Pol. J. Environ. Stud. Vol. 33, No. 4 (2024), 4071-4082
DOI: *10.15244/pjoes/177435* ONLINE PUBLICATION DATE: 2024-05-20

Original Research

Effect of Phosphate Rock Powder, Active Minerals, and Phosphorus Solubilizing Microorganisms on the Phosphorus Release Characteristics of Soils in Coal Mining Subsidence Areas

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Received: 12 October 2023
Accepted: 21 December 2023

Abstract

Coal mining causes surface subsidence, resulting in the loss of phosphorus from the soil surface and affecting crop growth. To provide a basis for the reclamation of soils in coal mining subsidence areas, using the Suntuan mine area in Huaibei City as an example, indoor soil cultivation experiments were conducted to investigate the effects of applying different types of phosphate rock powders (mechanically activated and nonmechanically activated), active minerals (zeolite, kaolin, bentonite) and phosphorus solubilizing microorganisms (*Pseudomonas fluorescens*, *Aspergillus niger*) on soil pH, available phosphorous content, and the conversion of soil phosphorus fractions. The results showed that the combined action of phosphate rock powder and phosphorus solubilizing bacteria could significantly improve the soil acidic environment. The application of phosphate rock powder, active minerals, and phosphorus solubilizing microorganisms can promote the release of available phosphorous in soil, and *Aspergillus niger* has a better effect than *Pseudomonas fluorescens*. Soil content of H_2O-P, NaOH-Pi, $NaHCO_3$-Pi, and HCl-P increased, NaOH-Po decreased, and some treatment groups had reduced Residual-P content. Together, phosphate rock powders, active minerals, and phosphorus solubilizing microorganisms can mitigate soil acidification, promote soil phosphorus release, result in the transformation of soil phosphorus fractions, and improve phosphate fertilizer utilization.

Keywords: phosphate rock powder, phosphorus solubilizing microorganisms, soil phosphorus, mechanical activation, active minerals

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Introduction

Phosphorus is one of the primary nutrients necessary for plant growth and development and plays a significant role in carbohydrate metabolism, nitrogen metabolism, energy metabolism, and the functional processes of plants [1, 2]. In the main coal-producing areas of eastern China, surface subsidence caused by well-working activities has led to a decline in the fertility of previously cultivated land, especially due to a loss of phosphorus nutrients, which has resulted in inadequate land fertility and poor crop yields after the reclamation of the subsided areas [3]. To expand crop production, excessive amounts of phosphate fertilizer are often applied to meet crop requirements for phosphorus. Nevertheless, only a tiny proportion of phosphate fertilizer effectively promotes plant growth [4, 5]. Most phosphate fertilizer is strongly adsorbed and further mineralized in the soil, where the phosphate ions in the fertilizer interact with Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+} cations in the soil, converting them into insoluble tricalcium phosphate, magnesium phosphate, iron phosphate, and aluminum phosphate complexes, which are unavailable for plant uptake [6, 7]. Phosphate ore is a non-renewable resource. China's phosphate ores mainly consist of low-grade phosphate, which account for more than 80% of the total phosphate ores [8, 9]. These phosphate ores are difficult to sort and process into high-concentration phosphate fertilizers or compound fertilizers [10]. As available phosphate ore and phosphate ore with development value continue to decrease, the discrepancy between the demand for phosphate and the supply of phosphate resources is becoming increasingly acute. Therefore, the use of low- and medium-grade phosphate ore to develop phosphate mine fertilizer has become an inevitable choice in many countries [1, 11]. However, due to its low solubility and reactivity, the direct application of phosphate fines does not promote the release of phosphorus from the soil well [12], and enhancing the effectiveness of phosphate fines has become an urgent problem to be solved.

Much of the current research into the effectiveness of phosphate rock powder has focused on the mechanical activation of ordinary phosphate rock powder or the addition of various phosphate activators [13], surface active minerals and microorganisms [9, 14]. Mechanical activation, as an essential measures of processing low-grade phosphate ores, is simple to implement, inexpensive and environmentally friendly and enhances apatite reaction properties [15]. Mechanical processing changes the crystal structure of the phosphate rock powder and increases its leaching rate, which promotes the release of available phosphorous from the soil and provides a prolonged fertilization effect of 5-7 years [16]. The combination of phosphorus solubilizing microorganisms and phosphate rock powder increases the available phosphorous content of soil phosphorus, converts soil phosphorus forms, and increases crop yields [17, 18]. Phosphorus solubilizing microorganisms dissolve phosphorus through secretions of organic acids or H^+ and their excretion into the surrounding soil environment, leading to acidification of the surrounding soil environment and the conversion of insoluble phosphorus compounds into phosphorus that is directly absorbable by the soil, facilitating the release of available phosphorous from the soil [19-21]. Silicate minerals such as zeolites and bentonites usually have good ion exchange capacity and a large specific surface area, offering the possibility for application in soil fertilizers [22]. For example, when NH_4^+-exchanged zeolite and phosphate powder were applied to sunflower cultivation soil, the pH of the soil solution decreased, while the phosphorus concentration increased fourfold [23]. The current activation method for phosphate rock powder is relatively straightforward, systematic experimental studies of combined activation methods are lacking, and the effects of multiple varieties of activation methods on soil phosphorus are neglected.

