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Understanding phosphorus mobilization mechanisms in acidic soil amended with calcium-silicon-magnesium-potassium fertilizer

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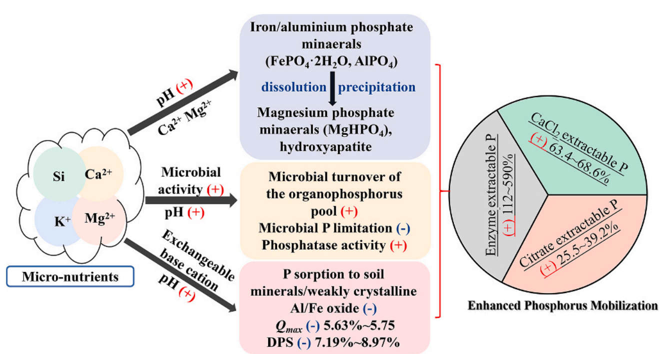
HIGHLIGHTS

- The acidic soil was amended by calcium-silicon-magnesium-potassium fertilizer
- The mobilization of phosphorus in acidic soil was enhanced after amendment
- Phosphorus mobilization attributed to both chemical and biological processes
- The decreased sorption and precipitation contributed to phosphorus mobilization
- Enzymatic stoichiometry mediated phosphorus mobilization in amended acidic soil

GRAPHICAL ABSTRACT



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ABSTRACT

Calcium-silicon-magnesium-potassium fertilizer (CSMP) is usually used as an amendment to counteract soil acidification caused by historical excessive nitrogen (N) applications. However, the impact of CSMP addition on phosphorus (P) mobilization in acidic soils and the related mechanisms are not fully understood. Specifically, a knowledge gap exists with regards to changes in soil extracellular enzymes that contribute to P release. Such a knowledge gap was investigated by an incubation study with four treatments: i) initial soil (Control), ii) urea (60 mg kg^{-1}) addition (U); iii) CSMP (1%) addition (CSMP) and iv) urea (60 mg kg^{-1}) and CSMP (1%) additions (U + CSMP). Phosphorus mobilization induced by different processes was distinguished by biologically based P

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extraction. The *Langmuir* equation, *K* edge X-ray absorption near-edge structure spectroscopy, and ecoenzyme vector analysis according to the extracellular enzyme activity stoichiometry were deployed to investigate soil P sorption intensity, precipitation species, and microbial-driven turnover of organophosphorus. Results showed that $CaCl_2$ extractable P (or citric acid extractable P) content increased by 63.4% (or 39.2%) in the soil with CSMP addition, compared with the study control. The accelerated mobilization of aluminum (Al)/iron (Fe)-bound P after CSMP addition, indicated by the reduction of the sum of $FePO_4{\cdot}2H_2O$ and $AlPO_4$ proportion, contributed to this increase. The decrease of P sorption capacity can also be responsible for it. The CSMP addition increased enzyme extractable P in the soil nearly 7-fold and mitigated the limitations of carbon (C) and P for soil microorganisms (indicated by the enzyme stoichiometry and ecoenzyme vector analysis), suggesting that microbial turnover processes also contribute to P mobilization in amended acidic soil. These findings indicate that the P mobilization in CSMP amended acidic soil not only attributed to both decreasing P sorption capacity and dissolving phosphate precipitation, but also to the increase of the microbial turnover of the organophosphorus pool.

1. Introduction

Globally, soil legacy phosphorus (P) mobilization has long been of interest because of its important role in agricultural production (Pavinato et al., 2020; Wang et al., 2023). Also, it is worth noting that excessive P release from agricultural soil can impact negatively on the environment, for instance via eutrophication (Fan et al., 2022a). In acidic soils, phosphate interacts with soil iron (Fe)/aluminum (Al) oxides (and/or hydroxides) along with clay minerals, forming various complexes (Nguyen et al., 2017; Celi et al., 2020). Microbial activities and mineralization-immobilization of soil organic P are also limited under acidic conditions (Cui et al., 2020). This leads to low bioavailability and mobility of P in such acidic soils. However, with soil P accumulation increasing under continuous fertilization, it becomes increasingly important to investigate the mobilization of P from different perspectives. In acidic soils both potential agronomic benefits and/or P loss to waters must be balanced.

