Optimizing Phosphorus Application Rate and the Mixed Inoculation of Arbuscular Mycorrhizal Fungi and Phosphate-solubilizing Bacteria Can Improve the Phosphatase Activity and Organic Acid Content in Alfalfa Soil

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Abstract

Alfalfa (*Medicago sativa* L.) is an important high-quality legume forage, and phosphorus is an important nutrient element for high-quality and high-yield of alfalfa. This study assessed the effects bacteria and phosphorus (P) use efficiency of alfalfa soil under different P applications. In this experiment, a two-factor complete randomized block design was used. Four bacterial treatments were as follows: *Funneliformis mosseae* (Fm), *Bacillus megaterium* (Bm), double inoculation (Fm × Bm) and no inoculation bacteria (CK). There are four levels of phosphorus treatment, namely: phosphorus application 0 (P_0), 50 (P_1), 100 (P_2), 150 mg·kg^{-1} (P_3). There were 16 treatments in total, and each treatment was repeated 6 times. The results showed that the effects of single inoculation and mixed inoculation were significantly higher than those of noninoculation ($P < 0.05$). With the increase in phosphorus application, each index increased first and then decreased. The alkaline phosphatase activity (AKP) and organic matter (SOM) content in soi lincreased with the increase of cutting times, and the content of organic matter in rhizosphere soil was higher than that in non-rhizosphere soil. Principal component analysis (PCA) shows that the top three treatments were $J_3P_2 > J_3P_1 > J_3P_3$. Therefore, when (P_2O_5) was 100 mg·kg^{-1}, the mixed inoculation of Fm × Bm could improve the phosphatase activity in alfalfa soil, promote the secretion of organic acids in rhizosphere soil and then improve the content of soil fertility.

Key Points

Alfalfa (*Medicago sativa* L.), is a perennial herb of leguminous alfalfa.

AMF and PSB are symbiotic microorganisms in nature.

Soil phosphatase can decompose lipid phosphorus and accelerate the hydrolysis process, thus increasing the content of available phosphorus in soil.

Introduction

Alfalfa (*Medicago sativa* L.), is a perennial herb of leguminous alfalfa. Because of its high yield, rich nutrition, huge production potential, and wide use (Zhang et al. 2020). Phosphorus is one of the most important nutrients for the normal development of plants, which are involved in the synthesis and metabolism of many important compounds in plants (Wang et al. 2018). Therefore, phosphorus has an important effect on the formation of alfalfa production performance.

Arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) are symbiotic microorganisms in nature (Kucey et al. 1983; Pandit et al. 2020). AMF can promote the uptake and utilization of phosphorus in soil by plants. The rate of P uptake by mycorrhizal plants is an important factor affecting the amount of P uptake by plants under a certain phosphorus concentration (Liu et al. 2019). Studies have shown that AM fungal hyphae can secrete organic acids, improve the pH value of the rhizosphere, and promote the transformation of organic phosphorus in soil into inorganic phosphorus that can be absorbed and utilized by plants (Bolan et al. 1991). At the same time, AMF can stimulate the secretion of plant phosphatase and enhance the activity of phosphatase, thus improving plant phosphorus nutrients (Javot et al. 2007). PSB plays an important role in the turnover and bioavailability of soil phosphorus. It can increase the dissolution and mineralization of insoluble inorganic phosphorus and organic phosphorus in soil by secreting protons, organic acids, and phosphatase (Rodriguez et al. 2007). It can be seen that the study of phosphatase activity and organic acid is an important means to study phosphate solubilizing microorganisms.

Soil phosphatase can decompose lipid phosphorus and accelerate the hydrolysis process, thus increasing the content of available phosphorus in soil (Xie et al. 2014). According to the optimal pH value of phosphatase for the dissociation of insoluble phosphate, it can be divided into hydrolytic acid phosphatase and specific alkaline phosphatase (AKP). Among them, alkaline phosphatase is a kind of specific enzyme of AMF and plant symbiosis system (Wang et al. 2014). The results showed that the acid phosphatase activity was significantly affected by PSB inoculation and phosphorus application (Gomezjurado et al. 2015). With the increase of organic acid content, insoluble phosphate in soil moved to the direction of effective dissociation (Liang et al. 2020); At the same time, due to the change of pH value in the soil, the phosphatase activity was indirectly affected; Organic acids can also chelate with Ca, Al and Fe plasma to release PO_4^{3-}, which can effectively increase the solubility of insoluble phosphate (Saxena et al. 2016). Therefore, through the study of the two, we can reveal the partial mechanism of phosphorus solubilization in the symbiotic system of phosphorus solubilizing microorganisms and plants, which are also one of the current research hotspots.

Interaction of AMF and PSB can enhance the ability of plants to obtain phosphorus. At present, a lot of studies mainly focus on inoculating AMF or PSB on alfalfa plants alone. However, there are relatively few studies on the effects of the interaction between AMF and PSB on the absorption of phosphorus and the secretion of phosphatase by alfalfa, as well as the relationship between various indicators, especially the effects of AMF and PSB on soil phosphatase activity. Therefore, the effects of AMF and PSB on phosphatase activity, organic acid, pH value, and organic matter content of alfalfa under different phosphorus application levels were studied to provide a theoretical basis for the efficient utilization of phosphorus fertilizer and the development of microbial fertilizer.

Materials And Methods

Experimental materials

In this experiment, AMF *Funneliformis mosseae* (Fm) was selected, which was given by the Qingdao Agricultural Mycorrhizae Research Institute of China. The inoculant was rhizosphere soil, comprising host plant root, mycorrhizal fungal spore, and ectomycorrhizal mycelium. Spore density: 25-35 g. The host plant alfalfa variety tested was WL354HQ.

For the PSB, *Bacillus megaterium* (Bm) was taken from the Agricultural Culture Collection of China (ACCC, WDCM 572, 10011).

Experimental design

The experiment was conducted in a split plot based on a two-factor random block design and consisted of bacterial application and phosphorus application. Four bacterial treatments were as follows: *Funneliformis mosseae* (Fm), *Bacillus megaterium* (Bm), double inoculation (Fm × Bm) and no inoculation bacteria (CK), respectively labeled as J_1, J_2, J_3, and J_0. There are four levels of phosphorus treatment, namely: phosphorus application 0 (P_0), 50 (P_1), 100 (P_2), 150 mg·kg^{-1} (P_3), repeat 6 times for each treatment.