Therefore, to address problems associated with the declining phosphorus content of soils in coal mining subsidence areas, the combined effects of mechanical activation, phosphorus solubilizing microorganisms and active minerals on soil pH, available phosphorous, and soil Hedley phosphorus forms in coal mining subsidence areas were investigated through indoor soil cultivation experiments to provide a basis for the targeted use of phosphate resources and promote sustainable agricultural development.

Materials and Methods

Experimental Materials

The test soil was collected from the Suntuan mining area in Huaibei City, Anhui Province, naturally dried, ground, and passed through a 2 mm sieve. The test soil had a pH of 5.66, a total nitrogen of 1.05 g/kg, an organic matter content of 25.13 g/kg, an available phosphorous content of 9.88 mg/kgn and a fast-acting potassium content of 180.74 mg/kg. The phosphate rock powder was purchased from Shandong Chuangye Chemical Co. The primary chemical properties of the inactivated phosphate powder were obtained from XRF tests, as shown in Table 1. *Pseudomonas fluorescens* and *Aspergillus niger* were purchased from the Beijing Biological Conservation Centre.

Culture medium: (1) LB: beef paste 3.0 g, peptone 5.0 g, NaCl 5.0 g, agar 15.0 g, distilled water 1.0 L, adjust pH to 7-7.2, autoclave sterilization. (2) PDA: potato extract 1.0 L, glucose 20.0 g, agar 15.0 g, autoclave sterilization.

Experimental Design

The mechanical activation equipment consisted of a planetary ball mill (from Nanjing Boynton Instruments Technology Co., LTD) with a grinding speed of 500 revolutions per minute (rpm). The grinding time was set

Table 1. Elemental composition of phosphate rock powder (%).

Samples	CaO	P_2O_5	SiO_5	Fe_2O_3	BaO	SO_3	Al_2O_3	F	SrO	TiO_2	MgO
PR	70.92	20.22	2.18	1.38	1.34	1.11	0.72	0.57	0.52	0.37	0.33

to 60 minutes (min). The particle size after grinding is 10.59 μm.

Three different active minerals (zeolite, kaolin and bentonite) were added to the phosphate rock powder for mixing and grinding. A mass ratio of 1:1 of active minerals to phosphate rock powder was first weighed and then ground for 60 min on a planetary ball mill at 500 revolutions per minute (rpm).

A total of 18 treatment groups were set up in an indoor soil cultivation experiment, and each treatment group was replicated three times, as shown in Table 2. Each pot was filled with 500 g of air-dried 2 mm sieved soil. 500g of air-dried soil with 2 mm sieve was packed in each pot, and 2 g of phosphorus rock powder was added, and *Pseudomonas fluorescens* and *Aspergillus niger* were inoculated into the corresponding medium (LB or PDA) according to 1:100 to expand the culture, and 10 mL of PSM cell suspension was inoculated into the soil, and the water was replenished through weighing method, so as to maintain the soil water content at about 30%. The soil water content was kept at about 30% and incubated at a constant temperature of 25ºC. Samples were taken after 30 days of incubation, and then the soil pH, available phosphorous and the contents of different phosphorus fractions were determined.

Measurement Indicators and Analytical Methods

Soil pH was determined by leaching with a 2.5:1 ratio of water to soil, shaking and leaving for 30 minutes before determining the pH of the suspension with a pH meter. Soil available phosphorous was determined by leaching with $NaHCO_3$ solution and molybdenum blue colorimetric method.

To analyze different phosphorus fractions in the soil, the Hedley soil phosphorus grading method modified by Sui et al. was used [24]. Soil phosphorus forms were extracted by weighing 0.5 g of sieved soil sample in a 50 mL centrifuge tube and adding 30 mL of deionized water, 0.5 mol/L $NaHCO_3$, 0.1 mol/L NaOH, and one mol/L HCl to extract the soil phosphorus forms at each level, i.e., H_2O-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Pi, and HCl-P. The residual soil was determined by high-temperature digestion with

Table 2. Experimental design treatment groups.

Treatment group		Microorganisms
CK	-	-
PR	Phosphate rock powder	-
MP	Mechanical activation of phosphate rock powder for 60min	-
MPB	Activation of phosphate rock powder to bentonite mass ratio 1:1 for 60 min	-
MPK	Activation of phosphate rock powder to kaolin mass ratio 1:1 for 60 min	-
MPZ	Activation of phosphate rock powder to zeolite mass ratio 1:1 for 60 min	-
PF	-	PF
PR-P	Phosphate rock powder	PF
MP-P	Mechanical activation of phosphate rock powder for 60min	PF
MPB-P	Activation of phosphate rock powder to bentonite mass ratio 1:1 for 60 min	PF
MPK-P	Activation of phosphate rock powder to kaolin mass ratio 1:1 for 60 min	PF
MPZ-P	Activation of phosphate rock powder to zeolite mass ratio 1:1 for 60 min	PF
An	-	An
PR-A	Phosphate rock powder	An
MP-A	Mechanical activation of phosphate rock powder for 60min	An
MPB-A	Activation of phosphate rock powder to bentonite mass ratio 1:1 for 60 min	An
MPK-A	Activation of phosphate rock powder to kaolin mass ratio 1:1 for 60 min	An
MPZ-A	Activation of phosphate rock powder to zeolite mass ratio 1:1 for 60 min	An

sulfuric acid-perchloric acid for residual-P. During each extraction, the volume of the extract was 30 mL, and the samples were shaken on a shaker for 16 h. Then the samples were centrifuged for 10 min, and the soil and supernatant were separated, and the $NaHCO_3$ and NaOH extracts were divided into two groups for the determination of whole phosphorus and inorganic phosphorus (determined by molybdenum-blue colorimetric method). The content of organic phosphorus (Po) at each level was equal to the content of total phosphorus at each level minus the content of inorganic phosphorus (Pi) at each level.