Incorporation of alkaline amendments plays an essential role in agricultural activities to elevate soil pH and enhance exchangeable base cation contents in acidic soil. Fan et al. (2022b) have demonstrated that the increased soil pH significantly affected the P mobilization in acidic soil. Dolomite amendment addition has been shown to enhance labile P content >40% in an acidic red soil, and the transformation between Fe/Al-bound P and Calcium(Ca)-bound P was the main contributor (Fan et al., 2019). Zeolite amendment increases P uptake of crop in acidic soils by inhibiting P fixation of soil (Johan et al., 2021). Many studies have found that the increase of soil pH mainly contributed to the enhanced P mobilization in biochar-amended acidic soil (Glaser and Lehr, 2019; Peng et al., 2022). The joint action of precipitation-dissolution, sorption-desorption, and microbial immobilization-mineralization is generally regarded as the key process which regulates P mobilization (Zhang et al., 2022). Among the mentioned processes, the physicochemical processes such as the desorption of adsorbed P and the dissolution of phosphate-precipitation, are commonly reported to mainly govern the mobilization of P in acidic soils after alkaline amendments (Peng et al., 2022). Furthermore, it has been shown that soil microorganisms and their turnover through extracellular enzymes enhanced organic matter decomposition, thereby increasing the corresponding organophosphorus mineralization (Ge et al., 2017; Park et al., 2022). This would contribute to the mobilization of P. However, whether this process also occurs in acidic amended soils and contributes to enhanced P mobilization remains a knowledge gap.

Soil extracellular enzymes activity is essentially basic information required to connect environmental nutrient mobilization and microbial metabolic requirements. Generally, microorganisms express their nutrient requirements and improve their nutrient acquisition by changing soil extracellular enzymes activities (Waring et al., 2014; Tian et al., 2020). Vector length and angle analysis of enzyme activities obtained from the relationship between C/(C + N) versus C/(C + P) have been used as a quantitative method to determine the limitation and shifts in relative nutrient requirements of microorganisms (Zuccarini et al., 2020; Shen et al., 2023). The alkaline amendment predictably changes enzyme activities, therefore affecting P mobilization by modifying enzymatic allocations. For example, the microbial biomass distribution and the solubility of substrates in soils was affected by the increase of pH, thus affecting soil enzyme activities and stoichiometry, which positively changed soil P mobilization (Yan et al., 2016; Chen et al., 2022).

Calcium-silicon-magnesium-potassium fertilizers (CSMP), which are produced by calcination of a mixture of industrial wastes, have the potential to ameliorate soil acidification, suppress the occurrence of various soil-borne diseases and supply abundant micro-nutrients elements as a low-cost alkaline amendment (Zhao et al., 2020; Kong and Lu, 2023). However, whether the CSMP amendment influences soil P mobilization and the corresponding mechanisms involved remains a knowledge gap, especially with regards to change in soil extracellular enzymes.

The purpose of this research is to demonstrate the effect of CSMP amendment on P mobilization in an acidic soil and explore the corresponding mechanisms. An incubation experiment was conducted with an acidic soil to fill this knowledge gap. The soil P sorption characteristics, precipitation species and vector characteristics of extracellular enzymatic stoichiometry in response to CSMP addition were investigated. It was hypothesized that CSMP addition enhances P mobilization not only by decreasing P sorption capacity and promoting the dissolution of phosphate precipitation, but also by changing the microbial turnover of the organophosphorus pool. The present research will promote optimized application of CSMP amendments in agriculture and environmental management.