The experiment was conducted in the experimental base of Shihezi University (44°18'N, 86°03'E) from 2019 to 2020. The soil was sterilized in the autoclave for 2 h and then air-dried for standby (at 121℃). A pot experiment was carried out in a nutrient bowl with an upper diameter of 23 cm, bottom diameter of 15 cm, and height of 16 cm. Each pot contained 3 kg sterilized air-dried soil. In the treatment group J_1 treatment, Fm was inoculated 5 cm below the surface of the soil in the pot, and 10 g of bacteria was applied in each pot to promote the colonization of alfalfa roots. In the J_2 treatment group, 10 mL (Bm) bacterial solution was applied to each pot. In the J_3 treatment group, alfalfa seeds were soaked for 12 h, and then the seeds and 5 mL of Bm bacterial solution were planted in the flowerpot with 5 g (about 8500 inoculum potential units) of Fm. In non inoculated J_0 treatment group, the same amount of inactivated bacteria was added as the J_3 treatment. Select full and uniform alfalfa seeds, disinfect them with 10% H_2O_2 for 10 min, then wash them repeatedly with distilled water, and sow them on May 1, 2019, with 10 seeds in each pot. The same amount of water was supplied every day, and then the seedlings were thinned after sowing(the growth period was three-leaf stages). Five alfalfa seedlings with uniform growth were kept in each pot, and each treatment was repeated 6 times. To keep the same daylighting, the flower pots were randomly placed. The phosphate fertilizer used was mono ammonium phosphate (containing 52% P and 11% N). To keep the same content of N in each treatment, urea (containing 46% N) as added. Fertilizer is applied twice a year, on June 18 and September 19, 2019; The fertilizer will be applied on June 25 and September 27, 2020, respectively, and the fertilizer will be applied with water drop. Alfalfa is mowed twice a year, all at the initial flowering stage (5-10%) and on August 2 and October 12 in 2019; It will be cut on August 12 and October 16 in 2020, and the stubble height will be 2 cm.

The specific fertilization scheme is shown in Table 1:

Soil sample collection

The shaking method was used to collect the rhizosphere and non-rhizosphere soil. The soil directly shaking off was regarded as non-rhizosphere soil, and the soil brushed from the root with a brush was regarded as rhizosphere soil. The soil was packed in self-sealed bags and brought back to the laboratory. Transfer the soil sample to the aluminum box and dry it at 65℃ to constant weight. Grind the dried soil sample and screen out fine soil with 100 mesh sieves for standby.

Measurement index and method

Determination of alkaline phosphatase activity (AKP) in the soil

The AKP test kit was used to test the AKP in the soil, which was provided by Beijing Solarbio Technology Co., Ltd. The determination principle is as follows: AKP decomposes disodium phenyl phosphate to produce free phenol and phosphoric acid. Phenol reacts with 4-amino antipyrine in an alkaline solution and oxidizes with potassium ferricyanide to produce red quinone derivatives. The enzyme activity can be determined according to the red color (Wang et al 2016).

Determination of organic acid content

The contents of organic acids (citric acid, malic acid, oxalic acid, and acetic acid) in rhizosphere soil were determined by HPLC (Ma et al. 2020).

PH value

The ratio of water to soil is 5:1, and the pH meter is used for detection.

Soil organic matter (SOM)

The potassium dichromate hydration heat method was used (Liu et al. 2019).

Data processing and analysis

Excel 2010 was used for data processing. SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Duncan's method was used for multiple comparisons after two-way ANOVA. Origin 8.5 software (OriginLab OriginPro, USA) was used for mapping. Canoco 5.0 was used for principal component analysis (PCA) of the soil microbial community. The average value of the measured data is used ± Standard deviation.

Pearson correlation coefficient is a method to measure the degree of correlation between two variables. It is a value between 1 and -1, in which 1 is a completely positive correlation, 0 is not a correlation, and -1 is a completely negative correlation (Chatterjee et al. 2020).

Results

The AKP in the soil

Under the same treatment, with the increase of phosphorus application, the AKP in soil increased first and then decreased (Table 2). The P_2 treatment was significantly higher than other treatments under the J_0 condition ($P < 0.05$), except for the first crop AKP in 2019, and the second crop AKP in 2020 reached the highest value under the P_1 treatment. The P_2 treatment was significantly higher than other treatments under the condition of J_1 ($P < 0.05$). Except that the AKP of the P_1 treatment was significantly higher than that of the P_0 treatment in 2020 ($P < 0.05$). The other treatments the maximum value in P_1 treatment and reached the maximum value under the condition of J_2, except for the first crop AKP in 2020 P_2 treatment was significantly higher than other treatments ($P < 0.05$). Under the J_3 condition, except for the rhizosphere soil in 2019, the P_2 and P_3 treatments were significantly higher than that of the P_0 treatment ($P < 0.05$). Under the same phosphorus application conditions, the treatment with bacteria application was significantly higher than that without bacteria application ($P < 0.05$). With the increase of mowing times, alkaline phosphatase in soil increased. In each crop, the AKP in the soil was significantly different under the J, P, and J × P treatments ($P < 0.01$).

Soil organic acid content

Under the same conditions, except for the oxalic acid content in 2020, the contents of malic acid, oxalic acid, and acetic acid in soil increased first and then decreased with the increase of phosphorus application (Fig. 1). Under J_0 condition, except that the oxalic acid content increased gradually in 2020, the oxalic acid content of P_2 treatment was significantly higher than that of P_0 treatment ($P < 0.05$), and there was no significant difference between P_2 treatment and P_3 treatment in 2019 ($P > 0.05$). Under the condition of J_1, the P_3 treatment was significantly higher than the P_0 treatment in 2019 ($P < 0.05$), and the P_2 treatment was significantly higher than the P_0 treatment in 2020 ($P < 0.05$). Under the J_2 condition, acetic acid and malic acid reached the maximum at the P_3 treatment,

while oxalic acid and organic acid reached the maximum at the P_2 treatment. Under the J_3 condition, the P_3 treatment was significantly higher than the P_0 treatment in 2019 ($P < 0.05$). However, in 2020, herbicide P_2 and P_3 treatments were significantly higher than those in the P_0 treatment ($P < 0.05$), and there was no significant difference between P_2 treatment and P_3 treatment ($P > 0.05$). In other treatments, herbicide P_2 treatment was significantly higher than that in P_0 treatment ($P < 0.05$).