Data Processing

All statistical analysis and plotting were performed using R (version 4.1.2) and Origin 2021 software. Significant variations of pH, available phosphorous, and soil phosphorus fractions of the different treatments and the correlation of different phosphorus fractions were performed in R using the "vegan" packages.

Results

Effect of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms on Soil Ph

Application of different types of phosphate rock meal improved soil pH to varying degrees (Fig. 1). In the phosphorite powder only treatment group (Fig. 1a), the application of both plain phosphorus rock powder (PR) and mechanically activated phosphorus rock powder (MPR) increased soil pH compared to CK, but the differences were not significant, being 2% and 3% higher than CK, respectively. The addition of kaolin (MPK), bentonite (MPB), and zeolite (MPZ) to the mechanical activation of phosphorus rock powder also increased soil pH, but the differences were not significant, being 4%, 4%, and 3% higher than CK, respectively.

As shown in Fig. 1b), application of *Pseudomonas fluorescens* significantly ($P<0.05$) increased soil pH by 11% compared to CK. Soil pH was significantly increased ($P<0.05$) when different types of phosphorus rock powder were paired with *Pseudomonas fluorescens*, with MPB-P, MPK-P, MPZ-P, MPR-P, and PR-P treatments having 28%, 22%, 16%, 19%, and 17% higher soil pH than CK, respectively.

As shown in Fig. 1c), application of *Aspergillus niger* (An) only significantly increased soil pH ($P<0.05$) by 26% compared to CK. Soil pH was significantly increased ($P<0.05$) when different types of phosphorus rock powder were paired with *Aspergillus niger*, with MPB-A, MPK-A, MPZ-A, MPR-A, and PR-A treatments having 31%, 28%, 25%, 26%, and 23% higher soil pH than CK, respectively.

It can be seen that phosphorus rock powder and phosphorus solubilizing microorganisms can regulate soil acidity and alkalinity, and adding active minerals to phosphate meal significantly affects pH enhancement in the treatment group. Compared with the application of phosphorus rock powder alone, the combined effect of phosphorus solubilizing microorganisms and phosphorus rock powder was more effective in alleviating soil acidification, and *Aspergillus niger* was more effective than *Pseudomonas fluorescens* in regulating acidic soil.

Effect of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms on Soil Available Phosphorous

The application of different types of phosphorus rock powder and phosphorus solubilizing microorganisms could increase soil available phosphorous content to different degrees (Fig. 2). In the treatment group of phosphorus rock powder only (Fig. 2a), the application of un-mechanically activated phosphorus rock powder and mechanically activated phosphorus rock powder both increased the soil available phosphorous content, which was 39% and 93% higher than that of CK, respectively, but the difference was not significant. The addition of zeolite (MPZ) and kaolin (MPK) to the mechanical activation of phosphorus rock powder significantly increased soil available phosphorous content ($P<0.05$) by 90% and 69%, respectively, compared with CK.

As shown in Fig. 2b), soil available phosphorous content increased with the application of *Pseudomonas*

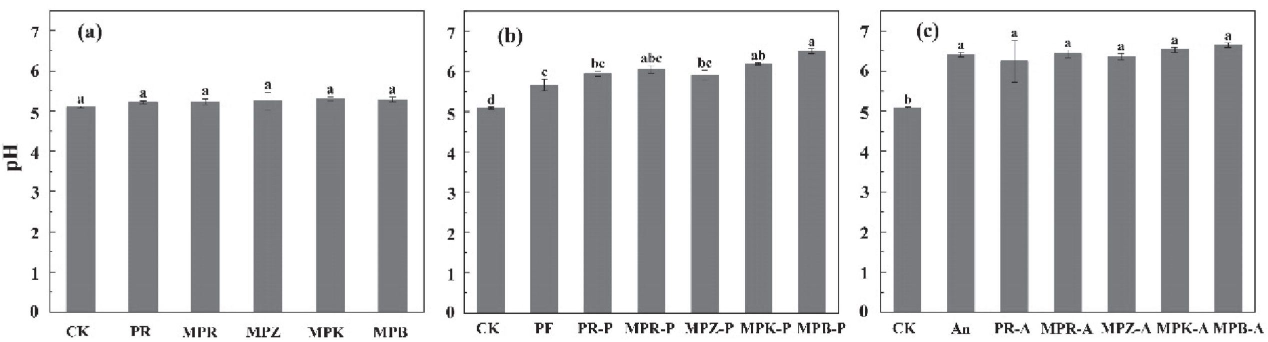

Fig. 1. Soil pH in different treatment groups.

