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RESEARCH ARTICLE



## Effects of phosphate-solubilizing bacteria application on soil phosphorus availability in coal mining subsidence area in Shanxi

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### ABSTRACT

The effect of phosphate-solubilizing bacteria (PSB) application on phosphorus (P) availability in reclaimed soil in coal mining subsidence region was investigated. Seven treatments were carried out including control, chicken manure (CM), PSB, PSB + tricalcium phosphate (TCP), CM + TCP, PSB + ground phosphate rock (GPR) and CM + GPR. The results showed soil Olsen-P concentration and phosphatase level as well as the yield of pakchoi (*Brassica chinensis* L.) were significantly higher in PSB application treatments compared to the corresponding CM application treatments. Soil phosphatase, invertase and urease contents were increased most significantly in PSB treatment, 1.18-, 1.31- and 2.32-fold higher than those in the control, respectively. Soil Ca<sub>2</sub>-P, Ca<sub>8</sub>-P, Fe-P and Al-P concentrations exhibited the greatest increases in PSB + TCP treatment, while occluded-P showed minor changes in different treatments. Application of PSB fertilizer reduced the transformation of Olsen-P to Ca<sub>10</sub>-P, thus increasing P availability in reclaimed soil of coal mining subsidence area.

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### KEYWORDS

Phosphate-solubilizing bacteria; soil reclamation; phosphorus fractionation; phosphorus adsorption-desorption; coal mining subsidence area

## 1. Introduction

Large-scale coal mining has inevitably led to the destruction of the farmland. The destroyed cropland in coal mining subsidence areas is characterized by low fertility (Chen et al. 1996), poor structure and serious compaction (Wei et al. 2001), poor soil productivity and low crop yields (Hu et al. 1997). Soil reclamation and utilizations can be a solution which helps recover soil function and improve eco-environment in coal mining subsidence area. However, improving soil fertility is a critical factor that determines the success of land reclamation in coal mining subsidence areas (Li et al. 2007; Liu & Lu 2009). Phosphorus (P) is one of essential macro-minerals for the growth and development of plant (Schachtman et al. 1998), it plays an importance role as the component of DNA, RNA, ATP and the phospholipids (Rodríguez & Fraga 1999). Soil P concentration is one of the most important limiting factors for soil fertility (Hu & Wei 2003). Generally, the P content of soil is ~0.05%, but only 0.1% of them can be utilized by plants (Scheffer & Schachtschabel 1992). About 56.17% of total arable land had low P concentration in soil that is, 5 mg/kg in Shanxi Province (Liu & Zhang 1992), and there may be a more inferior soil P concentration in coal mining subsidence areas. Therefore, it is necessary to improve soil available P concentration and to study the mechanism of soil P fractionation in coal mining subsidence areas.

Phosphate-solubilizing bacteria (PSB) play a crucial role in soil P solubilization (Abd-Alla 1994), increasing the bioavailability of soil P for plants (Zhu et al. 2011). PSB can transform insoluble P to available P in the soil, so as to improve fertilizer use efficiency and crop yield (Hao et al. 2006; Jiang et al. 2012; Hu et al. 2012). Application of PSB into reclaimed soil is a biotechnical practice for comprehensive management and

improvement of reclaimed soil. This practice can promote the ripening of soil and increase soil available P content and other nutrients, thus shortening the rehabilitation period. Previous studies regarding the application of PSB in reclaimed soils have primarily focused on increasing soil P availability and biological activity. Liang et al. (2010) assessed the effect of different fertilizer treatments on the ripening of reclaimed soil in a coal mining subsidence area in Jincheng, Shanxi Province, China. The results presented that organic manure + chemical fertilizer + PSB biofertilizer was most beneficial to soil ripening (Liang et al. 2010). Li et al. (2014) conducted pot experiments to assess the effect of different doses of PSB biofertilizer on the biochemical capacity of soil microorganisms and relevant enzyme activities in coal mining subsidence area. The results showed that the application of PSB biofertilizer markedly enhanced the biochemical capacity and enzyme activities in the reclaimed soil. Thus far, few studies have assessed the effect of PSB on P adsorption-desorption and the fractionation of P forms in reclaimed soils. To address the above issue, this study was planned to assess the effect of PSB on the fractionation and desorption characteristics of inorganic P forms in reclaimed soil, in order to provide a reference for increasing P availability and guidance for applying PSB biofertilizer in reclaimed soil.

