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Kinetics of microbial immobilization of phosphorus in a weathered subtropical soil following treatment with organic amendments and *Pseudomonas* sp.

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Abstract To understand the role of microbial processes in phosphorus (P) immobilization in a weathered subtropical soil, the effects of application of a phosphate-solubilization microorganism strain (*Pseudomonas* sp. 2VCP1) on P availability in soil, dynamics in microbial biomass P (*B_p*), microbial biomass C (*B_c*) and Olsen-P were investigated during a 60-d laboratory incubation. The included treatments were *P. sp.* inoculums at $\times 10^6$ cfu·g⁻¹ soil (CKM); glucose at 5 g·kg⁻¹ soil (G); G with *P. sp.* inoculum (GM); rice straw at 5 or 10 g·kg⁻¹ soil (5S or 10S); 5S and 10S with *P. sp.* inoculum (5SM and 10SM). The results indicated that the amount of soil *B_c* increased about 3.2, 1.7, and 2.6 times for G, 5S and 10S compared to the control (no organic amendment and *P. sp.*; CK), respectively. The amount of soil *B_p* for G and 10S almost doubled during the first 7 d, then remained relatively steady. The amount of Olsen-P in G, 5S and 10S showed a significant decrease (0–5.4 mg P·kg⁻¹ soil) during the 60-d incubation compared to CK. However, changes in soil *B_p* between the treatments inoculated with *P. sp.* (CKM, G, 5SM, 10SM) and the uninoculated controls (CK, G, 5S,

10S) were not significant during the 60-d incubation period, whereas a small increase in *B_p* of the GM treatment was seen up to day 11. The amount of soil *B_c* in CKM, GM, 5SM and 10SM had increased but not greater than 20% compared to their corresponding uninoculated control. The amount of Olsen-P increased but not greater than 0.88 mg P·kg⁻¹ soil. The result illustrated that there were a few effects on soil P immobilization following inoculation with *P. sp.* in the soil, whereas organic amendments can significantly motivate the soil native microorganisms to immobilize phosphorus.

Keywords soil microbial biomass, phosphorus-solubilization microorganism, organic substance

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1 Introduction

Phosphorus (P) is an essential element for plant nutrition but can only be assimilated by plants as a phosphate ion. However, if the soil phosphate ion concentration remains lower than a certain value, which varies by crop, a plant shortage of P may result. In natural conditions, P can be tightly bound with calcium, iron, or aluminum, leading to precipitation of P, and unavailable to plants (Li et al., 2003). The consequence is that P is commonly a limiting nutrient for plant growth in many soils, including highly weathered subtropical soils.

Highly weathered soils (mainly Ultisols, USDA Soil Taxonomy) cover a major area of the subtropical regions in China (Shi et al., 2006) and are an important land resource for agriculture. Highly weathered subtropical soils are characterized by a low total and available P content and a high P retention capacity (Friesen et al., 1997). Although large amounts of soluble P have been applied to soil as fertilizers, plants are able to use only a small portion of the applied phosphate, and the remainder is rapidly immobilized and becomes less available to plants. Enhancement of

the availability of P in soil is essential to rationalize the use of P fertilizers. This is especially important for highly weathered soils such as Oxisols and Ultisols with low crop uptake efficiency of fertilizer P (Oberson et al., 1999). In recent years, biologic resolution of P deficiency, as addressed by improving P availability and P application efficiency, has been highlighted as a good practice for P management for growing pasture grasses on highly weathered and P-deficient soils in tropical regions (Oberson et al., 1999; Oberson et al., 2001). Many intensive studies have been made to understand P transformations in highly weathered and P-deficient tropical soils, and these have consistently confirmed that P availability in these soils depends more on biologically mediated organic P transformation (mainly through microbial activities) than on the release of adsorbed inorganic P through chemical processes (Gijssman et al., 1997; Oberson et al., 1999; Buehler et al., 2002; Bünemann et al., 2004; Agbenin and Adeniyi, 2005; George et al., 2006).