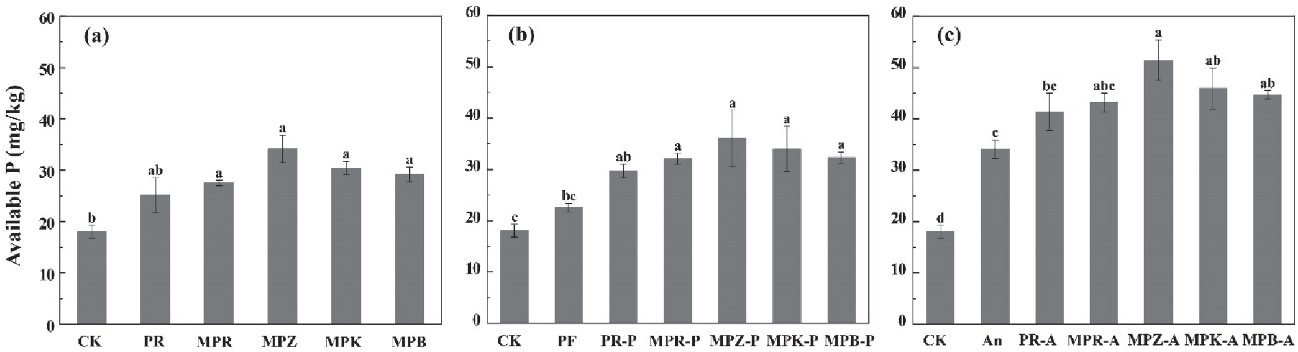

Fig. 2. Soil available P in different treatment groups.

fluorescens (PF) alone, but the difference with CK was not significant. When different types of phosphorus rock powder were applied with *Pseudomonas fluorescens*, all of them increased soil available phosphorous content, among which MPR-P, MPZ-P, MPK-P, and MPB-P treatments showed significant differences (P<0.05) compared with CK, which increased 78%, 100%, 88%, and 79%, respectively, and among which MPZ-P treatment showed the largest increment in available phosphorous in the soil.

As shown in Fig. 2c), soil available phosphorous content was significantly (P<0.05) increased by 89% when *Aspergillus niger* (An) was applied alone compared to CK. When different types of phosphorus rock powder and *Aspergillus niger* were co-applied to the soil, all of them significantly increased (P<0.05) the soil available phosphorous content, of which PR-A, MPR-A, MPZ-A, MPK-A and MPB-A treatments increased by 129%, 139%, 185%, 155%, and 148%, respectively, compared to CK.

It can be seen that phosphorus rock powder and phosphorus solubilizing microorganisms alone can increase the soil available phosphorous content, in which the treatment effect of mechanically activated phosphorus rock powder and the addition of the active mineral phosphorus rock powder was better than that of non-mechanically activated phosphorus rock powder. Compared with the application of phosphorus powder alone, the combined application of phosphorus solubilizing microorganisms and phosphorus rock powder significantly increased the soil available phosphorous content. *Aspergillus niger* was more effective than *Pseudomonas fluorescens*.

Effect of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms on the Phosphorus Fraction of Soils

(1) Phosphorus in the Active State

As can be seen from Fig. 3, the H_2O-P content in the control soil was 1.48 mg/kg, and different treatments improved soil H_2O-P content to different degrees, but there was no significant difference between the treatment group with phosphorus rock powder application and CK. The soil H_2O-P content was enhanced when different types of phosphorus rock powder were mixed with *Pseudomonas fluorescens* for soil application, in which the soil treated with MPB-P, MPK-P and MPZ-P differed significantly (P<0.05) from CK, and the soil H_2O-P content was in the order of MPB-P>MPZ-P>MPK-P from high to low. The application of *Aspergillus niger* alone elevated soil H_2O-P content, but the difference with CK was not significant. Different types of phosphorus rock powder significantly enhanced soil H_2O-P content when applied in mixture with *Aspergillus niger*, and the differences with CK were significant (P<0.05), and the soil H_2O-P content was MPB-A>MPK-A>MPZ-A>MPR-A>PR-A in the order of from high to bottom.

The $NaHCO_3$-Pi content of the control soil was 21.09 mg/kg (Fig. 3), and the soil $NaHCO_3$-Pi content increased with the application of phosphorus rock powder, but the difference was not significant compared with CK, in which the mechanically activated phosphorus rock powder with zeolite added (MPZ) treatment had the largest increment in soil $NaHCO_3$-Pi, which was 21% higher than that of CK. Soil $NaHCO_3$-Pi content increased in *Pseudomonas fluorescens* treatment, but none of the differences were significant compared with CK, and soil $NaHCO_3$-Pi content was in the order from high to low, MPZ-P>MPR-P>PR-P>MPK-P>MPB-P>PF. Soil $NaHCO_3$-Pi content increased when *Aspergillus niger* was applied alone, but the difference with CK was not significant, and when *Aspergillus niger* was applied to soil together with different types of phosphorus rock powder, all of them significantly elevated the soil $NaHCO_3$-Pi content compared to CK (P<0.05), and the soil $NaHCO_3$-Pi content was in the following order, from high to low: MPZ-A>MPR-A>PR-A>MPB-A >MPK-A.

The $NaHCO_3$-Po content of the control soil was 21.09 mg/kg (Fig. 3), and the application of phosphate rock flour increased soil $NaHCO_3$-Po content in all cases compared with CK, but there was no significant difference, among which the MPB treatment had the most tremendous increase in soil $NaHCO_3$-Po content, which was 34% higher than that of CK. Soil $NaHCO_3$-Po content was also increased when *Pseudomonas fluorescens* was mixed with different types of