2. Materials and methods

2.1. Experimental soil and calcium-silicon-magnesium-potassium fertilizer descriptions

The paddy soil used in the present study was sampled from a field in Zhuzhou City (27°49′53″N, 113°24′55″E), Hunan Province, China. The undisturbed soil samples were sampled using cutting rings for water holding capacity (WHC) determination as in Dai et al. (2021). A brief description of this method is outlined in the Supplemental Material. The collected soil was sieved (< 2 mm), fully mixed, and air-dried. The acidic soil has a pH of 4.8. Detailed information of the tested soil is shown in Table S1. The CSMP amendment with pH 10.3 and acid buffering capacity 618 kg t^{-1} used in the present study was prepared from a calcination of phosphogypsum and potassium feldspar. The CSMP and urea used in the present study were obtained from Kingenta Ecological Engineering Group Co., Ltd. (Shandong province, China), and Guoyao, Co., Ltd. (Shanghai, China), respectively. Table S2 shows the contents of the main elements that the tested CSMP amendment contains.

2.2. Microcosm experiment

The microcosm incubation experiment was conducted with four treatments as follows: i) initial soil (Control), ii) urea (60 mg kg^{-1}) was added into initial soil (U); iii) CSMP (1%) was added to initial soil (CSMP) and iv) both urea (60 mg kg^{-1}) and CSMP (1%) were added into initial soil (U + CSMP). Each treatment had three replicates. For treatments iii) and iv), the CSMP was fully mixed with the soil before the experiment started, and for treatments ii) and iv), the urea (analytically pure) was dissolved in deionized water and applied at the beginning of the experiment.

For the incubation, treatments iii) and iv) were filled with 101 g consisting of a mixture of the initial soil and amendment into 442 mL containers (80 mm diameter and 88 mm height) ($n = 6$). For the treatments i) and ii), 100 g air-dried soil was placed into 442 mL containers with 80 mm diameter and 88 mm height (n = 6). The soil moisture was then adjusted up to 60% of the field water holding capacity by deionized water for treatments i) and iii), and by adding urea solution for treatments ii) and iv). Subsequently, all of the containers were covered with parafilm, which was perforated (about 0.7 mm in diameter) with a sterile syringe needle in 10 places including the four corners and the center. The covered containers were incubated in darkness for 45 days in an artificial climate chamber with a constant temperature of 25 °C and a constant humidity of 70%. Mobilization of P induced by biological processes and physicochemical processes requires an incubation period of between 40 and 45 days (i.e. same as present study) (Arenberg and Arai, 2021; Ding et al., 2023). To avoid the impact of light-sensitive microbial activities on soil P transformation the incubations were conducted in darkness (Siegenthaler et al., 2020; Chen et al., 2021). During the incubation period, about 1 mL of deionized water was added to each container every 2 to 3 days in order to maintain moisture of incubated soils over time.

2.3. Soil properties analysis

The soil was sampled for analysis after a 45-day incubation period. The electrode method was performed for the soil pH measurement with a 1:2.5 soil/water suspension. Phosphorus mobilization in acidic soils induced by multiple process was investigated using the biologically-based P extraction (BBP) method proposed by DeLuca et al. (2015), which can assign soil P mobilization induced by a specific process to the corresponding P fractions. For example, the size of the enzyme-P pool shows the P fraction with the potential that can be mobilized by phosphatases in the soil (Chen et al., 2018). The procedures of the BBP method was applied as follows: 0.5 g of soil were separately put into four 15 mL centrifuge tubes and shaken in 10 mL extractants. The four extractants were 0.01 M calcium chloride ($CaCl_2$) solution, 0.01 M $C_6H_8O_7$ (citric acid), 0.02 EU ml^{-1} phosphatase solution in 50 mM sodium acetate buffer and 1 M hydrochloric acid solution.