## 2. Materials and methods

### 2.1. Site description

The experiment was carried out in farmland from coal mining subsidence in Xiangyuan Country (113.02°35.21'E, 36.28°13.28'N) of Shanxi Province, China. The collapse-fissure in the coalmining subsidence area was filled and leveled

as reclaimed farmland to cultivate vegetable in March 2013. The site has a continental climate with an average annual temperature of 9.5°C and an average annual rainfall of 532.8 mm. The pakchoi (*Brassica chinensis* L.) was sown in 10 May 2014 in the study. Principal chemical properties of soil at 0–20 cm depth before sowing were as follows: 9.45 g/kg of soil organic matter, 0.31 g/kg of total nitrogen, 17.8 g/kg of total potassium, 0.43 g/kg of total phosphorus, 18.74 mg/kg of available nitrogen, 4.35 mg/kg of available P and 8.21 of pH. In addition, soil inorganic P fractionation were as follows: 5.01 mg/kg of Ca<sub>2</sub>-P, 10.5 mg/kg of Ca<sub>8</sub>-P, 37.4 mg/kg of Al-P, 21.4 mg/kg of Fe-P, 32.8 mg/kg of occluded-P and 195.3 mg/kg of Ca<sub>10</sub>-P.

## 2.2. Experimental design

The experiment was laid out according to a randomized complete block design with three replications, and the plot size of 225 m<sup>2</sup>. Seven fertilizers treatments included a control with no fertilizer (Control), phosphate-solubilizing bacteria fertilizer (PSB, dosage: 1500 kg/ha), chicken manure (CM, dosage: 1500 kg/ha), phosphate-solubilizing bacteria fertilizer with tricalcium phosphate (PSB + TCP, dosage: 1500 kg/ha, 750 kg/ha, respectively), chicken manure with tricalcium phosphate (CM + TCP, dosage: 1500 kg/ha, 750 kg/ha, respectively), phosphate-solubilizing bacteria fertilizer with ground phosphate rock (PSB + GPR, dosage: 1500 kg/ha, 750 kg/ha, respectively) and chicken manure with ground phosphate rock (CM + GPR, dosage: 1500 kg/ha, 750 kg/ha, respectively). Cultivar of the pakchoi was Siyue-man with a growth period of 40 days. The pakchoi was sown on 10 May 2014 and harvested on 20 June 2014. Composted CM included 54.8% of organic matter, 2.01% of N, 1.06% of P<sub>2</sub>O<sub>5</sub> and 1.91% of K<sub>2</sub>O. Total P contents of GPR (Ji'nan Hongju Chemical Co. Ltd.) and TCP (Lianyungang Kexin Chemical Co. Ltd.) were 19.8% and 20%, respectively. In addition, the PSB fertilizer was manufactured by the following methods. Three phosphate-solubilizing bacteria were isolated and purified from a calcareous cinnamon soil in Taiyuan, Shanxi Province, China, which were supplied by Shanxi Agricultural University. All the three isolates were identified to be *Pseudomonas fluorescens* according to the morphological, physiological and biochemical properties and the analysis of the 16S RNA gene sequence. The isolates exhibited no antagonistic activity against each other. Three PSB isolates were cultured by mixed fermentation in a fermenter. The culture broth was prepared with the beef extract-peptone medium. After fermentation, the PSB fertilizer was obtained through mixing up PSB culture and composted CM at a ratio of 1: 9 (v/w).

## 2.3. Soil sampling and analysis

After harvest of pakchoi, plant samples were collected to determine the yield. Meanwhile, soil sample were collected from a depth of 20 cm using a coring tube (5 cm in diameter), mixed and passed through a <2 mm sieve to discard the plant stubbles and pebbles from the soil sample. Some of the soil samples were stored at 4°C in a mobile refrigerator to determine relevant enzyme activity, others were air-dried to determine Olsen-P and inorganic P fractionations. Soil urease, phosphatase and invertase activities were measured by colorimetry using phenol sodium, disodium phenyl phosphate and

dinitrosalicylic acid, respectively (Guan 1986). Soil Olsen-P concentration was determined by 0.5 mol/L NaHCO<sub>3</sub> extraction and Mo-Sb colorimetry.