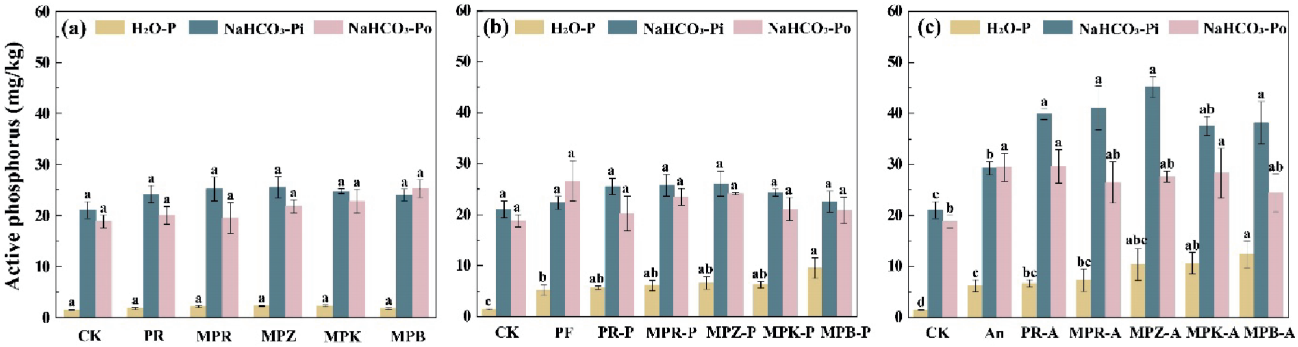

Fig. 3. Active phosphorus content in different treatment groups.

phosphorus rock powder application, in which the PF treatment had the most significant increase in soil $NaHCO_3$-Po, which was elevated by 42% compared with CK, but the difference was not significant. The soil $NaHCO_3$-Po content increased when *Aspergillus niger* was mixed with different types of phosphorus rock powder, and its increase was higher than that of the phosphorus rock powder treatment group and the *Pseudomonas fluorescens* phosphorus rock powder treatment group, in which the soil $NaHCO_3$-Po content of the An and PR-A treatments was significantly increased (P<0.05), which was 57% and 58% higher than that of the CK, respectively.

(2) Moderately Active Phosphorus

The NaOH-Pi content in the control soil was 11.05 mg/kg (Fig. 4), and compared with CK, the application of phosphorus rock powder all increased the soil NaOH-Pi content, but the difference was not significant, and the soil NaOH-Pi content was in the following order from high to low: MPZ>MPK>MPB>PR>MPR. Mixed application of *Pseudomonas fluorescens* and different types of phosphorus rock powder increased soil NaOH-Pi content, which was not significantly different from CK. Mixed application of *Aspergillus niger* and different types of phosphorus rock powder dust significantly increased soil NaOH-Pi content (P<0.05), and soil NaOH-Pi content was MPZ-A>MPK-A>MPR-A>MPB-A>PR-A>An in the order of high to low.

The NaOH-Po content in the control soil was 33.09 mg/kg (Fig. 4), and compared with CK, in the phosphorus rock powder-only treatment group, soil NaOH-Po content decreased in the PR, MPK, and MPZ treatments, and increased in the MPR and MPB treatments, but none of the differences were significant. *Pseudomonas fluorescens* reduced soil NaOH-Po content when mixed with different types of phosphorus rock powder, with the MPB-P treatment showing a significant (P<0.05) reduction in soil NaOH-Po, which was 32% lower than that of CK. In all the treatment groups of different types of phosphorus rock powder mixed with *Aspergillus niger*, the NaOH-Po content of the soil was reduced compared to CK, but the difference was insignificant.. The reduction in NaOH-Po was lower in *Pseudomonas fluorescens*-treated soils than in *Aspergillus niger*-treated soils.

(3) Phosphorus in the Low Activity State

The HCl-P content in the control soil was 2.89 mg/kg (Fig. 5), and the application of different types of phosphorus rock powder increased the soil HCl-P content compared to CK, but the difference was not significant. Soil HCl-P content increased significantly (P<0.05) when *Pseudomonas fluorescens* was mixed with different types of phosphorus rock powder, and the soil HCl-P content was PR-P>MPB-P>MPK-P>PF>MPR-P>MPZ-P in descending order. Soil HCl-P content also increased significantly (P<0.05) when *Aspergillus niger* was mixed with different types of phosphorus rock

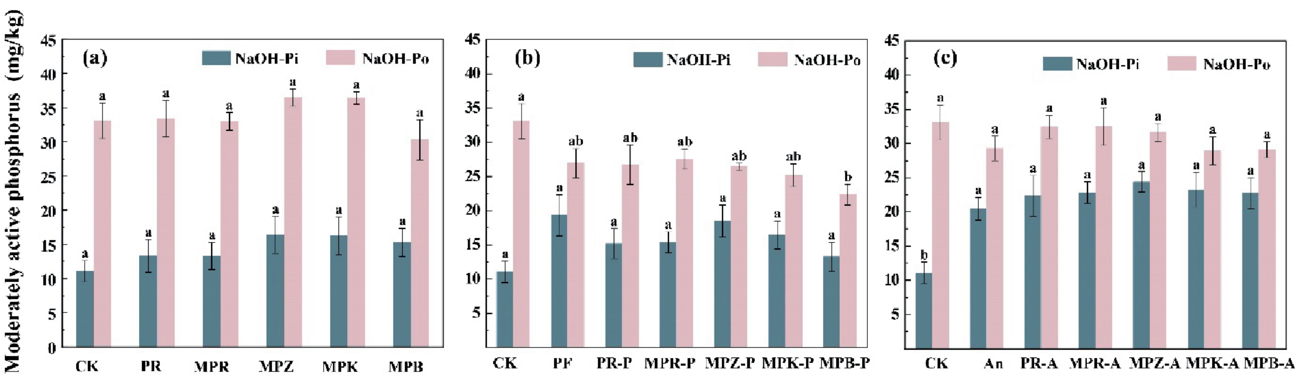

Fig. 4. Moderately active phosphorus content in different treatment groups.