The pH value in soil

Under the same bacteria treatment, with the increase of phosphorus application rate, the soil pH value first increased and then decreased (Table 3). Under the J_0 condition, the pH value of the first crop rhizosphere soil had no significant difference with the increase of phosphorus application rate ($P > 0.05$), but the pH value of P_2 and P_3 treatment of non-rhizosphere soil was significantly higher than that of P_0 treatment ($P < 0.05$), and there was no significant difference between P_2 treatment and P_3 treatment ($P > 0.05$). Under the condition of J_1, there was no significant difference between the treatments of phosphorus application in 2019 ($P > 0.05$), but in 2020, except for the treatment of P_2 and P_3, the treatment of P_1 was significantly higher than that of P_0 ($P < 0.05$). Under the condition of J_2, the pH value of P_2 treatment was significantly higher than that of P_0 treatment in 2019 ($P < 0.05$), and that of P_1 treatment was significantly higher than that of the P_0 treatment in 2020 ($P < 0.05$). Under the J_3 condition, P_1 and P_2 treatments were significantly higher than the P_0 treatments in the second crop of two years ($P < 0.05$). There was no significant difference in pH value among treatments under the same phosphorus application ($P > 0.05$). The pH values of soil decreased with cutting times, and there was no significant difference between rhizosphere soil and non-rhizosphere soil. In each crop, the pH value was significantly different under the J, P, and J × P treatments ($P < 0.01$).

SOM

Under the same conditions, the content of SOM increased first and then decreased with the increase of phosphorus application (Table 4). Under J_0 condition, except that the SOM content of P_2 was significantly higher than that of P_1 and P_0 treatments ($P < 0.05$), the SOM content of P_1 was significantly higher than that of P_0 treatment ($P < 0.05$), and the SOM content of P_2 was significantly higher than that of P_1 and the P_0 treatment ($P < 0.05$) in 2019. Under the condition of J_1, the SOM content of P_1 was significantly higher than that of the P_0 treatment in 2019 ($P < 0.05$), except that the SOM content of P_2 was significantly higher than that of other treatments ($P < 0.05$). In 2020, the SOM content of P_1 was significantly higher than that of other treatments ($P < 0.05$), and the SOM content of P_2 was significantly higher than that of P_0 treatment in other treatments ($P < 0.05$). Under the condition of J_2, the P application was significantly higher than that of no P application ($P < 0.05$). Except for the treatment of P_1 in 2020, the SOM content of the second rhizosphere soil was significantly higher than that of the P_0 treatment ($P < 0.05$), and the SOM content of other treatments was significantly higher than that of other treatments ($P < 0.05$). Under the same P application, the J_3 treatment was significantly higher than other treatments ($P < 0.05$). With the increase of mowing times, the content of SOM in rhizosphere soil was higher than that in non-rhizosphere soil. In each crop, the SOM content was significantly different under the J, P, and J × P treatments ($P < 0.01$).

Correlation analysis of each index

Pearson correlation analysis showed that AKP in the soil was significantly positively correlated with malic acid, oxalic acid, total organic acid and, pH value ($P < 0.01$), and significantly positively correlated with acetic acid, and SOM content ($P < 0.05$) (Table 5). Malic acid was positively correlated with oxalic acid, total organic acid, and SOM ($P < 0.01$). Oxalic acid was positively correlated with acetic acid, total organic acid, and SOM ($P < 0.01$). There was a significant positive correlation between total organic acids and SOM ($P < 0.01$). There was a significant positive correlation between pH value and SOM ($P < 0.01$).

Principal component analysis (PCA)

Based on the relevant analysis, we analyzed the main components of the total amount of alkaline phosphatase, organic matter, pH value in soil, malic acid, oxalic acid, acetic acid, and organic acid in the rhizosphere soil (Fig. 2). The results show that the variance contribution rate of axis 1 (Principal component 1, PC1) is 79.55%, that of axis 2 (Principal component 1, PC2) is 15.30%, and the cumulative contribution rate of PC1 and PC2 is 94.85%. Therefore, it can represent the original 7 indexes, the related indexes of PC1 are pH value in soil, organic matter, and AKP, and the indexes related to PC2 include malic acid, oxalic acid, acetic acid, and organic

acid. The PC1 eigenvalues were sorted as J_3P_2 > J_3P_3 > J_3P_1 > J_3P_0. According to the characteristic value of each treatment on two factors and the contribution rate of the factor, the comprehensive evaluation model is $Y=0.796Y_1$ (PC1)+$0.153Y_2$ (PC2). The greater the “Y” value indicates that the treatment has the best effect on soil fertility. The top three are J_3P_2 > J_3P_1 > J_3P_3 for processing.

Discussion

Effects of inoculating AMF and PSB on phosphatase activity in soil under different phosphorus applications