The P sorption isotherms were determined referring to a previous study (Zhang et al., 2022). In summary, the soil and the phosphate solution were put in a polypropylene centrifuge tube at a ratio of 1:15. The P concentration of the phosphate solution were 0, 1, 5, 10, 25 and 50 mg P L^{-1}. The KH_2PO_4 was dissolved in the 0.01 M $CaCl_2$ solution, and then gradually diluted to the required concentration. All of the polypropylene centrifuge tubes were shaken for 24 h at 25 °C, and then these polypropylene centrifuge tubes were centrifuged (4000 rpm) to obtain the suspensions and filtered (< 0.45 μm). The P concentration in each suspension was determined by the molybdate blue method (Murphy and Riley, 1962). The contents of P adsorbed by the soil during the shaking period were measured by subtracting equilibrated P concentrations of supernatants from the initial P solution concentrations, together with ammonium oxalate extracted-P content (the P initially adsorbed in the soil matrix before shaking) represented the total amount of soil adsorbed P. The P concentration in suspension (mg L^{-1}) and total amount of P adsorbed by the soil (mg kg^{-1}) at equilibration were fitted by *Langmuir* and *Freundlich* models, respectively. The *Langmuir* and the *Freundlich* models are as in Eqs. (1) and (2), respectively.

$$Q = \frac{K_L Q_{max} C}{(1 + K_L C)} \tag{1}$$

$$Q = K_F C^{1/n} \tag{2}$$

Both the *Langmuir* and the *Freundlich* models can be transformed into linear models shown in Eqs. (3) and (4) (Yan et al., 2018; Zhang et al., 2022).

$$\frac{C}{Q} = \frac{1}{K_L Q_{max}} + \frac{C}{Q_{max}} \tag{3}$$

$$ln Q = ln K_F + \frac{1}{n} ln C \tag{4}$$

where C and Q are the concentration of P in supernatants (mg L^{-1}) and the total amount of P adsorbed by the soil (mg kg^{-1}) at equilibration. The Root Mean Squared Error of the *Langmuir* and *Freundlich* fitting are $(6.86 \pm 1.57)*10^{-4}$ and $(4.54 \pm 0.459)*10^{-2}$, respectively. The corresponding P values of the F-test of the regression model and the t-test of the regression coefficient are both significant, indicating that the linear regression model fits well. The coefficients obtained using Eq. (3) were used to calculate the binding energy (K_L, L mg^{-1}), the maximum adsorption capacity (Q_{max}, mg kg^{-1}) and the P buffer capacity (PBC, L kg^{-1}) (Indiati et al., 1999). The coefficients obtained using Eq. (4) were used to calculate the sorption constant (K_F, mg kg^{-1}) and nonlinearity constant (1/n) (Ding et al., 2023).

The poorly crystalline Fe/Al oxides (Fe_{ox} and Al_{ox}) and Fe_{ox}/Al_{ox} bound P (P_{ox}) were extracted by acid ammonium oxalate solution (pH 3) in the dark and analyzed by ICP-OES (Perkin Elmer Optima 7300 V, USA) after filtration. The degree of P saturation (DPS) was calculated using Eq. (5):

$$DPS\% = \frac{P_{OX}}{\alpha(Feox + Alox)} \tag{5}$$

where α is an empirical coefficient and the value is 0.5 (van Doorn et al., 2023). The soil P precipitation species was conducted using the P K-edge XANES spectroscopy at 4B7A beamline at the Synchrotron Radiation Facility, Beijing, China. Previous studies have introduced the Synchrotron Radiation Facility and explained the steps of sample measurements in detail (Yan et al., 2018). As standard materials, Fe-phosphate ($FePO_4 \cdot 2H_2O$), berlinite ($AlPO_4$), hydroxyapatite [$Ca_5(PO_4)_3OH$, HAP] and newberyite ($MgHPO_4$) were purchased and used for phosphate associated with Fe, Al, Ca and Mg, respectively. All soil samples were freeze-dried and sieved to 0.15 mm particle size, then each sample was scanned by turning in total electron yield mode and fluorescence mode, respectively.