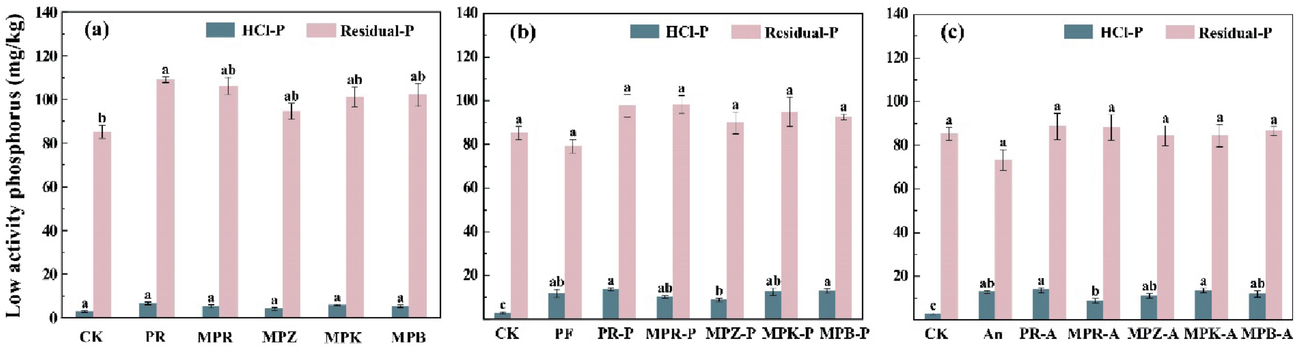

Fig. 5. Low active phosphorus content in different treatment groups.

powder compared to CK, in descending order from high to low, PR-A>MPK-A>An>MPB-A>MPZ-A>MPR-A.

Residual-P content of the control soil was 85.20 mg/kg (Fig. 5), and the application of different types of phosphorus rock powder increased the soil Residual-P content compared to CK, where the difference in soil Residual-P was significant in the PR treatment, which was 28% higher than that of CK, and the difference in the rest of the treatment groups was not significant. Soil Residual-P content in descending order was PR>MPR>MPB>MPK>MPZ>CK. Application of *Pseudomonas fluorescens* alone reduced soil Residual-P content compared to CK, and soil Residual-P content was higher than CK when *Pseudomonas fluorescens* was co-applied with phosphate meal, but lower than that of the soil treated with phosphorus rock powder only. The soil Residual-P content of *Aspergillus niger* only was lower than that of CK, and when different types of phosphorus rock powder were mixed with *Aspergillus niger*, the soil Residual-P content of MPZ-A and MPK-A treatments was lower than that of CK, and the soil Residual-P content of PR-A, MPR-A, and MPB-A treatments was higher than that of CK but lower than that of phosphorus rock powder only treatments. The results indicated that both *Pseudomonas fluorescens* and *Aspergillus niger* could activate soil insoluble phosphorus, and *Aspergillus niger* activated insoluble phosphorus better than *Pseudomonas fluorescens*.

Correlation Analysis of Different Phosphorus Fractions in Soils with Soil Available Phosphorous

The stronger the correlation between soil available phosphorous and a particular form of phosphorus, the more effective that form of phosphorus is. As shown in Fig. 6, the correlations between available phosphorous and $NaHCO_3$-Pi, H_2O-P, NaOH-Pi were significant and positive, and the strongest correlation was with $NaHCO_3$-Pi (Fig. 6), indicating that $NaHCO_3$-Pi is an effective source of phosphorus for plants [25].

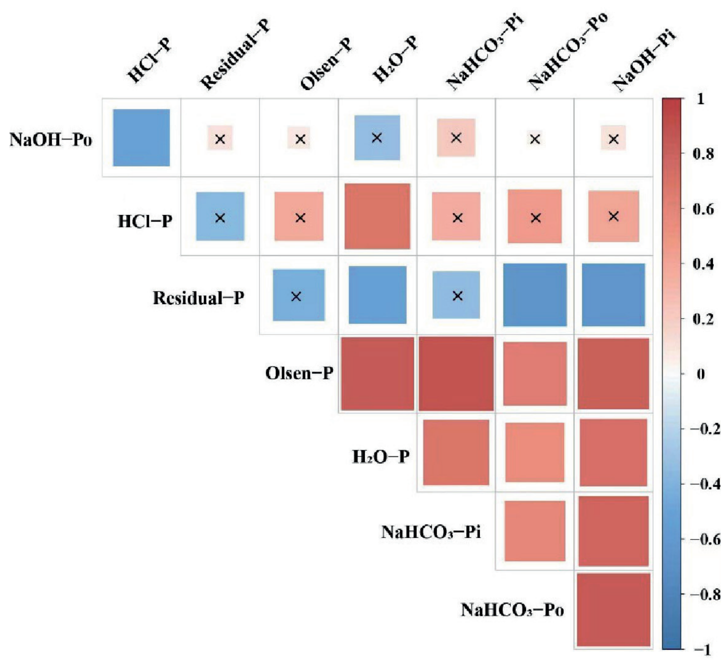

Fig. 6. Correlation analysis of soil fast-acting phosphorus and different phosphorus fractions in soils *Note:× Indicates not significant.