Enzyme activity in the soil is not only one of the important indicators to evaluate the quality of soil microbial ecosystem (Elfstrands et al. 2007), but also an effective biological index to characterize soil microorganisms and soil fertility of alfalfa grassland (Liang et al. 2018). At the same time, the soil enzyme activity can reflect the soil biological activity and the soil biochemical reaction intensity (Tischer et al. 2014). The AKP is the key enzyme in the soil phosphorus cycle and directly affects the effectiveness of soil phosphorus (Shaw et al. 2020). Among them, alkaline phosphatase is the key enzyme in the soil phosphorus cycle, and directly affects the availability of soil phosphorus (Shaw et al. 2020). The results showed that the soil alkaline phosphatase activity increased first and then decreased with the increase of phosphorus application rate under the same bacteria treatment (Table 2). The results showed that the addition of AMF and PSB could increase the content of organic matter and nutrients in the soil, promote the metabolism of alfalfa root exudates, and make the microbial life activities more vigorous, thus improving the activities of various soil enzymes (Ghorchiani et al. 2018). In different soils, AMF has different effects on phosphatase activity in the soil and AKP. Inoculation with AMF can significantly enhance phosphatase activity in the soil and then can significantly improve its phosphorus utilization rate. For instance, inoculation with *Glomus intraradices* can enhance the activities of acid phosphatase and AKP of *Ipomoea carnnea* (Amaya-Carpio et al. 2009). Inoculating *Glomus* on soybean (*Glycine max* L.) could enhance phosphatase activity in rhizosphere soil (Abdel-Fattah et al. 2014). Mycorrhizal maize plants also enhanced phosphatase activity in soil (Wang et al., 2013). In *Citrus* rhizosphere soil, the activity of soil phosphatase increased significantly after inoculation with AMF (Wu et al. 2011). In rhizosphere soil, the carbon source provided by plant root exudates promotes the reproduction of phosphatase-producing microorganisms (Zhang et al. 2016), thus improving the phosphatase activity of rhizosphere soil. Therefore, inoculation plays a key role in improving phosphatase activity in the soil.

Effects of inoculating AMF and PSB on organic acid content under different phosphorus applications

Phosphorus solubilizing microorganisms mainly produce organic acids, phosphatase, and hydrogen protons, and the main way is to produce organic acids. These organic acids can chelate with calcium, aluminum, and iron plasma while reducing the pH value of the reaction solution, so that insoluble phosphorus can be transformed into effective phosphorus for plant use (Wen et al. 2019). The results showed that malic acid, oxalic acid, and acetic acid in rhizosphere soil increased at first and then decreased with the increase of phosphorus application rate under the same phosphorus application rate. Moreover, under the same phosphorus application rate, the amount of malic acid, oxalic acid, and acetic acid in rhizosphere soil under the same phosphorus application rate were higher than that under the no phosphorus application rate (Fig. 1). Among them, the order of organic acid content in rhizosphere soil was: acetic acid > malic acid > oxalic acid > citric acid. Citric acid was not detected in soil, which may be due to the low use efficiency of phosphorus in root exudates, and the different types of organic acids secreted by different plants to the medium are different (Oliveira et al. 2020). Meanwhile, the phosphatase activity in soil was significantly positively correlated with malic acid, oxalic acid, acetic acid, total organic acid, and pH value in the soil (Table 5), indicating that many physiological metabolic processes in plants were closely related to malic acid, citric acid, oxalic acid and acetic acid (Aoki et al. 2012). With the increase of organic acid content, the microbial population, structure, and enzyme activity in the rhizosphere soil also changed significantly, which improved the phosphorus absorption conditions, thus increased the phosphorus availability in the rhizosphere soil and enhanced the phosphorus absorption capacity of plants (Tewari et al. 2007; Li et al. 2009). Studies have shown that soybean root exudates can significantly promote the growth of rhizosphere bacteria, and the presence of bacteria can also promote the secretion of root organic acids (Saeki et al. 1996). Meanwhile, fungi can also change the composition and content of organic acids in soil (Bavaresco et al. 2000). It can be seen that organic acids secreted by roots and microbial activity are mutually utilized and promoted.

Effects of inoculating AMF and PSB on pH value and SOM content in soil under different phosphorus applications

The pH value is not only an index reflecting pH intensity in the soil but also one of the important factors affecting phosphorus absorption in soil and utilization. The results showed that with the increase of the P application rate, the pH value in soil increased

first and then decreased under the same bacteria treatment (Table 3). This was because the AM mycelium activity directly affected the secretion of roots, resulting in the change of pH value in rhizosphere soil, and indirectly stimulated the reproduction of phosphorus bacteria and fungi in the rhizosphere. Thus, the absorption, utilization, and transformation of insoluble phosphorus in soil can be greatly accelerated (Lin et al. 2016). At the same time, physical or chemical changes in the surrounding environment will directly affect the mycorrhiza, resulting in changes in the pH value of the rhizosphere and even the microbial flora in the rhizosphere (Wen et al. 2019). Therefore, AMF and dephosphorizing bacteria affect the organic acids secreted by roots, and then affect pH value in soil.

The SOM is an important indicator of soil fertility. As the largest carbon pool of the terrestrial ecosystem, it not only provides nutrients for crop growth and development but also provides energy for soil microbial decomposition activities. It can be seen that SOM content is crucial for soil respiration (Amit et al. 2020). The results showed that under the same bacteria treatment, with the increase of phosphorus application, the content of SOM increased first and then decreased, and the content of SOM in rhizosphere soil was higher than that in non-rhizosphere soil (Table 4). This may be because the organic acids and enzymes secreted by microorganisms after inoculation promote the release of soil nutrients, promote the physiological metabolism of plants and the growth and development of plants, enhance the transpiration, and promote the activation and migration of nutrients in the soil and the enrichment in the rhizosphere (Rodriguez et al. 2007).

After applying phosphate fertilizer, the interaction between AMF and PSB directly or indirectly affected the content of SOM, pH value in the soil, AKP, and organic acid. The specific performance was as follows: mixed inoculation was better than single inoculation, and single inoculation was better than non-inoculation (Fig. 2). The main reason is that the inoculated plants can absorb more NH_4^+ than the non-inoculated plants, the cells can assimilate ammonia, and the H^+ exudates, which reduces the pH value, thus affecting the bioavailability of insoluble phosphorus in minerals (Matse et al. 2020). Due to the adsorption of cation and anion in soil solution, the pH value of rhizosphere soil is different, which changes the plant availability of phosphorus adsorption (Wan et al. 2019). At the same time, organic acids secreted by roots, as nutrients of microorganisms, can stimulate the growth and reproduction of acid phosphatase-producing microorganisms in organic fertilizer, and then improve the acid phosphatase content in rhizosphere soil (Giles et al. 2018).

In conclusion, the present study showed that the appropriate phosphorus application and inoculation with AMF and PSB could significantly promoted fertility in the soil field of alfalfa. The AKP in the soil, organic acid, pH value, and content of SOM increased first and then decreased with the increase of phosphorus application under the same bacteria treatment. The order of organic acid content in rhizosphere soil was acetic acid > malic acid > oxalic acid > citric acid. The results showed that the optimum phosphorus application rate (P_2O_5, 100 mg·kg^{-1}) and the mixed inoculation of AMF (Fm) and PSB (Bm) could increase the phosphatase activity in alfalfa soil, promote the secretion of organic acids in rhizosphere soil, and then increase the content of SOM in alfalfa soil and improve soil fertility.