Microbial biomass represents a dynamic pool, which is a major source of labile P in soil. Microorganisms play a central role in the biochemical transformation process of P from organic to inorganic forms to facilitate plant uptake. Many microorganisms possess phosphate-solubilizing ability and can convert insoluble phosphatic compounds into soluble forms in soil and make them available to crops. Application of phosphate-solubilizing microorganisms (PSM) has been reported to increase P uptake and plant growth. Particularly important is the role of ectotrophic mycorrhiza on plant roots aiding in mineralizing organic P in soil to increase plant available P; the main explanation for this is that the ectotrophic mycorrhiza can increase the root phosphate-absorbing sites (Bolan, 1991). Since certain PSM strains (such as *Aspergillus niger*, *Penicillium bilai*, *Azospirillum* sp., *Pseudomonas* sp., etc) are unstable with regard to their phosphate-solubilizing activity, once efficient phosphate-solubilizing organisms are selected, they are tested for their ability to solubilize insoluble phosphate in a liquid culture medium. Finally, the selected efficient phosphate-solubilizing cultures are used for making the inoculants, and their performance under green house or field conditions is tested against various crops (Cunningham and Kuiack, 1992; Chuang et al., 2007). The inoculated PSM that colonize the soil are actively engaged in P transformation in soil and transport phosphate to the plants (Zaidi et al., 2003, 2004; Zaidi and Khan, 2005).

However, microbial survival following introduction into natural soils depends on both abiotic and biotic factors, such as soil composition, physiologic status, temperature, pH, moisture content, the presence of recombinant plasmids, competition with native microbes, etc. The most critical factor among these is the ability to survive and multiply in soil; many inoculated microorganisms were often limited to colonize roots and survive in soil.

Therefore, the dynamics in microbial biomass P (B_p), microbial biomass C (B_c) and Olsen-P in a highly weathered subtropical soil following inoculation with PSM were investigated. We expect that our research will provide a reference for assessing the effects of application of PSM and organic matter on P availability.

2 Materials and methods

2.1 Soil, organic matter and PSM

A typical Ultisol (clay loam) developed from a quaternary red-earth parent material was selected from the site (E111°28'; N29°10') located in the middle of the subtropical region of China, with an annual mean temperature of 16.8°C and rainfall of 1330 mm. Samples were taken from the top 20 cm of soil and plant debris. All living tissues and any visible soil animals were carefully removed by hand. The soil was sieved (<2 mm) and adjusted to approximately 40% of field water holding capacity (WHC), pre-conditioned at 25°C and 100% humidity for 10 d, and then stored at 4°C until use. The soil contained 7.33 g·kg⁻¹ organic C, 0.96 g·kg⁻¹ total N, and 0.53 g·kg⁻¹ total P, and had a pH of 5.23 (in 1 mol·L⁻¹ KCl).

Rice straw was dried at 35°C and ground to <0.15 mm. It contained 439.9 g·kg⁻¹ C, 682.6 g·kg⁻¹ total N, 1.28 g·kg⁻¹ total P and 136.0 g·kg⁻¹ K. Glucose (contained 400 g C·kg⁻¹) was ground to <0.15 mm. Rice straw was used as the major source of organic material (OM) since it enhances microbial immobilization and transformations of P in soil (Wu et al., 2007). Glucose was used as a suitable carbon source for stimulating a rapid build-up of soil microbial biomass (Wu et al., 1993).

The phosphate solubilizing microorganism used was *P. sp.* 2VCP1 which had been previously identified as able to dissolve the mineral phosphate. The bacteria were isolated from the phosphorus microbial fertilizer provided by Water Science Department of China Agricultural University, cultured in an LB liquid medium for 5 d, centrifuged for 15 min at 2500 r·min⁻¹, washed five times with 0.85% NaCl solution, and then used as liquid inoculum. This inoculum contained 7.3×10^7 cfu·mL⁻¹ solution, as measured by plate counting using 0.85% NaCl solution.

2.2 Experiment design, incubation and sampling

Four replicates of eight treatments were established in a randomized block design; see Table 1.