2.4. Enzyme assays and calculation of microbial nutrient limitation

Seven soil enzymes activities (β-1,4-glucosidase, BG; β-D-cellobiosidase, CBH; α-1,4-glucosidase, AG; β-xylosidase, XYL; β-1,4-*N*-acetyl-glucosaminidase, NAG; L-leucine aminopeptidase, LAP; acid phosphatase, AP) were determined (Saiya-Cork et al., 2002) by the standard fluorimetric method and experimental procedure details can be found in the study of Cui et al. (2019). The relative nutrient limitation for soil microorganisms was quantified through vector characteristics analysis of soil enzymatic stoichiometry. The details of this calculation are shown in the supplementary material (S2).

2.5. Statistical analysis

All data were pre-processed by Microsoft Excel 2016. Statistical analyses were all conducted with IBS SPSS Statistics 20, using one-way analysis of variance (ANOVA). The significance of the results across the treatments was estimated using the Least Significant Difference (LSD) test at $P < 0.05$ level. The parameters obtained using Eqs. (3) and (4) were tested for normality ("*skewness*" and "*kurtosis*" calculation) and homogeneity of variances (Levene's test) to ensure that they meet relevant assumptions before deploying the LSD test. The absolute *Z-scores* calculated based on the "*skewness*" and "*kurtosis*" of each parameter obtained by fitting Eqs. (3) and (4) are all <1.96, indicating that these parameters conform to a normal distribution. Similarly, the *P* value obtained by Levene's test are all >0.05, indicating that these parameters show homogeneity of variances. Graphs were drawn in Sigmaplot (version 12.5). R software (Version 3.3.3) was used to conduct the heatmap of Spearman correlation between the P fractions, sorption parameters fitted by *Langmuir* and *Freundlich*, phosphatase and microbial nutrient limitation.

3. Results

3.1. Soil P fractions

Phosphorus fractions in the soil were detected by the BBP method. The calcium chloride extracted P ($CaCl_2$-P) was used to indicate the soluble inorganic P which crops can use or lose directly from soil. It was found that the $CaCl_2$-P content was noticeably enhanced by 63.4% and 68.6% in the CSMP and U + CSMP treatments, in line with the study control (Fig. 1a). These two treatments also showed 39.2% and 25.5% increase in the content of phosphate extracted by citric acid (citric acid-P), respectively, compared with the study control (Fig. 1b). In soil amended only with CSMP, the P extracted by phosphatase (enzyme-P) was the highest, which was about 7 times higher than in the study control (Fig. 1c). The HCl-P content between the study control and the other treatments showed no remarkable difference in this study (Fig. 1d).

3.2. Vector characteristics of extracellular enzymatic stoichiometry

The soil phosphatase activity increased by 109% and 75.3% respectively after CSMP and U + CSMP additions, in line with the study control ($P < 0.05$, Fig. 2a). Based on data shown in Fig. 2b, the calculated vector degrees in the four treatments were >45°. It was also found that the vector length of the CSMP and U + CSMP treatments were reduced by 16.5% and 17.0%, compared with the study control respectively (Fig. 2c).

3.3. Soil P sorption characteristics and precipitation species

In the present study, soil Q_{max}, K_L and PBC were calculated via *Langmuir* isotherms (Fig. 3). Table 1 shows the parameters of fitting curves from *Langmuir* model, which exhibited high determination coefficients ($R^2 > 0.98$). Both *Freundlich and Langmuir* models were fitted to sorption data. Result showed that the *Langmuir* model produces a better fit to the data and therefore is more suitable for describing the adsorption characteristics of soil P in the present study. Therefore, the parameters of fitting curves from *Freundlich* model were not shown. The