Abbreviations

AMF: Arbuscular mycorrhizal fungi;

AKP: Alkaline phosphatase activity;

Bm: *Bacillus megaterium*;

Fm: *Funneliformis mosseae*;

PSB: Phosphate solubilizing bacteria;

P: Phosphorus;

SOM: Soil organic matter.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Please contact the authors for all requests.

Competing interest

The authors declare that they have no competing interests.

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Authors'contributions

Q-B Z and J-Y L designed the study. J-Y L interpreted the results. X-X A, J-Y L, and X-S L performed the experiments. X-X A and J-Y L participated in writing the manuscript: W-H L and C-H M supervised the study. All authors read and approved the final manuscript for publication.

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Tables

Table 1 Fertilizer application scheme

Number	Treatments	$NH_4H_2PO_4$ (mg/pot) (Containing N 12.2%)	CN_2H_4O (mg/pot) (Containing N 46%)	*Funneliformis mosseae* (g/pot)	*Bacillus megaterium* (mL/pot)
1	J_0P_0	0	105.3	0	0
2	J_0P_1	35.1	72.9	0	0
3	J_0P_2	72.9	35.1	0	0
4	J_0P_3	105.3	0	0	0
5	J_1P_0	0	105.3	10	0
6	J_1P_1	35.1	72.9	10	0
7	J_1P_2	72.9	35.1	10	0
8	J_1P_3	105.3	0	10	0
9	J_2P_0	0	105.3	0	10
10	J_2P_1	35.1	72.9	0	10
11	J_2P_2	72.9	35.1	0	10
12	J_2P_3	105.3	0	0	10
13	J_3P_0	0	105.3	5	5
14	J_3P_1	35.1	72.9	5	5
15	J_3P_2	72.9	35.1	5	5
16	J_3P_3	105.3	0	5	5

Note: P_0, P_1, P_2, and P_3 represent 0 $mg{\cdot}kg^{-1}$, 50 $mg{\cdot}kg^{-1}$, 100 $mg{\cdot}kg^{-1}$, and 150 mg P kg^{-1}, respectively. J_0, J_1, J_2, and J_3 represent CK, Fm, Bm, and Fm×Bm, respectively.

Table 2 Alkaline phosphatase activity in rhizosphere soil and non-rhizosphere soil under different treatments ($U{\cdot}g^{-1}$)

Treatments	Rhizosphere soil AKP in 2019		Non-rhizosphere soil AKP in 2019		Rhizosphere soil AKP in 2020		Non-rhizosphere soil AKP in 2020	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
J_0P_0	2.76±0.04 Cc	6.03±0.09 Cd	3.32±0.06 Cc	6.70±0.09 Bb	1.84±0.01 Cd	1.08±0.03 Dd	1.09±0.02 Dc	1.11±0.04 Dd
J_0P_1	3.18±0.08 Ba	6.62±0.08 Bc	3.63±0.05 Bb	7.11±0.03 Aa	2.16±0.02 Dc	2.11±0.05 Da	1.86±0.06 Da	1.88±0.08 Db
J_0P_2	2.93±0.04 Bbc	6.81±0.03 Ca	3.77±0.05 Aa	7.13±0.16 Aa	2.34±0.01 Da	1.84±0.03 Db	1.80±0.03 Dab	2.59±0.07 Da
J_0P_3	2.84±0.06 Cb	6.53±0.06 Bb	3.27±0.09 Bc	6.88±0.3 Bab	2.23±0.02 Cb	1.49±0.06 Dc	1.72±0.03 Bb	1.65±0.01 Dc
J_1P_0	3.18±0.02 Ab	6.29±0.04 Bb	3.72±0.04 Ab	7.19±0.16 Aa	2.17±0.07 Bd	1.87±0.05 Bc	1.51±0.05 Bc	2.15±0.07 Bd
J_1P_1	3.20±0.04 Bb	6.32±0.12 Cb	3.84±0.06 Aa	7.21±0.18 Aa	2.84±0.03 Ba	2.97±0.03 Bb	2.37±0.08 Ca	5.21±0.05 Aa
J_1P_2	3.33±0.08 Aa	7.19±0.01 Ba	3.35±0.08 Bc	7.13±0.09 Aa	2.46±0.01 Cb	3.72±0.06 Ba	2.31±0.09 Ca	3.31±0.06 Bb
J_1P_3	3.23±0.01 Ab	7.17±0.01 Aa	3.34±0.05 Bc	6.89±0.17 Bb	2.26±0.08 Cc	3.01±0.06 Bb	1.79±0.04 Bb	2.87±0.04 Bc
J_2P_0	3.06±0.04 Bbc	6.44±0.13 ABb	3.19±0.02 Db	6.91±0.15 Ba	2.10±0.06 Bd	1.72±0.04 Cc	1.36±0.01 Cd	1.54±0.03 Cd
J_2P_1	3.30±0.06 Aa	6.63±0.08 Ba	3.29±0.04 Ca	7.07±0.08 Aa	2.62±0.02 Cb	2.32±0.03 Ca	2.65±0.02 Bb	3.10±0.04 Ca
J_2P_2	3.09±0.05 Bb	6.36±0.11 Db	3.38±0.03 Ba	6.85±0.25 Ba	2.69±0.02 Ba	2.29±0.02 Ca	3.25±0.06 Ba	2.90±0.02 Cb
J_2P_3	2.97±0.06 Bc	5.22±0.10 Cc	3.31±0.06 Ba	6.83±0.17 Ba	2.34±0.03 Bc	1.83±0.02 Cb	1.77±0.01 Bc	2.21±0.08 Cc
J_3P_0	3.10±0.03 ABb	6.60±0.02 Ab	3.52±0.06 Bd	6.77±0.02 Bb	2.26±0.01 Ad	2.27±0.06 Ad	3.47±0.05 Ac	3.04±0.06 Ad
J_3P_1	3.36±0.06 Aa	6.83±0.17 Ac	3.71±0.08 Bc	7.09±0.08 Aa	2.93±0.06 Ab	3.47±0.07 Ab	3.51±0.11 Ac	4.56±0.07 Bb
J_3P_2	3.33±0.07 Aa	7.89±0.20 Aa	3.83±0.02 Ab	7.25±0.04 Aa	5.00±0.01 Aa	3.99±0.05 Aa	5.29±0.13 Aa	5.43±0.05 Aa
J_3P_3	3.14±0.09 Ab	7.09±0.10 Ab	4.22±0.02 Aa	7.22±0.06 Aa	2.76±0.05 Ac	3.08±0.04 Ac	4.92±0.06 Ab	3.27±0.12 Ac
J	**	**	**	**	**	**	**	**
P	**	**	**	**	**	**	**	**
J×P	**	**	**	**	**	**	**	**