Four replicates of pre-conditioned moist soil were weighed (1 kg, oven-dry) into plastic jars, and the soil, organic matter and *Pseudomonas* sp. inoculum were mixed thoroughly as per the requirements of each treatment. All samples were amended with (NH₄)₂SO₄ solution (50 g N·L⁻¹) to give 50 mg N·kg⁻¹ soil, and the soil moisture content was adjusted to approximately 45% of field water-holding

Table 1 Experiment design in this study

treatment	type and quantity ^a of amendment		
	rice straw/ (g·kg ⁻¹ soil)	glucose/ (g·kg ⁻¹ soil)	<i>Pseudomonas</i> sp./ (×10 ⁶ cfu·g ⁻¹ soil)
CK	0	0	0
CKM	0	0	1
G	0	5	0
GM	0	5	1
5S	5	0	0
5SM	5	0	1
10S	10	0	0
10SM	10	0	1

Note: ^a indicated the dry weight of rice straw and glucose applied in the soil, *Pseudomonas* sp. was applied as inoculum.

capacity (WHC) with distilled water and maintained at this level throughout the experiment. The jars (opened) were placed in polypropylene barrels (65 × 39 × 66 cm³) with 500 mL water to maintain 100% humidity and 100 mL 1 mol·L⁻¹ NaOH to trap CO₂ evolved from the soil. The barrels were sealed, and the soil jars were incubated at 25±1°C for 60 d. At 0, 3, 7, 11, 14, 21, 31, 45 and 60 d of incubation (respectively referred to as D0, D3, D7, D11, D14, D21, D31, D45 and D60); barrels were opened, and a sub-sample was taken from each jar to measure the amount of soil microbial biomass P (*B_p*) and C (*B_c*), and extractable P (Olsen-P). The jars were aerated at sampling time to maintain adequate O₂ levels.

2.3 Treatment

To determine soil microbial biomass C (*B_c*), the fumigation and extraction procedure was modified from Vance et al. (Vance et al., 1987). Briefly, portions of moist soil (20 g on an oven-dry basis) were fumigated by exposing soil to alcohol-free CHCl₃ vapor for 24 h in a vacuum desiccator. After CHCl₃ fumigation for 24 h, the CHCl₃ vapor was removed by vacuum extraction; the fumigated portions were extracted with 80 mL of 0.5 mol·L⁻¹ K₂SO₄ by shaking at 250 r·min⁻¹ for 30 min. The suspension was filtered using a filter paper. Also, equivalent portions of unfumigated soil were directly extracted with 80 mL 0.5 mol·L⁻¹ K₂SO₄ by shaking at 250 r·min⁻¹ for 30 min. Organic C in the extract was analyzed using a TOC (total organic carbon) analyzer (Phoenix-8000, Teledyne Tekmar, USA). The amount of soil *B_c* was calculated from the difference in the amount of organic C extracted from the fumigated soil and non-fumigated soil, using a conversion factor of 0.45 (Wu et al., 1990).

Soil microbial biomass P (*B_p*) was measured following the fumigation-extraction method (Brookes et al., 1982). Briefly, portions of moist soil (4 g on an oven-dry basis) were fumigated by exposing soil to alcohol-free CHCl₃ vapor for 24 h in a vacuum desiccator. After CHCl₃ was

removed by vacuum extraction, the fumigated portions were extracted with 80 mL 0.5 mol·L⁻¹ NaHCO₃ (pH 8.5) by shaking at 250 r·min⁻¹ for 30 min. Equivalent portions of unfumigated soil were directly extracted with 80 mL 0.5 mol·L⁻¹ NaHCO₃ (pH 8.5) by shaking at 250 r·min⁻¹ for 30 min. The suspension was filtered using a filter paper. Inorganic P in the extract was analyzed by colorimetric procedures according to Murphy and Riley (Murphy and Riley, 1962). We calculated the amount of soil *B_p* from the amount of extractable P in fumigated soil minus that in non-fumigated soil using the recovery efficiency of *B_p* (0.29) as the conversion factor (Wu et al., 2007). The recovery efficiency of *B_p* was determined by spiking soil (at 45% WHC; six replicates) with a suspension (1 mL; providing 25 mg biomass-P·kg⁻¹ soil) of cultivated microorganisms produced according to Wu et al. (2000). After spiking, the soil was fumigated and extracted, and inorganic P extracted was analyzed by colorimetric procedures.

Organic C in soil and rice straw was measured by a K₂CrO₇-H₂SO₄ oxidation procedure (Kalembasa and Jenkinson, 1973). Total N was estimated using the Kjeldahl method (Bremner, 1965), and total P was measured by NaOH fusion and colorimetric determination (Olsen and Somers, 1982). Soil WHC was measured by percolation tests, and pH was determined in 1 mol·L⁻¹ KCl at a soil-to-solution ratio of 1:2.5 (w/v).