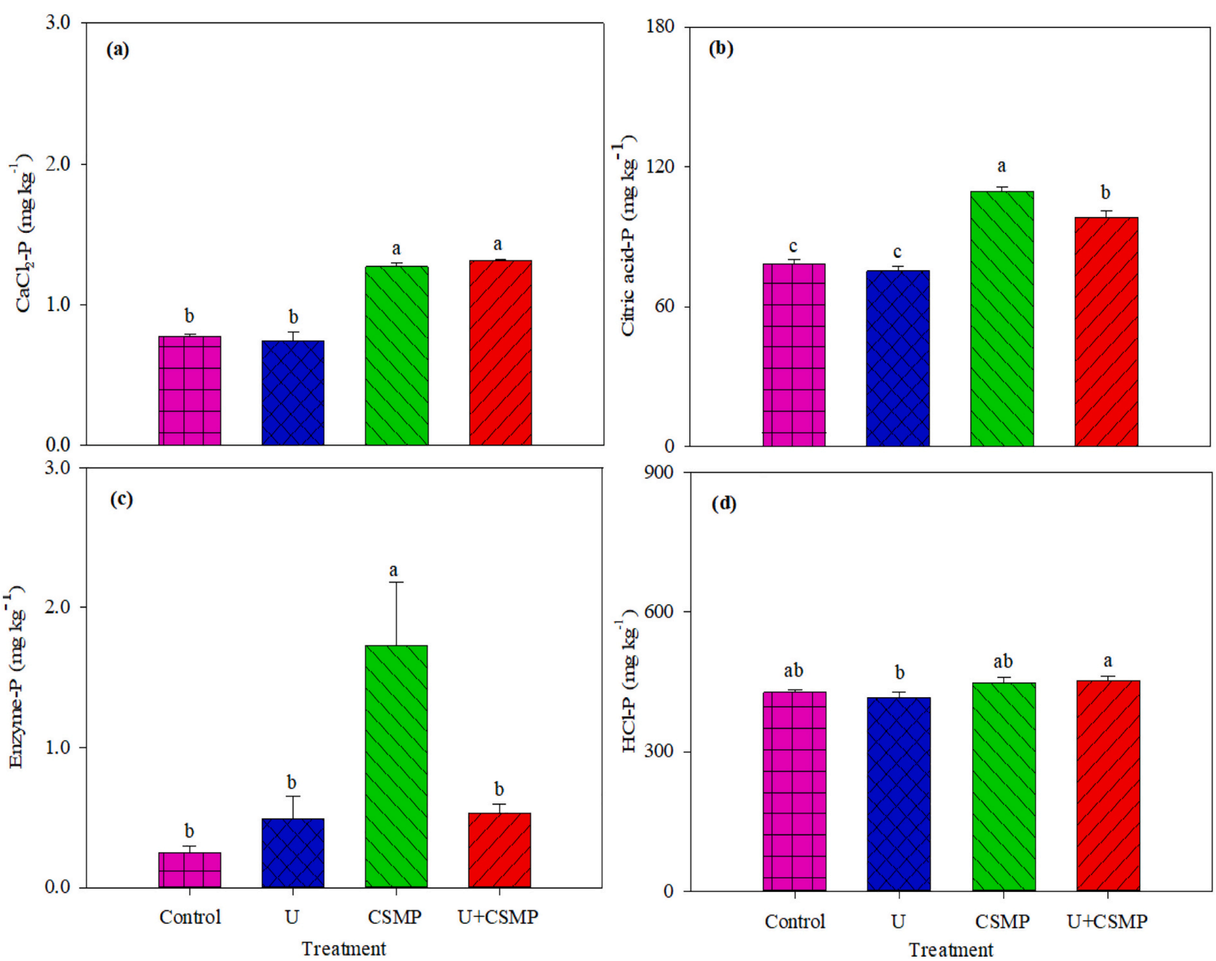

Fig. 1. Soil P bioavailability across treatments. $CaCl_2$-P (a), Citric acid-P (b), Enzyme-P (c) and HCl-P (d). Values are the means ± standard error ($n = 3$). The same lowercase letters indicate no significant differences among the treatments according to the LCD at $P < 0.05$. U, urea addition; CSMP, CSMP addition; U + CSMP, CSMP and urea addition.