Note: Different capital letters in the same column indicated significant difference in different bacteria treatments under the same phosphorus application conditions (P<0.05), differences small letters in the same column mean significant difference under the same bacteria application conditions (P<0.05).

P_0, P_1, P_2, and P_3 represent 0 mg·kg^{-1}, 50 mg·kg^{-1}, 100 mg·kg^{-1}, and 150 mg P kg^{-1}, respectively. J_0, J_1, J_2, and J_3 represent CK, Fm, Bm, and Fm×Bm, respectively.

Table 3 The pH value of rhizosphere soil and non-rhizosphere soil under different treatments

Treatments	The pH value of rhizosphere soil pH in 2019		The pH value of non-rhizosphere in 2019		The pH value of rhizosphere soil in 2020		The pH value of non-rhizosphere soil in 2020	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
J_0P_0	8.48±0.11 Aa	8.24±0.06 Ab	8.33±0.13 Bb	8.38±0.16 Ac	8.09±0.01 Ab	7.87±0.04 Bc	8.26±0.04 Ab	7.79±0.02 Ac
J_0P_1	8.61±0.04 Aa	8.40±0.11 Aa	8.42±0.01 Bb	8.55±0.01 Aa	8.18±0.02 Aa	8.16±0.04 Aa	8.27±0.04 ABb	8.27±0.04 Aa
J_0P_2	8.65±0.03 Ba	8.43±0.03 Aa	8.60±0.08 Ba	8.42±0.01 Bbc	8.19±0.01 Aa	8.09±0.01 Bb	8.36±0.04 Ba	7.89±0.03 Bb
J_0P_3	8.64±0.35 Ba	8.42±0.01 Aa	8.70±0.35 Aa	8.52±0.02 Aab	8.16±0.04 Aa	8.07±0.03 Bb	8.28±0.02 Ab	6.39±0.01 Bd
J_1P_0	8.55±0.29 Ab	8.28±0.06 Ac	8.33±0.01 Bc	8.34±0.11 Ab	7.97±0.03 Bb	7.90±0.01 Ac	8.19±0.01 Bb	6.58±0.02 Bb
J_1P_1	8.55±0.05 Ab	8.36±0.04 ABb	8.55±0.02 Bb	8.35±0.01 Bb	8.08±0.02 Ba	8.09±0.01 Bb	8.26±0.04 Da	7.88±0.02 Ba
J_1P_2	8.90±0.01 Aa	8.44±0.08 Aa	8.92±0.02 Aa	8.58±0.01 Aa	8.09±0.01 Ba	8.18±0.02 Aa	8.09±0.01 Ac	6.40±0.01 Dc
J_1P_3	8.89±0.08 Aa	8.40±0.02 Aab	8.66±0.17 Ab	8.34±0.08 Bb	8.06±0.04 Ba	8.16±0.04 Aa	8.08±0.02 Bc	6.38±0.02 Bd
J_2P_0	8.57±0.05 Abc	8.13±0.01 Bc	8.17±0.08 Cc	8.32±0.04 Ab	8.09±0.01 Ac	7.79±0.02 Cc	8.27±0.04 Ab	6.49±0.02 Cd
J_2P_1	8.60±0.34 Ab	8.29±0.03 Bb	8.49±0.02 Ba	8.51±0.04 Aa	8.20±0.01 Aa	8.16±0.04 Aa	8.29±0.02 Aa	7.98±0.02 Bb
J_2P_2	8.88±0.01 Aa	8.45±0.04 Aa	8.35±0.15 Cab	8.54±0.01 Aa	8.16±0.04 Ab	8.17±0.03 Aa	8.17±0.03 Cc	8.07±0.04 Aa
J_2P_3	8.36±0.03 Cc	8.08±0.05 Bc	8.27±0.03 Bbc	8.45±0.10 Aa	8.08±0.02 Bc	8.08±0.02 Bb	8.08±0.02 Bd	6.69±0.01 Ac
J_3P_0	8.62±0.35 Aa	8.09±0.03 Bb	8.62±0.01 Ab	8.29±0.14 Ab	7.87±0.03 Cc	7.59±0.01 Dc	8.19±0.01 Bc	6.47±0.03 Cc
J_3P_1	8.64±0.06 Aa	8.36±0.06 ABa	8.75±0.02 Aab	8.25±0.20 Bb	8.09±0.01 Bb	7.90±0.01 Cb	8.27±0.04 ABb	7.97±0.03 Ca
J_3P_2	8.65±0.06 Ba	8.33±0.06 Ba	8.79±0.08 Aa	8.47±0.01 ABa	8.16±0.04 Aa	8.00±0.01 Ca	8.58±0.02 Aa	7.79±0.02 Cb
J_3P_3	8.60±0.11 Ba	8.00±0.15 Cc	8.76±0.10 Aab	8.31±0.02 Bb	8.06±0.05 Bb	7.98±0.02 Ca	7.98±0.02 Cd	6.27±0.03 Cd
J	**	**	**	**	**	**	**	**
P	**	**	**	**	**	**	**	**
J×P	**	**	**	**	**	**	**	**

Note: Different capital letters in the same column indicated significant difference in different bacteria treatments under the same phosphorus application conditions (P<0.05), differences small letters in the same column mean significant difference under the same bacteria application conditions (P<0.05).