2.4 Statistical analysis

There were four replicates per treatment, and two sets of analyses were performed per replicate, resulting in eight values for mean determination. Data were processed using Excel 2000 for the means, and the significance of the difference between means for different treatments at the same time or for different incubation times with the same treatment was analyzed by the ANOVA test at 0.05 and 0.01 probability using SPSS 11.5 software (SPSS Inc., Chicago, Illinois, USA).

3 Results

3.1 Changes in soil microbial biomass C

During the 60-d incubation period, the amount of *B_c* in the CK treatment was essentially constant (about 140–190 mg C·kg⁻¹ soil; Fig. 1). The amount of soil *B_c* in G, 5S, and 10S treatments increased 3.15, 1.74, and 2.63 times, respectively, as compared to the CK treatment by the D3. Following the initial increase in the first 3 d, the amount of soil *B_c* decreased from 510 to 270 mg C·kg⁻¹ soil ($P < 0.01$) between 3 and 14 d for G, and from 425 to 330 mg C·kg⁻¹ soil ($P < 0.01$) between 3 and 7 d for 10S, respectively, but no significant decline occurred in 5S, and the amount of soil *B_c* in G, 5S, and 10S remained generally constant during the remaining incubation period.

The amount of soil *Bc* in treatments with *Pseudomonas* sp. 2VCP1 inoculum had a significant increase at 30.22 mg C·kg⁻¹, 75.43 mg C·kg⁻¹, 36.30 mg C·kg⁻¹ and 22.89 mg C·kg⁻¹ soil for CKM, GM, 5SM, and 10SM, respectively, compared to their corresponding uninoculated control (CK, G, 5SM, 10SM) during the first 3 d ($P < 0.05$). The *Bc* levels in the CKM treatment remained constant (19.18 to 3.61 mg C·kg⁻¹ soil) after the initial increase compared to CK during the remaining incubation period. The amount of *Bc* in GM, 5SM and 10SM occurred a sharp decline and then demonstrated a constant increase to the level of uninoculated control (G, 5S, 10S) during the first 20 d, respectively, and then remained steady during the remaining incubation period.

3.2 Changes in soil microbial biomass P

The amount of soil *Bp* in CK gradually declined from 6.7 to 5.6 mg P·kg⁻¹ soil ($P < 0.05$) during the 60-d incubation period (Fig. 2). The amount of soil *Bp* in 5S remained generally constant during the 60-d incubation period but increased to two times larger than CK (4.5 mg P·kg⁻¹ soil; $P < 0.01$) by the end of incubation. During the first 3 d, the amount of soil *Bp* in G almost doubled, but no significant increase ($P > 0.05$) was found in 5S and 10S. However, the amount of soil *Bp* 10S doubled at D7; thereafter, the amount of soil *Bp* in both G and 10S remained almost constant throughout the 60-d incubation period.

Changes in soil *Bp* between treatments inoculated with

Pseudomonas sp. 2VCP1 (CKM; GM; 5SM; 10SM) and uninoculated controls (CK; G; 5S; 10S) showed a few significant differences during the 60-d incubation period except for GM ($P > 0.01$, Fig. 2). The amount of soil *Bp* increased about 2.67 mg P·kg⁻¹ soil for GM by 3 d, and a significant decrease (2.19 mg P·kg⁻¹ soil, $P < 0.01$) occurred in *Bp* between 3 and 14 d for GM, and thereafter remained almost steady throughout the 60-d incubation period, at a level equal to the uninoculated control (G).

3.3 Changes in Olsen-P

During the 60-d incubation period, the amount of Olsen-P in CK remained essentially constant at the level of about 12 mg P·kg⁻¹ soil. The amount of Olsen-P in G decreased by 3.5 mg P·kg⁻¹ ($P < 0.01$) in the first 3 d (Fig. 3), then maintained relatively constant at a level of about 8.2 mg P·kg⁻¹ during the remaining incubation period. The amount of Olsen-P in 5S and 10S remained at levels comparable to that of CK throughout the 60-d incubation period.

The amount of Olsen-P increased but not greater than 0.88 mg P·kg⁻¹ for CKM, GM, 5SM and 10SM, respectively, compared to their corresponding uninoculated controls (CK, G, 5S, 10S) throughout the 60-d incubation period; therefore, there was no significant difference ($P > 0.05$) between the treatments with *Pseudomonas* sp. 2VCP1 inoculum (CKM, GM, 5SM, 10SM) and the uninoculated controls (CK, G, 5S, 10S).