Phosphorus in the soil will be affected by the soil type and environmental factors, changing the original form of phosphorus, and the transformation of each form of phosphorus into each other, thus changing the content and effectiveness of each form of phosphorus. HCl-P showed a significant negative correlation with NaOH-Po. Residual-P showed a significant negative correlation with $NaHCO_3$-Po, H_2O-P and NaOH-Pi, indicating that phosphorus rock powder, reactive minerals and phosphorus solubilizing microorganisms could activate the insoluble phosphorus into phosphorus that was more easily absorbed and utilized by plants, which improved the soil phosphorus effectiveness.

Discussion

Effect of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms a on Soil Ph

Soil pH is an essential indicator of soil acidity and alkalinity, affecting not only plant absorption of soil nutrients but also the valence state, migration and transformation of metallic elements such as Cu, Zn, Fe and Mn in the soil [26–28]. The soil used in this experiment is acidic and contains high levels of iron, aluminum and manganese, which cause manganese and aluminum toxicity in plants, as well as loss of calcium and magnesium from the soil, affecting the growth and development of most plants [26]. The application of phosphate rock powder reduces soil acidity (Fig. 1a) because the CaO in phosphate rock powder depletes the H^+ in acidic soils, and the release of $H_2PO_4^-$ and F^- from the dissolved phosphate rock powder substitutes for the hydroxyl groups on the surface of soil colloids, reducing soil acidity. The more significant effect of mechanically activated phosphate rock powder observed on soil pH regulation may be due to the increased specific surface area of phosphate rock powder particles after mechanical activation of phosphate rock powder, which results in a larger contact surface with H^+, more H^+ consumption and better soil acidity mitigation [29, 30]. When phosphate rock powder and active minerals are mechanically activated in combination and applied to the soil, the soil pH tends to rise (Fig. 1a), probably for two reasons: first, the active minerals such as zeolite, bentonite and kaolin contain strong alkaline substances, and second, the silicates in the active minerals react with the H^+ and Al mononuclear hydroxyl compounds in the soil, reducing soil acidity [31]. Phosphorus solubilizing microorganisms secrete organic acids and H^+ when dissolving insoluble phosphate, lowering soil pH [32]. Nevertheless, some studies have found that inoculating soil with phosphorus solubilizing microorganisms can raise soil pH [33]. The significant increase in soil pH by inoculation with phosphorus solubilizing microorganisms (Fig. 1b, Fig. 1c) may be due to the fact that the decomposition of soil organic matter as well as the dissolution of insoluble phosphorus in the soil consumes H^+ in the soil, and inoculation with phosphorus solubilizing microorganisms increases the soil microbial abundance, and microorganisms will decompose more organic matter during their growth, thus increasing soil pH.

Effect Of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms on Soil Available Phosphorous

Soil available phosphorous can be directly absorbed and used by plant roots and is an important indicator of the effectiveness of soil phosphorus [34]. Fannana et al. used a planetary ball mill to grind sedimentary and metamorphic phosphorite for 30 min and 21 min, respectively, and the maximum phosphorus dissolution rates were 57.51% and 46.53%, respectively [35]. Huang et al. shown that the available phosphorous content of ultrafine pulverized phosphate rock powder increased by 40%, the active phosphorus content increased by more than 3.5 times, and the accumulation of soluble phosphorus increased by 24 times in six consecutive extractions [36]. The application of phosphate rock powder can promote the release of available phosphorous in the soil. The effect of the mechanical activation of phosphate rock powder on available phosphorous is better than that of ordinary phosphate rock powder (Fig. 2a), which may be mainly due to two reasons. One is that mechanical force results in the breakage of Ca-P bonds in the lattice of phosphate rock powder, enhancing the activity of PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$. Second, the particle size of mechanically activated phosphate rock powder decreases, and the larger its surface area is, the greater the contact between the phosphate rock powder and the soil, which means greater contact with the H^+ present in the soil, and therefore, the phosphate release rate of the phosphate rock powder increases [37]. Gao Shang et al. conducted leaching experiments on phosphate rock powder using Bacillus subtilis and achieved a high phosphorus leaching rate of 51.76% under optimal cultivation conditions [38]. Compared with phosphate ore powder alone, inoculation with the *Pseudomonas fluorescens* (PF) and *Aspergillus niger* (An) had a more significant effect on the promotion of available phosphorous in soil (Fig. 2b, Fig. 2c), probably because phosphorus solubilizing microorganisms secrete organic acids and other substances with the ability to complex Fe, Al, Ca and other metal ions, promoting the dissolution of insoluble phosphorus such as $FePO_4$, $AlPO_4$, $Ca_3(PO_4)_2$ and the release of PO_4^{3-} [39]. The addition of active minerals such as zeolite, kaolin and bentonite during the mechanical activation of phosphate rock powder also facilitates the release of available phosphorous from the soil compared to non-mechanically activated phosphate rock powder (Fig. 2a). This is because these highly surface-active minerals are silica-aluminate-based minerals, and silica-aluminate is negatively charged[40]. To maintain electrical neutrality, these minerals need to adsorb K^+, Na^+, Ca^{2+} and other cations to balance the negative charge. Different cations can be freely exchanged, so these active minerals usually have good ion exchange capacity, while the active minerals typically have a large specific surface area, so they have a large adsorption capacity [41, 42]. When combined with phosphate rock powder, the active minerals adsorb Ca^{2+} from the phosphate rock powder, releasing HPO_4^{2-}, which in turn promotes the release of available phosphorous from the soil. In this study, the MPZ-A treatment had the highest soil available phosphorous content, indicating that the co-application of phosphorus solubilizing microorganisms with mechanically activated phosphate rock powder with zeolite addition can have a synergistic effect on the ability to dissolve phosphorus.