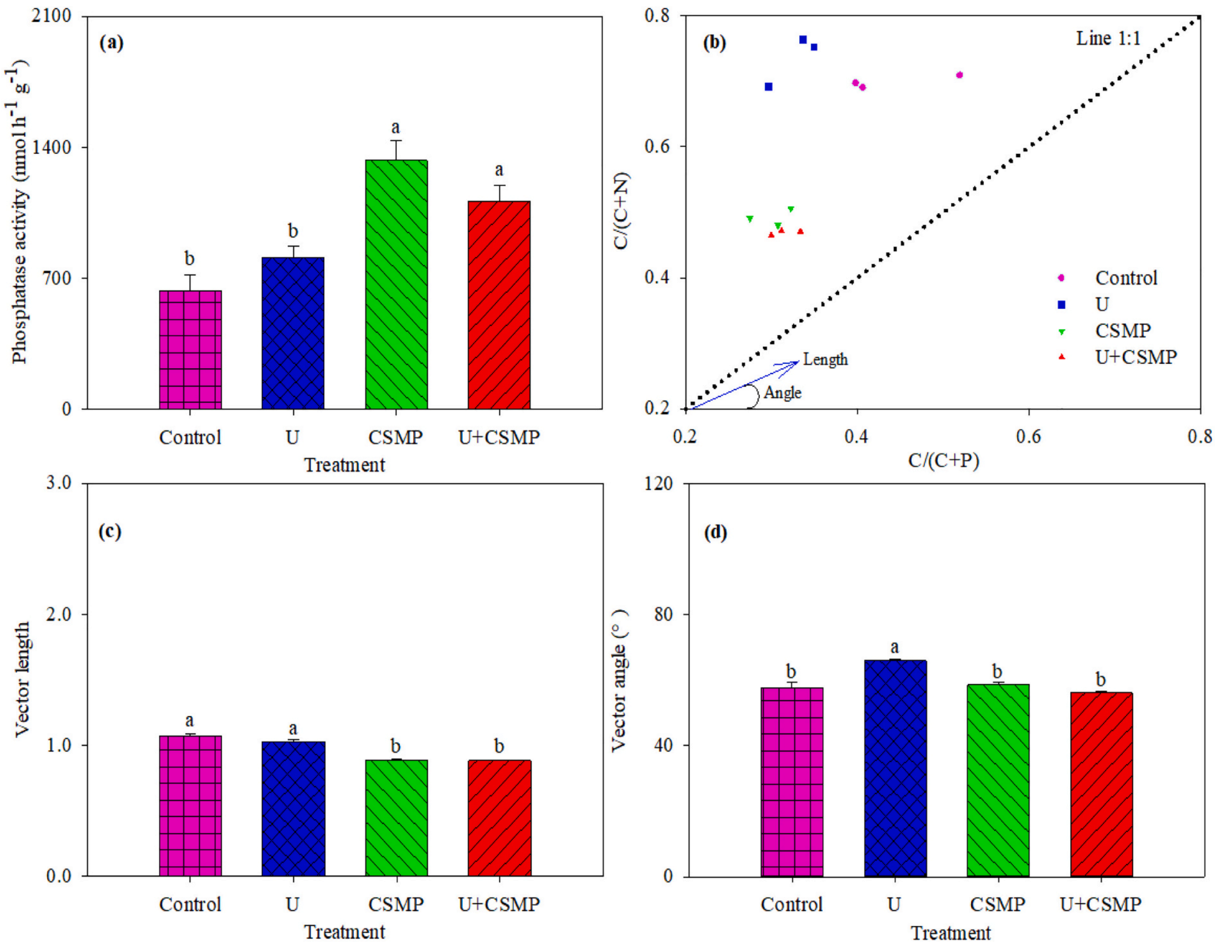

Fig. 2. Phosphatase activity (a), enzymatic stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (b), the variation of vector length (c) and angle (d) across treatments. The same lowercase letters indicate no significant differences among the treatments according to LSD at $P < 0.05$. U, urea addition; CSMP, CSMP addition; U + CSMP, CSMP and urea addition.