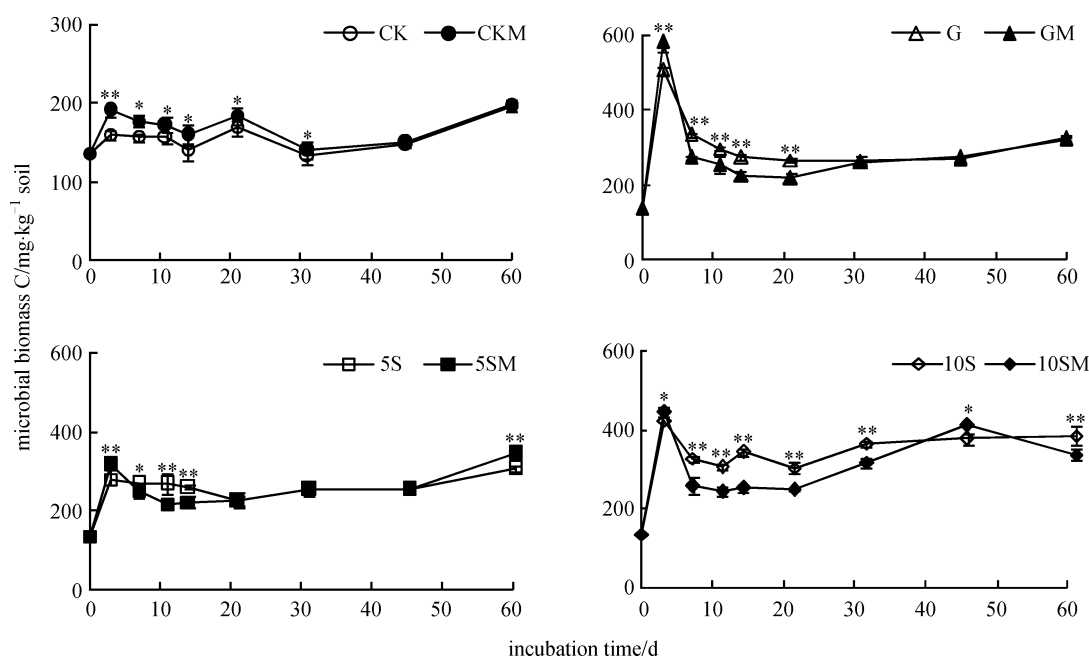


Fig. 1 Changes in soil microbial biomass C in the subtropical soil between the treatments with *Pseudomonas* sp. 2VCP1 (CKM, GM, 5SM, 10SM) and corresponding controls (CK, G, 5S, 10S) during 60-d incubation period

Note: Bars indicate the standard deviation of the mean, the same below. *, ** indicated significant difference between treatments amended with and without *Pseudomonas* sp. 2VCP1 at 0.05 and 0.01 level, respectively, the same below.