The fractionation of soil inorganic P was performed in accordance with the calcareous soil classification scheme proposed by Jiang and Gu (1989). P adsorption isotherm studies were performed under conditions of initial P concentration ranging in 0–150 mg/L, reaction time 25 h (oscillated for 1 h and stand for 24 h), at 28°C. All batch experiments were conducted in 50 mL centrifuge tubes by taking 200 mg (dry weight) of 1-mm-sieved soil. Toluene-3,5-d was added into each tube to inhibit soil microbial activity.

P concentration in the equilibrium solution was measured by Mo-Sb colorimetry. Difference between the P added and the P in the equilibrium solution was obtained as the amount of soil P adsorption. The maximum P adsorption capacity and adsorption energy constant were obtained by fitting Langmuir equations.

For P desorption experiment, the liquid in the centrifuge tubes was decanted and the soil at the bottom of the tubes was washed twice with 25 mL of saturated NaCl solution. Care was taken to make sure that the soil was stirred from the bottom of the centrifuge tube. After two washes, each centrifuge tube was added with 25 mL of 0.01 mol/L CaCl<sub>2</sub> solution, followed by toluene-3, 5-d. The mixture was oscillated at 28°C for 1 h and allowed to stand for 24 h before centrifugation (10 min, 3500 r/min). The supernatant was collected to measure P concentration by Mo-Sb colorimetry. The amount of soil P desorption was calculated according to the difference of P concentration in supernatant between P adsorption isotherm experiment and P desorption experiment. Then, the ratio of P desorption to P adsorption was obtained as the desorption rate of soil P. All experimental were run in triplicate.

## 2.4. Statistical analysis

Analysis of variance (ANOVA) and correlation were performed using the SAS V8.1 (SAS Institute). A one-way ANOVA was conducted to test the effects of fertilizer treatments on the yield, Olsen-P, enzyme activities and inorganic P fractionation. The LSD (least significant difference) method ( $P < .05$ ) was used to assess the differences among different fertilizer treatments. The correlation analysis was used to study the significance of relationships between inorganic P forms and available P concentration. Differences were considered significant if  $P < .05$ .

## 3. Results

### 3.1. Effect of PSB application on Olsen-P and pakchoi yield in reclaimed soil

The Olsen-P concentration and rape yield in reclaimed soil under different fertilizer application treatments is shown in Table 1. The Olsen-P concentration was significantly higher as compared to the control. The increase of Olsen-P concentration was 35.11% in PSB treatment, indicating that PSB application effectively increased P availability in reclaimed soil. The highest Olsen-P concentration occurred in PSB + TCP treatment, 23.31 mg/kg, which was 28.5% higher than that in PSB treatment.

Pakchoi yield was higher with fertilizer application relative to the control. The increase of pakchoi yield was more

**Table 1.** The Olsen-P concentration and pakchoi yield in reclaimed soil under different fertilizer treatments.

Treatment	Soil Olsen-P (mg/kg)	Pakchoi yield (kg/ha)
Control	4.27 ± 0.19e	1601.6 ± 117.9f
CM	10.81 ± 1.26cd	1862.0 ± 154.0ef
PSB	16.66 ± 1.31b	2217.6 ± 141.4cd
PSB + TCP	23.31 ± 1.42a	2844.8 ± 167.5a
CM + TCP	12.34 ± 0.71c	2626.4 ± 133.8ab
PSB + GPR	11.83 ± 0.26c	2377.2 ± 117.9bc
CM + GPR	10.33 ± 1.01d	2038.4 ± 97.4de

Note: Control, no fertilizer; CM, chicken manure; PSB, phosphate-solubilizing bacteria fertilizer; TCP, tricalcium phosphate; GPR, ground phosphate rock. Different lowercase letters following the digit in the same column indicate significant difference at  $P < .05$  among treatments.

