supply to plants. The annual flux of P through soil biota can be roughly estimated by measuring Bp and its turnover rate. The amounts of Bp were 12, 17, and 31 mg kg⁻¹, respectively, for the three soils (soil No. 1, No. 2, and No. 3), which are equivalent to 33.6, 47.6, and 86.8 kg P ha⁻¹ at soil depth of 20 cm (assume a soil bulk density of 1.4 g cm⁻³). The corresponding turnover rates of Bp in the three soils were 130, 190, and 217 days. The calculated annual P fluxes through microbial biomass were 94, 91, and 146 kg P ha⁻¹, respectively. The amount of P removed by harvested crops from these soils is approximately 30 kg ha⁻¹ (data not shown). Therefore, microbial biomass turnover could provide a pool of potentially available P with the size of 3–5 times greater than that removed by the annually harvested crops from the soils. This suggests that the turnover of Bp could play a very important role in the plant-availability and cycling of soil P.

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