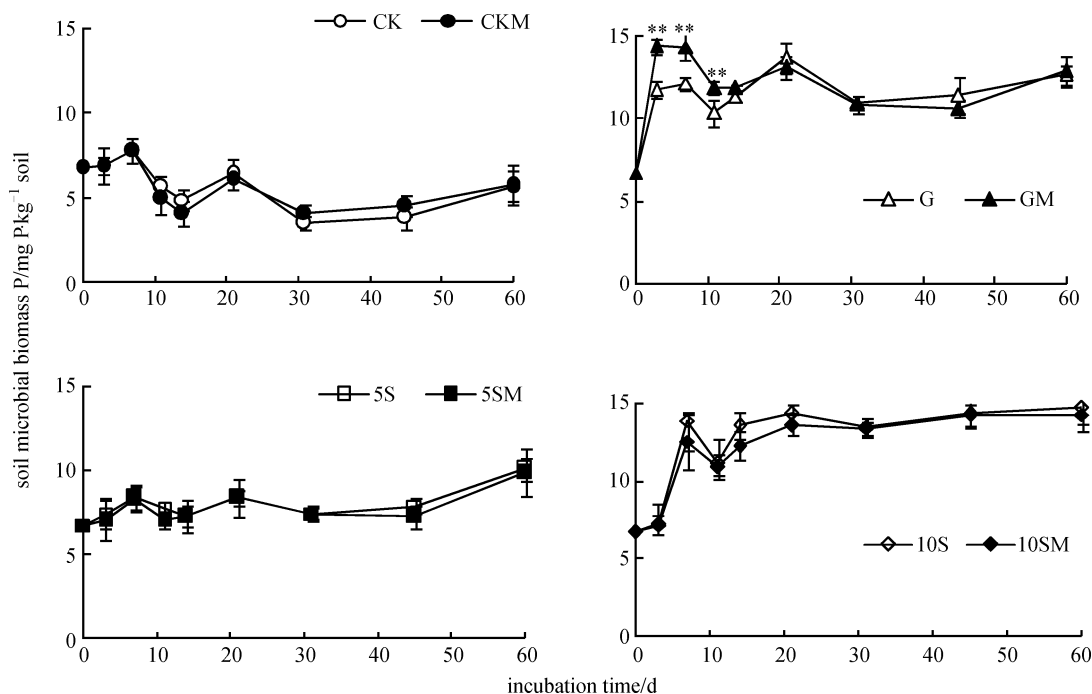


Fig. 2 Changes in soil microbial biomass P in the subtropical soil between the treatments with *Pseudomonas* sp. 2VCP1 (CKM, GM, 5SM, 10SM) and corresponding controls (CK, G, 5S, 10S) during 60-d incubation period

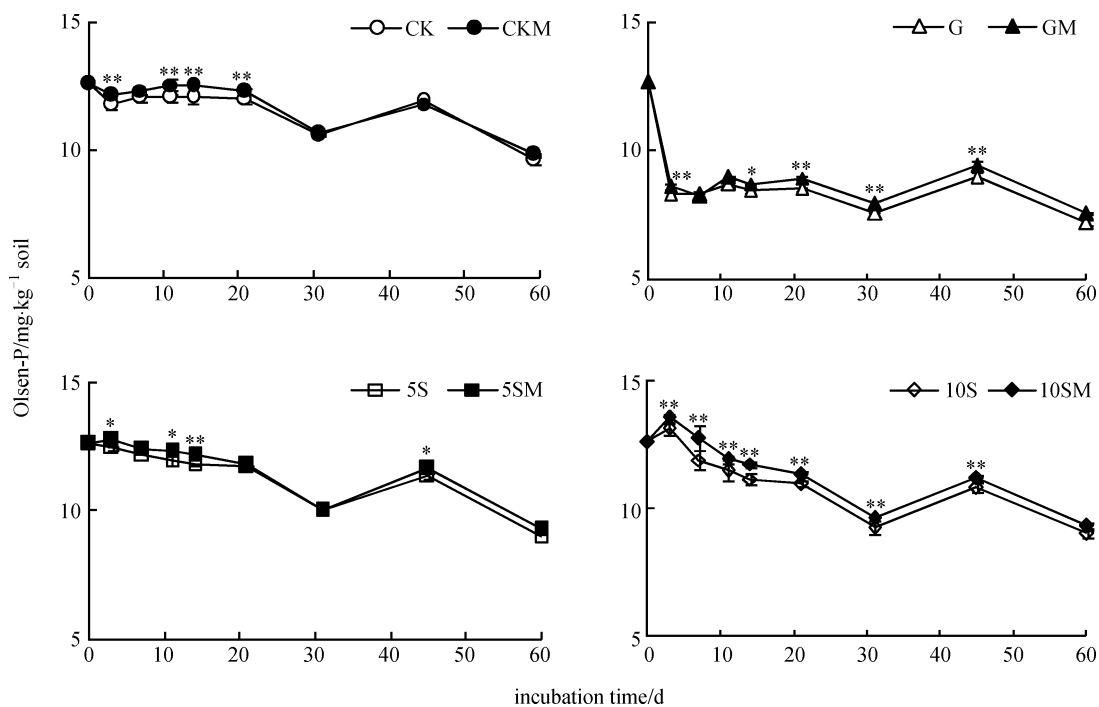


Fig. 3 Changes in Olsen-P in the subtropical soil between the treatments with *Pseudomonas* sp. 2VCP1 (CKM, GM, 5SM, 10SM) and corresponding controls (CK, G, 5S, 10S) during 60-d incubation period

3.4 Changes in C to P ratio of microbial biomass

The C-to-P ratio of microbial biomass implicates the potential to release or immobilize P. A biomass with a wide