**Table 2.** The Phosphatase, invertase and urease levels in reclaimed soil under different fertilizer treatments.

Treatment	Phosphatase (mg $P_2O_5$ 100 $g^{-1}$ 2 $h^{-1}$ )	Invertase (mg glucose $g^{-1}$ 24 $h^{-1}$ )	Urease (mg $NH_3-N$ $g^{-1}$ 24 $h^{-1}$ )
Control	1.08 ± 0.15c	8.69 ± 1.83e	0.17 ± 0.02d
CM	1.88 ± 0.17b	13.68 ± 2.00bc	0.40 ± 0.03b
PSB	2.36 ± 0.27a	18.52 ± 1.90a	0.56 ± 0.08a
PSB + TCP	1.68 ± 0.19b	11.45 ± 0.87cd	0.43 ± 0.06ab
CM + TCP	1.13 ± 0.09c	9.79 ± .83de	0.32 ± 0.04c
PSB + GPR	1.68 ± 0.19b	11.80 ± 0.51c	0.51 ± 0.07a
CM + GPR	1.26 ± 0.11c	13.33 ± 0.65b	0.30 ± 0.03c

Note: Control, no fertilizer; CM, chicken manure; PSB, phosphate-solubilizing bacteria fertilizer; TCP, tricalcium phosphate; GPR, ground phosphate rock. Different lowercase letters following the digit in the same column indicate significant difference at  $P < .05$  among treatments.

significant in PSB treatment than in CM treatment alone, and the former gained a 16.3% increase compared to the latter. The highest pakchoi yield occurred in PSB + TCP treatment, 2844.8 kg/ha. Pakchoi yield in different treatments followed the order of PSB + TCP > CM + TCP > PSB + GPR > PSB > CM + GPR > CM > Control.

### 3.2. Effect of PSB application on soil enzyme activities in reclaimed soil

The enzyme levels in reclaimed soil under different treatments are shown in Table 2. Upon PSB application, soil phosphatase level exhibited a significant increase in PSB treatment (25.2%) versus CM treatment, as well as in PSB + TCP treatment (49.2%) versus CM + TCP treatment. Moreover, there was a 33.5% increase of soil phosphatase level in PSB + GPR treatment versus CM + GPR treatment. Soil phosphatase levels in different treatments of PSB application were higher than those in the corresponding treatments of CM application, indicating that PSB bio-organic fertilizer significantly increased alkaline phosphatase level in reclaimed soil. The highest soil phosphatase level was 2.36 mg  $P_2O_5$  100  $g^{-1}$  2  $h^{-1}$  in PSB treatment.

Soil invertase level in different fertilizer treatments followed the order of PSB > CM > CM + GPR > PSB + GPR > PSB + TCP > CM + TCP > Control. Higher levels of soil invertase were obtained with fertilizer application compared to the control. The highest value occurred in PSB treatment, 18.52 mg glucose  $g^{-1}$  24  $h^{-1}$ , which was significantly higher (35.4%) than that in CM treatment. As can be seen the application of PSB fertilizer effectively increased soil invertase level.

Soil urease level was also significantly higher with fertilizer application compared to the control. The highest soil urease level was found in PSB treatment (0.56 mg  $NH_3-N$   $g^{-1}$  24  $h^{-1}$ ) followed by PSB + GPR (0.51 mg  $NH_3-N$   $g^{-1}$  24  $h^{-1}$ ). Different treatments of PSB application obtained higher levels of soil urease than the corresponding treatments of CM application. These results showed that PSB application effectively increased soil urease level during reclamation.

### 3.3. Effect of PSB application on P desorption characteristics in reclaimed soil

The adsorption and desorption constants of soil P are listed in Table 3. Among different treatments, the maximum adsorption capacity and maximum buffer capacity of soil P in the control (909 mg/kg and 62.72, respectively) were significantly higher than those in the other treatments. The same trend was observed in the adsorption constant (0.069 for control), indicating that the reclaimed soil had high capacity to fix and adsorb exogenous P sources.