P_0, P_1, P_2, and P_3 represent 0 mg·kg^{-1}, 50 mg·kg^{-1}, 100 mg·kg^{-1}, and 150 mg P kg^{-1}, respectively. J_0, J_1, J_2, and J_3 represent CK, Fm, Bm, and Fm×Bm, respectively.

Table 4 Organic matter content of rhizosphere soil and non-rhizosphere soil under different treatments (mg·kg^{-1})

Treatments	Organic matter content of rhizosphere soil in 2019		Organic matter content of non-rhizosphere soil in 2019		Organic matter content of rhizosphere soil in 2020		Organic matter content of non-rhizosphere soil in 2020	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
J_0P_0	24.31±0.33 Cd	41.32±0.36 Bc	20.88±0.09 Dc	38.84±0.27 Cc	25.78±0.33 Cd	19.18±0.12 Bb	23.52±0.88 Cb	16.04±0.55 Dd
J_0P_1	25.25±0.41 Cc	42.9±0.28 Cab	22.78±0.33 Db	42.64±0.69 Cb	30.68±0.64 Ba	20.51±0.16 Ca	28.57±0.32 Ca	20.07±0.69 Cc
J_0P_2	33.66±0.30 Ca	43.53±0.52 Da	23.94±0.31 Da	43.69±0.69 Ca	29.10±0.64 Db	21.04±0.24 Da	29.31±0.93 Da	25.04±0.71 Ca
J_0P_3	30.89±0.48 Cb	42.32±0.95 Cab	22.25±0.05 Db	42.9±0.51 Dab	27.62±0.84 Dc	20.88±0.18 Ba	28.26±0.27 Ca	22.83±0.29 Db
J_1P_0	29.61±0.12 Bd	44.64±0.50 Ab	23.25±0.37 Bd	40.37±0.87 Bc	28.05±0.32 Bd	21.37±0.50 Ad	25.25±0.69 Bd	20.04±0.57 Bc
J_1P_1	35.74±0.37 Ba	47.01±0.24 ABa	27.47±0.05 Ba	43.27±0.94 Cb	33.05±0.75 Aa	24.04±0.18 Bb	30.68±0.64 Bb	25.52±0.64 Ab
J_1P_2	34.68±0.48 Bb	47.64±0.29 Ba	26.54±0.20 Bb	50.54±0.99 Ba	31.15±0.46 Cb	25.52±0.55 Aa	35.63±0.55 Ba	29.03±0.71 Ba
J_1P_3	31.21±0.37 Cc	44.11±0.49 Bb	25.62±0.46 Bc	43.85±0.18 Cb	29.78±0.78 Cc	23.15±0.33 Ac	29.2±0.16 BCc	26.31±0.81 Bb
J_2P_0	29.63±0.46 Bc	41.11±0.33 Bd	22.41±0.50 Cd	44.80±0.46 Ac	28.84±0.45 Bd	19.83±0.09 Bc	24.04±0.57 Cb	18.04±0.51 Cd
J_2P_1	35.89±0.21 Ba	46.54±0.24 Ba	23.67±0.48 Cc	49.38±0.40 Ba	29.84±0.84 Bc	24.04±0.60 Ba	29.84±0.96 Ba	21.30±0.57 Bc
J_2P_2	34.26±0.18 Bb	45.32±0.28 Cb	25.73±0.36 Ca	49.91±0.45 Ba	36.05±0.33 Ba	22.04±0.14 Cb	30.57±0.46 Ca	28.22±0.50 Ba
J_2P_3	34.21±0.33 Bb	43.74±0.32 Bc	24.53±0.22 Cb	46.27±0.27 Bb	33.47±1.06 Bb	20.20±0.42 Bc	29.89±0.51 Ba	24.99±0.79 Cb
J_3P_0	36.42±0.46 Ac	44.96±0.55 Ad	25.89±0.43 Ac	45.48±0.47 Ad	30.57±0.46 Ad	22.09±0.47 Ad	29.20±0.15 Ac	23.09±0.37 Ad
J_3P_1	39.21±0.33 Aa	47.54±0.14 Ac	29.26±0.47 Aa	53.23±0.32 Ab	32.52±0.27 Ac	30.84±0.40 Aa	33.47±1.06 Ab	26.10±0.6 Ac
J_3P_2	37.47±0.52 Ab	59.60±0.48 Aa	27.68±0.33 Ab	54.96±0.79 Aa	37.20±0.55 Aa	24.13±0.58 Bb	39.58±1.46 Aa	36.84±0.95 Aa
J_3P_3	35.95±0.10 Ac	48.85±0.42 Ab	28.05±0.14 Ab	50.06±0.47 Ac	34.58±0.88 Ab	23.20±1.11 Ac	32.52±0.27 Ab	27.62±0.42 Ab
J	**	**	**	**	**	**	**	**
P	**	**	**	**	**	**	**	**
J×P	**	**	**	**	**	**	**	**

Note: Different capital letters in the same column indicated significant difference in different bacteria treatments under the same phosphorus application conditions (P<0.05), differences small letters in the same column mean significant difference under the

same bacteria application conditions (P<0.05).

P_0, P_1, P_2, and P_3 represent 0 mg·kg^{-1}, 50 mg·kg^{-1}, 100 mg·kg^{-1}, and 150 mg P kg^{-1}, respectively. J_0, J_1, J_2, and J_3 represent CK, Fm, Bm, and Fm×Bm, respectively.

Table 5 Correlation analysis of indexes of rhizosphere soil under different treatments in 2019-2020

Index	AKP	Malic acid	Oxalate	Acetic acid	Total organic acids	pH value
Malic acid	0.562**					
Oxalate	0.769**	0.579**				
Acetic acid	0.394*	0.180	0.670**			
Total organic acids	0.577**	0.984**	0.670**	0.200		
pH value	0.627**	0.094	-0.620	0.576**	0.147	
Organic matter	0.936*	0.613**	0.832**	0.647**	0.612**	0.618**

Note：*Significant correlation was found at the 0.05 level (bilateral)；**significant correlation was found at the 0.01 level (bilateral).