C-to-P ratio is poor in P and has high potential to immobilize P from soil, whereas a narrow C-to-P ratio is P-enriched and has high potential to turnover and release P (Kwabiah et al., 2003). The biomass C-to-P ratio in CK

increased significantly (from initially 20:1 to 35:1) after 60-d incubation (Table 2). However, the biomass C-to-P ratio in soil with organic amendments (G, 5S, 10S) declined continuously and then recovered to lower and steady levels (about 20:1 to 35:1) after the first 3 d remarkably increased ($P < 0.01$) (Table 2); the results may indicate that part of soil microbial biomass P in CK was lost during the incubation, and the organic amendment could improve P immobilization in this soil.

The C-to-P ratios in treatments with and without *Pseudomonas* sp. 2VCP1 inoculum had few differences. However, there was a significant decline ($P < 0.01$) in GM in contrast to the case in G between D3 and D11 (Table 2), suggesting that the soil microbial biomass in GM would have a stronger potential to immobilize P when a highly available carbon source was sufficient. This may also provide a good explanation as why a substantial amount of P was immobilized while the size of biomass declined significantly during this period.

4 Discussion

4.1 Microbial immobilization of P in weathered subtropical soil following organic amendments

Amendments with G or rice straw both caused a rapid build-up of the microbial biomass in the subtropical soil used in this work. As shown in Fig. 1, the initial increase in the microbial biomass induced by glucose was almost three times larger than the rice-only treatment of 5S and even 15% over the treatment of 10S. After time, *Bc* in the G declined to a much larger extent (about 47%) than that amended with rice straw (about 9% and 25% for 5S and 10S, respectively). The results are in accord with the previous studies which suggested that glucose was much more effective in building up soil microbial biomass than a slowly decomposable substrate such as plant residue and cellulose (Wu et al., 1993; Kouno et al., 2002; Bünemann et al., 2004).

Previous studies have shown that the amount of *Bp* is

generally correlated with *Bc* in a wide range of soils (Brookes et al., 1984; Perrott et al., 1990). The substantial increase in *Bp* by glucose amendment (G) in the first 3 d (Fig. 2) was concomitant with the rapid build-up of the microbial biomass C (Fig. 1) as expected in the subtropical soil used in our work. However, this was not the case for 5S and 10S. The soil *Bp* in 10S remained unchanged in the first 3 d while the *Bc* in 10S increased, then *Bp* in 10S increased substantially (by 2.1 times) during D3 and D7, while *Bc* was decreasing (Fig. 1). The amount of *Bp* in 5S did not significantly increase (Fig. 2), although this rate supplied the same amount of organic C as G. In addition to this, P assimilated by microbial biomass in the subtropical soil used in this work following amendments with glucose and rice straw was maintained within the biomass throughout the 60-d incubation period (Fig. 2), while the initial build-up of the *Bc* in G and 10S decreased after D7 and D14. These results clearly indicate that amendments with plant residues (as rice straw used this study) in soil are not necessarily linked with the multiplication of the microbial biomass and P assimilation.

The prolonged immobilization of P in the biomass suggested that the microbial biomass has strong abilities to recycle P and to protect this part of P from physico-chemical fixation in the subtropical soil. Earlier observations made by Kouno et al. (2002) showed that the turnover rate of *Bp* in the temperate soil was three times faster than that of *Bc*, but the amount of the *Bp* remains almost constant throughout a 60-d period. Bünemann et al. (2004) indicated that the amount of the microbial biomass P in tropical soils remains relatively constant for over 60 d after the addition of organic materials as also reported by Oehl et al. (2001) and Kwabiah et al. (2003).