The maximum adsorption capacity and adsorption constant of soil P in CM treatment were significantly lower than those in the control by 354 mg/kg and 0.016, respectively, indicating that CM effectively reduced P adsorption and fixation in the reclaimed soil. The maximum adsorption capacity was the lowest in PSB treatment, 526 mg/kg. This value was significantly lower (29 mg/kg) than that in CM treatment, indicating that PSB application further reduced the adsorption and fixation of exogenous P sources and thus improved the utilization rate of P fertilizer in reclaimed soil.

The average desorption rate of soil P was significantly higher with fertilizer application compared to the control. Different treatments followed the order of PSB + TCP > PSB > CM + TCP > PSB + GPR > CM > CM + GPR > Control. The rate in PSB treatment was 4.8% and 1.3% higher than those of the control and CM treatment, indicating that PSB application significantly increased the P adsorption capacity of reclaimed soil. The highest average desorption rate of soil P was observed in PSB + TCP treatment, 17.3%, which showed significant increases compared to the other treatments. Clearly, combined application of PSB and TCP significantly enhanced the P desorption capacity in reclaimed soil.

**Table 3.** P adsorption and desorption parameters in reclaimed soil under different fertilizer treatments.

Treatment	Langmuir adsorption isotherm	Max adsorption capacity (mg/kg)	Adsorption constant	Max. buffering capacity (mL/g)	Max desorption rate (%)	Average desorption rate (%)
Control	$y = 0.0011x + 0.016$	909a	0.069a	62.72a	15.0d	9.9e
CM	$y = 0.0018x + 0.034$	555c	0.053bc	29.42cd	18.5c	13.4cd
PSB	$y = 0.0019x + 0.041$	526c	0.047c	24.72de	27.6a	14.7b
PSB + TCP	$y = 0.0016x + 0.049$	625b	0.033d	20.63e	22.8b	17.3a
CM + TCP	$y = 0.0016x + 0.033$	625b	0.048c	30.00cd	17.1cd	14.4bc
PSB + GPR	$y = 0.0015x + 0.028$	667b	0.053bc	35.35bc	23.7b	14.3bc
CM + GPR	$y = 0.0015x + 0.024$	667b	0.063ab	42.02b	16.6cd	13.1d

Note: Control, no fertilizer; CM, chicken manure; PSB, phosphate-solubilizing bacteria fertilizer; TCP, tricalcium phosphate; GPR, ground phosphate rock. Different lowercase letters following the digit in the same column indicate significant difference at  $P < .05$  among treatments.

**Table 4.** Inorganic P forms and concentrations in reclaimed soil under different fertilizer treatments.

Treatment	Inorganic P forms and concentrations (mg/kg)					
	Ca <sub>2</sub> -P	Ca <sub>8</sub> -P	Al-P	Fe-P	Occluded-P	Ca <sub>10</sub> -P
Control	3.33 ± 0.85f	7.31 ± 0.59f	20.51 ± 0.54e	25.71 ± 1.47d	33.24 ± 2.89b	194.86 ± 13.12c
CM	9.73 ± 1.31de	13.07 ± 1.59e	27.99 ± 2.99d	33.13 ± 1.65c	35.19 ± 2.44b	203.47 ± 14.10c
PSB	18.16 ± 2.04b	24.66 ± 2.12d	51.33 ± 0.94c	45.92 ± 2.35b	33.91 ± 1.97b	184.15 ± 11.73c
PSB + TCP	23.47 ± 2.48a	105.14 ± 2.48a	94.86 ± 0.42a	60.61 ± 3.21a	60.64 ± 6.18a	223.33 ± 10.72b
CM + TCP	11.63 ± 2.27cd	41.19 ± 2.27b	70.92 ± 3.43b	57.55 ± 2.86a	64.93 ± 6.65a	295.44 ± 14.11a
PSB + GPR	14.55 ± 1.13c	29.42 ± 2.46c	34.25 ± 3.36d	35.65 ± 1.71c	35.23 ± 3.71b	296.67 ± 2.09a
CM + GPR	8.10 ± 1.30e	9.69 ± 3.03f	30.34 ± 3.08d	34.49 ± 3.37c	33.98 ± 2.21b	310.88 ± 9.91a

Note: Control, no fertilizer; CM, chicken manure; PSB, phosphate-solubilizing bacteria fertilizer; TCP, tricalcium phosphate; GPR, ground phosphate rock. Different lowercase letters following the digit in the same column indicate significant difference at  $P < .05$  among treatments.