Effect of Phosphate Rock Powder and Phosphorus Solubilizing Microorganisms on the Phosphorus Fractions in Soils

The active forms of phosphorus consist of water-soluble phosphorus and sodium bicarbonate extracted-phosphorus in the soil. These forms can be directly absorbed and used by crops after application to the soil [43]. We found that soil H_2O-P content increased in soils treated with phosphate rock powder, active minerals, and phosphorus solubilizing microorganisms, and soil $NaHCO_3$-Pi and $NaHCO_3$-Po content increased in soils treated with phosphorus-enhancing microorganisms (Fig. 3). This may be due to the fact that phosphorus-enhancing microorganisms mineralized soil phosphorus by decomposing soil organisms and dissolving phosphorite powder, thus increasing $NaHCO_3$-Pi content. Phosphorus solubilizing microorganisms increased soil $NaHCO_3$-Po content, probably because soil microbial residues return cellular phosphorus to the soil during decomposition, and microbial phosphorus is considered to be the main source of $NaHCO_3$-Po [44].

NaOH-Pi and NaOH-Po are phosphorus that readily binds to iron and aluminum oxides in the soil and, under certain circumstances, can be converted into phosphorus that can be taken up and used by plants [45,46]. We found that the content of NaOH-Pi increased in soils with the addition of kaolin and bentonite (Fig. 4), probably because these active minerals can bind to iron and aluminum oxides in the soil, reducing the adsorption of phosphorus to the soil. The addition of phosphorus solubilizing microorganisms to the soil increased the NaOH-Pi content, probably because the organic acid anions released by the phosphorus solubilizing microorganisms occupied adsorption sites on the Fe and Al surfaces, reducing the adsorption capacity of Fe and Al oxides for soil phosphorus [47]. A decrease in NaOH-Po content of the soil with the application of phosphorus solubilizing microorganisms was found in this study (Fig. 4), which may be due to the mineralization of organic phosphorus into other forms of phosphorus by the action of the inoculated phosphorus solubilizing microorganisms and the microorganisms in the soil [48].

HCl-P refers to calcium-bound phosphorus and is a potential source of phosphorus [48,49]. The increase in soil HCl-P content after the application of phosphate dust and phosphorus solubilizing microorganisms may be caused by the partial application of undissolved phosphate rock powder to the soil, which has a persistent residual effect, in agreement with a study by Omenda et al. [50]. HCl-P content increased in PSM-applied soil, and HCl-P was negatively correlated with NaOH-Po and Residual-P, suggesting that HCl-P may be derived from the conversion of other forms of phosphorus. Residual-P is usually a relatively stable phosphorus fraction that cannot be leached by extractants such as resins, sodium bicarbonate, sodium hydroxide and hydrochloric acid and is difficult to absorb and use by crops but can be converted to an effective phosphorus fraction in the soil after long-term weathering and mineralization [50, 51]. Rivaie [52] found that the application of phosphate rock powder did not affect residual phosphorus in the soil. Nevertheless, in this study, the content of residual-P in the soil increased significantly when only phosphate rock powder was applied (Fig. 4), probably because the phosphate rock powder particles applied to the soil were encapsulated by oxides such as iron and aluminum in the soil and became challenging to leach by extractants and remained in the residual phosphorus fraction of the soil. Soil Residual-P content was reduced after phosphate meal was dosed with phosphorus solubilizing microorganisms (Fig. 5), indicating that the *Aspergillus niger* was able to convert insoluble phosphorus into active phosphorus available for crop uptake through enzymatic and acidolytic action [53].

Conclusion

To improve the effectiveness of phosphorus and increase the utilization of phosphate rock powder, the effects of applying different combinations of phosphate rock powder, active minerals and PSM on soil pH, available phosphorous content and soil phosphorus conversion were investigated in an indoor soil incubation experiment. The main conclusions are as follows: the application of phosphate rock powder or inoculation with phosphorus solubilizing microorganisms can increase soil pH and alleviate soil acidification, and the effect is more pronounced when phosphate rock powder, active minerals and phosphorus solubilizing microorganisms are combined. Phosphate rock powder, active minerals and phosphorus solubilizing microorganisms were all able to promote an increase in the available phosphorous content of the soil, with mechanically activated phosphate rock powder treatment being better than ordinary phosphate rock powder treatment and the *Aspergillus niger* producing better activation than the *Pseudomonas fluorescens*. Soil available phosphorous increase was more significant when phosphate rock powder, active minerals and phosphorus solubilizing microorganisms were combined. Soil content of H_2O-P, NaOH-Pi, $NaHCO_3$-Pi, and HCl-P increased, NaOH-Po decreased, and some treatment groups had reduced Residual-P content. The application of phosphate rock powder, reactive minerals, and phosphorus solubilizing microorganisms altered phosphorus morphology and improved phosphorus fertilizer utilization. In addition, the effects of mechanically activated minerals and phosphorus solubilizing microorganisms on crop growth after application to the soil need further study.

Acknowledgments

This work was supported by the Anhui Provincial Natural Science Foundation Key Project (2023AH051225), the National Key Research and Development Program of China (grant number: 2022YFF1303303), the Research Foundation of Huainan Mining Group in 2023 and Research Foundation of Huaibei Mining Group in 2023.

Conflict of Interest

The authors declare no conflict of interest.

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