4.2 Microbial immobilization of P in weathered subtropical soil following organic amendments and *Pseudomonas* sp. 2VCP1

The results demonstrated that the increase in *Bc* for GM was greatest compared to G, followed by 5SM, CKM and 10SM as compared to 5S, CK and 10S, respectively,

Table 2 Dynamics of C-to-P ratio of microbial biomass during the 60-d incubation period in soil under different treatments (mean±SD)

treatment	incubation days							
	3	7	11	14	21	31	45	60
CK	23.7±1.1	20.5±0.4	28.4±1.6	29.3±1.8	26.8±2.0	38.9±3.2	39.1±0.5	34.8±0.2
CKM	28.0±1.8	23.2±1.9	35.5±2.5**	40.1±3.3**	30.7±2.5	35.3±2.6	33.8±0.9	35.0±1.3
G	43.3±2.5	27.6±1.5**	28.8±1.7**	24.2±1.6	19.5±1.7	24.4±2.0	23.6±1.5	25.8±1.9
GM	40.5±3.3	19.1±1.2	21.5±2.1	19.1±2.6	16.7±2.9	24.1±1.9	25.9±0.9	25.0±2.2
5S	37.9±3.9	31.8±0.5	34.4±3.6	37.3±1.2**	27.8±4.5	34.1±1.9	32.1±1.3	30.5±2.0
5SM	45.1±2.4	30.1±3.8	31.0±3.3	29.1±3.9	24.4±3.4	34.3±3.7	35.4±0.5	34.8±1.6
10S	58.7±2.0	23.6±2.1	27.6±2.6	25.2±3.8	21.2±2.2	27.0±3.0	26.2±3.0	26.1±2.8
10SM	62.5±3.3	20.6±2.4	22.4±3.3	20.7±2.3	18.3±0.8	23.7±3.3	28.9±0.5	23.7±4.0

Note: ** indicated significant difference between treatments amended with and without *Pseudomonas* sp. 2VCP1 at 0.01 level.

during the first 3 d as expected in the subtropical soil used in our work; then all declined to the control levels or lower. Such changes in the quantity of *Bc* may be attributed to different abilities in building up soil microbial biomass caused by differences in categories and quantity of carbon source input. The change in *Bc* of 10SM is a reflection of the dynamic in soil microbial communities, as some low molecular compounds, which may be easily soluble, would be assimilated by soil microorganisms initially, leading to a sharp increase in *Bc* quantity. Then, as the soluble compounds diminished, the *Bc* in soil declined. Later, the *Bc* showed a second increase due to the microbial immobilization of native microorganisms which could use large molecular organic compounds. The quantity of *Bc* in 10SM showed a second peak at D45 which may be attributed to the experimental error which needs further study.

Changes in soil *Bp* between treatments inoculated with *Pseudomonas* sp. 2VCP1 (CKM, GM, 5SM, 10SM) and control (CK, G, 5S, 10S) showed a few significant differences over the 60-d incubation period, whereas GM showed an increase during the first 11 d compared to G. Such changes in the amount of *Bp* may be attributed to the *Pseudomonas* sp. inoculum which had a small effect of building up *Bp*; however, glucose was a highly available carbon source for microbes, and *Pseudomonas* sp. 2VCP1 increased greatly using glucose at the early stages of incubation, then followed a steady decline as glucose was consumed. The amount of Olsen-p increased for the treatments with *Pseudomonas* sp., but did not exceed more than 0.88 mg·kg⁻¹ soil; therefore, inoculation with *Pseudomonas* sp. had a few effects on P assimilation by microbial biomass.

In this study, inoculation with *Pseudomonas* sp. 2VCP1 showed no significant effects on enhancing the P microbial immobilization possibly due to the following reasons: 1) difficulty in survival and colonization of inoculated *Pseudomonas* sp. in the soil; 2) competition with native microorganisms; 3) nature and properties of soils were unfit for the immobilization of inoculated *Pseudomonas* sp.; 4) insufficient nutrients in the soil to produce enough organic acids to solubilize phosphorus, and 5) inability of *Pseudomonas* sp. to solubilize soil phosphorus. It has been shown that at least two PSM showing phosphate solubilization in laboratory conditions were unable to release phosphate from alkaline vertisols even when supplemented with other nutrients (Gyaneshwar et al., 1998). Besides, it has been observed that phosphate-solubilizing bacteria, upon repeated sub-culturing, lose this phosphate-solubilizing activity (Kucey, 1983). It has been reported that the growth of introduced, freely suspended bacterial cells in soils characterized by unaffected microbial activity was a rare phenomenon, and inoculum strategies should include the application of carrier materials aimed at providing a protective niche together with the provision of nutrient sources (Vassilev et al., 2001).

In conclusion, there were a few effects on enhancing soil P microbial immobilization following inoculation with *Pseudomonas* sp. 2VCP1 in the native Ultisol. However, some organic amendments did significantly motivate the native soil microbes to immobilize phosphorus.

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