### 3.4. Effect of PSB application on inorganic P fractionation in reclaimed soil

The concentrations of various inorganic P forms in two-year reclaimed soil under different treatments are shown in Table 4. Ca<sub>2</sub>-P, Ca<sub>8</sub>-P, Al-P and Fe-P concentrations were significantly higher with fertilizer application than with the control. The values in PSB treatment were higher than those in CM treatment by 8.43, 11.59, 23.34 and 12.79 mg/kg, indicating that the PSB fertilizer was more effective than organic manure alone in facilitating the transformation of the four inorganic P forms.

Ca<sub>2</sub>-P, Ca<sub>8</sub>-P and Al-P concentrations in PSB + TCP treatment were significantly higher than those in the other treatments. The increases were 1.02-, 1.55- and 0.34-fold compared with CM + TCP treatment, suggesting that the combined application of PSB and TCP substantially improved soil P transformation towards to the three inorganic P forms. An exception was Fe-P concentration, which had no significant change between PSB + TCP and CM + TCP treatment.

No significant difference in occluded-P concentration was observed between PSB and CM treatments with or without TCP or GPR. This result indicated that PSB fertilizer had little effect on soil occluded-P concentration. As for Ca<sub>10</sub>-P concentration, PSB treatment was 19.32 mg/kg lower than CM treatment, indicating that PSB application reduced Ca<sub>10</sub>-P concentration in reclaimed soil. The possible mechanism is that the PSB could solubilize Ca<sub>10</sub>-P in the soil and thus prevent P transformation towards insoluble Ca<sub>10</sub>-P.

### 3.5. Correlation between inorganic P forms and available P in reclaimed soil

The correlation coefficients between inorganic P forms and available P concentrations in reclaimed soil are presented in Table 5. Olsen-P concentration was positively correlated with Ca<sub>2</sub>-P concentration at the highly significant level ( $r = 0.971$ ,  $P < .01$ ). Moreover, Olsen-P exhibited a significant correlation with Ca<sub>8</sub>-P ( $r = 0.864$ , Al-P ( $r = 0.862$ ) and Fe-P ( $r = 0.823$ ), but not with occluded-P ( $r = 0.543$ ) or Ca<sub>10</sub>-P ( $r = 0.097$ ).

**Table 5.** Correlation coefficients between inorganic P forms and available P concentration in reclaimed soil under fertilization treatments.

Olsen-P (mg/kg)	Ca <sub>2</sub> -P (mg/kg)	Ca <sub>8</sub> -P (mg/kg)	Al-P (mg/kg)	Fe-P (mg/kg)	Occluded-P (mg/kg)	Ca <sub>10</sub> -P (mg/kg)
Olsen-P (mg/kg)	0.971**	0.864*	0.862*	0.823*	0.543	0.097

\* $P < .05$ , \*\* $P < .01$ .

## 4. Discussion and conclusions

### 4.1. Effect of PSB on soil available P

Previous research has shown that functional strains in PSB fertilizer can facilitate the transformation of insoluble P to available P in the soil; this mechanism will increase available P concentration and soil P availability to crops, contributing to crop growth and yield (Gao et al. 2006; Hu et al. 2012). The results from the present study showed that soil available P (Olsen-P) concentration and pakchoi yield were significantly higher in different treatments of PSB application than the corresponding CM treatments. PSB fertilizer clearly increased soil Olsen-P concentration and pakchoi yield in reclaimed soil of coal mining subsidence area, and greatest increases were obtained by a combined application of PSB and TCP. Such an increasing effect was not observed with the application of PSB plus GPR compared to PSB application alone. This phenomenon agreed with the trend in the P-solubilization capacity of PSB strains in laboratory culture (TCP > GPR). Therefore, appropriate P source will help PSB strains to fulfill their P-solubilization capacity in the application of PSB fertilizer or PSB-associated organic manure for reclaimed soil.