Figures

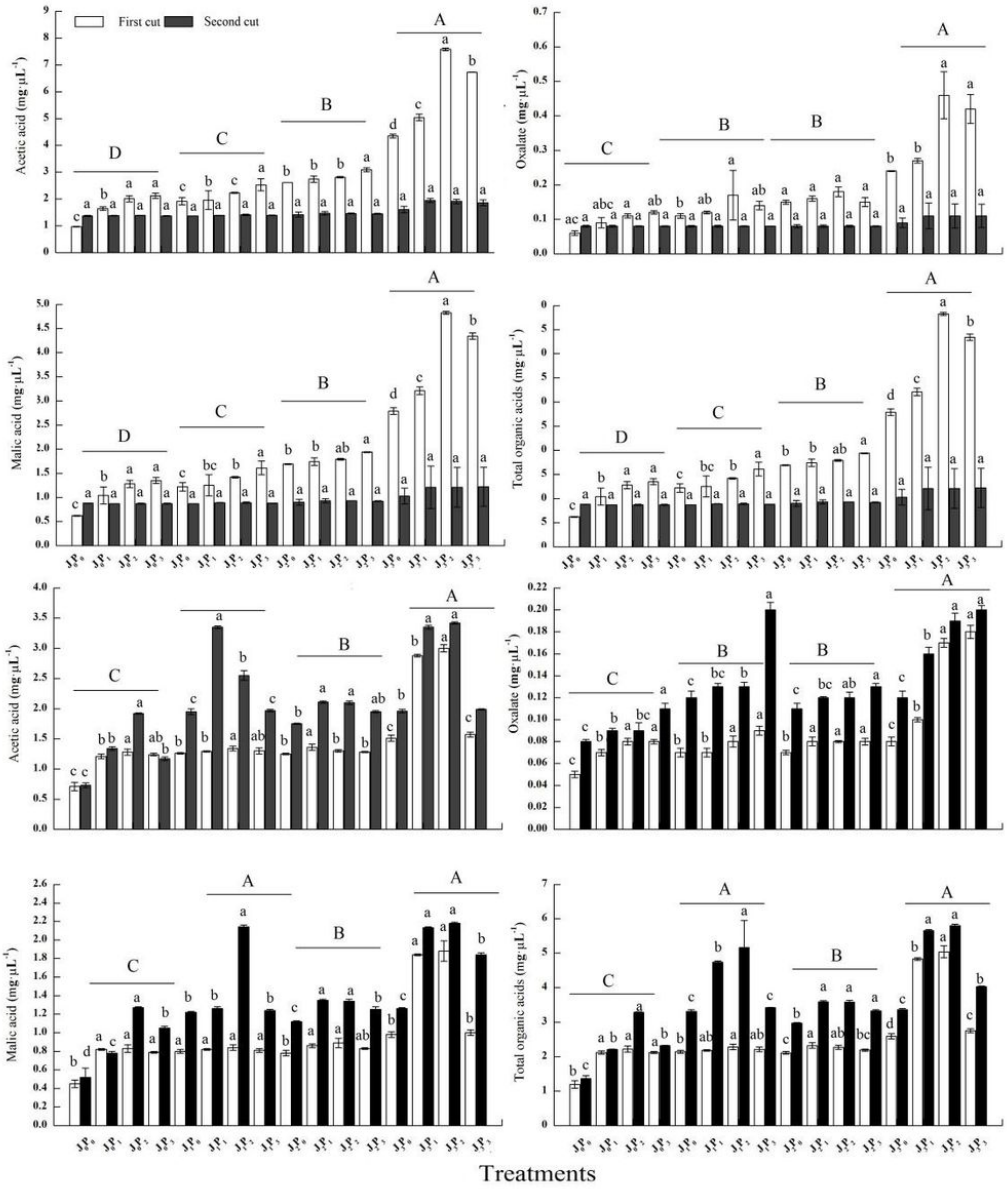

Figure 1

Organic acid content in rhizosphere soil under different treatments in 2019-2020 Note: P0, P1, P2, and P3 represent 0 mg·kg−1, 50 mg·kg−1, 100 mg·kg−1, and 150 mg P kg−1, respectively. J0, J1, J2, and J3 represent CK, Fm, Bm, and Fm×Bm, respectively.

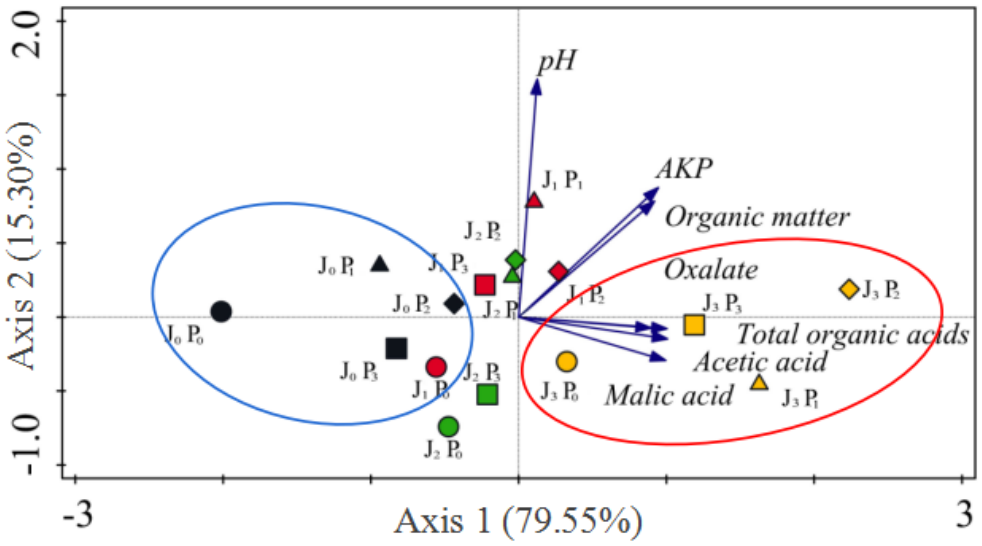

Figure 2

Principal component analysis of each index in 2019-2020 Note: P0, P1, P2, and P3 represent 0 mg·kg−1, 50 mg·kg−1, 100 mg·kg−1, and 150 mg P kg−1, respectively. J0, J1, J2, and J3 represent CK, Fm, Bm, and Fm×Bm, respectively.