### 4.2. Effect of PSB on soil inorganic P forms and P availability

PSB plays a positive role in transforming insoluble P to available P in the soil. This mechanism will inevitably affect the concentration of various P forms in the soil. Fan et al. (2004) showed that the Ca<sub>2</sub><sup>-32</sup>P and Ca<sub>8</sub><sup>-32</sup>P fractions were increased while the Ca<sub>10</sub><sup>-32</sup>P fraction was decreased in soil after application of a P-solubilizing *Penicillium oxalicum* strain. Liang (2008) suggested that PSB application resulted in higher Ca<sub>2</sub>-P, Al-P and Fe-P concentrations but lower Ca<sub>8</sub>-P and Ca<sub>10</sub>-P concentrations in a calcareous soil after, with little effect on soil occluded-P concentration. Moreover, Zhou et al. (2005) and Sun and Xiong (2002) applied PSB biofertilizer in calcareous soil and Shajiang black soil, which found that PSB strains present in the fertilizer facilitated the transformation of insoluble Ca<sub>10</sub>-P and slowly available Ca<sub>8</sub>-P towards available P, thus increasing soil concentrations of Ca<sub>2</sub>-P and Al-P. In the present study, Ca<sub>2</sub>-P, Ca<sub>8</sub>-P, Al-P and Fe-P concentrations in reclaimed soil of the coal mining subsidence area were higher with simple and combined PSB application compared to the corresponding CM application. On the other hand, occluded-P and Ca<sub>10</sub>-P concentrations in simple and combined PSB application treatments were lower than those in the corresponding CM treatments. These results suggested that PSB strains played a role in

increasing Ca<sub>2</sub>-P, Ca<sub>8</sub>-P, Al-P and Fe-P concentrations but decreasing insoluble occluded-P and Ca<sub>10</sub>-P concentrations in reclaimed soil. The Ca<sub>10</sub>-P concentration in CM + TCP treatment was 71.20 mg/kg higher than that in PSB + TCP treatment, suggesting that the activities of PSB strains inhibited the transformation of soil P towards Ca<sub>10</sub>-P.

Owing to their different solubilities, various inorganic forms of soil P have different availability to plants. According to Feng et al. (1996), the availability of various inorganic forms of soil P follows the order of Ca<sub>2</sub>-P > Al-P > Ca<sub>8</sub>-P > Fe-P > Ca<sub>10</sub>-P > occluded-P. Correlation analysis between inorganic P forms and available P (Olsen-P) concentration revealed that the availability of inorganic P forms in the reclaimed soil under different fertilizer treatments followed the trend Ca<sub>2</sub>-P > Al-P = Ca<sub>8</sub>-P > Fe-P > occluded-P > Ca<sub>10</sub>-P. Application of PSB fertilizer increased Ca<sub>2</sub>-P, Al-P, Ca<sub>8</sub>-P and Fe-P concentrations and thus improved soil P availability to crop.

#### 4.3. Effect of PSB on soil P adsorption and desorption

Zhao et al. (2014), Zhang et al. (1996) and Zhang et al. (2005) have indicated that soil application of organic manure can activate soil P and reduce P adsorption. This is because the decomposition of organic manure produces carbohydrates and organic matter, which will compete with P adsorption by masking the adsorption sites and thus reduce P adsorption. Meanwhile, these products could increase the saturation of P adsorption at the residual adsorption sites in the soil, thus decreasing the adsorption and binding energy of the adsorbed phosphate (Zhao & Lu 1991). Moreover, organic manure contains P element, so that manure application naturally increases soil available P in the soil. The combined use of PSB and CM as a bio-organic fertilizer could further reduce P adsorption in the soil. The PSB strains secrete organic acids in the soil and the latter compete with phosphate ions for the P adsorption sites, further reducing phosphate adsorption in the soil (Jones & Darrah 1994). These organic acid products also dissolve calcium salts in the soil and may make the P adsorption sites disappear. A study by Li et al. (2014) has suggested that the application of PSB fertilizer results in a reduction of the maximum adsorption capacity and adsorption constant, but an increase of the maximum desorption capacity and average desorption rate of soil P. The same conclusion was drawn in the present study. Moreover, the combined application of PSB biofertilizer and TCP (PSB + TCP) exerted a greater effect on soil P desorption.

#### Disclosure statement

No potential conflict of interest was reported by the authors.

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