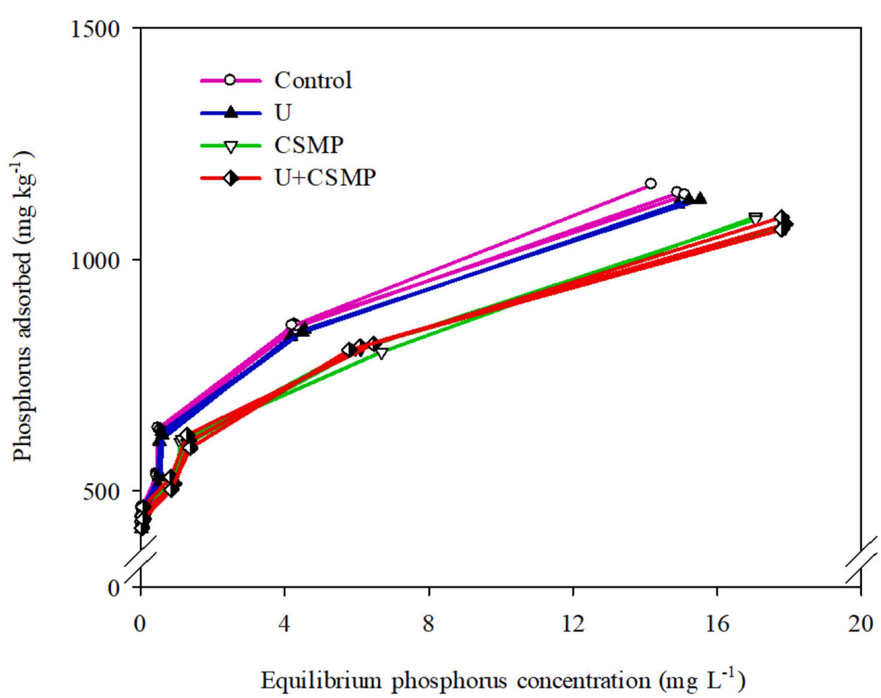

Fig. 3. The phosphorus sorption fitted by *Langmuir* model of soil among treatments. U, urea addition; CSMP, CSMP addition; U + CSMP, CSMP and urea addition.

Table 1
Langmuir fitted sorption parameters, degree of P sorption saturation of soil and soil pH among treatments.

Treatments[a]	*Langmuir* Fitting				DPS	pH
	Q_{max}[b] (mg kg^{-1})	K_L (L mg^{-1})	PBC (L mg^{-1})	R^2		
Control	1162 a[c]	2.08 a	2412 a	> 0.98	14.8 a	4.91 b
U	1141 b	1.99 a	2275 a	> 0.99	14.5 a	4.80 b
CSMP	1097 c	1.29 b	1410 b	> 0.98	13.4 b	6.10 a
U + CSMP	1095 c	1.26 b	1401 b	> 0.98	13.7 b	6.09 a

[a] U, urea addition; CSMP, calcium-silicon-magnesium-potassium fertilizer addition; U+CSMP, calcium-silicon-magnesium-potassium fertilizer and urea addition.
[b] Q_{max}, K_L, PBC, DPS are maximum P sorption capacity (mg kg^{-1}), binding energy (L mg^{-1}), P buffer capacity (L kg^{-1}) and degree of P saturation, respectively.
[c] The same lowercase letters indicate no significant differences among the treatments according to the LSD at $P < 0.05$.

relatively gentle sorption curve was found in the treatments amended by CSMP. The CSMP and U + CSMP treatments decreased Q_{max} by 5.63% and 5.75%, significantly decreased K_L by 38.1% and 38.4%, and decreased PBC by 41.6% and 41.9%, respectively, compared with the study control. Significantly lower DPS was also found in the soil amended by CSMP and U + CSMP among all treatments.

The soil P species in the study control and CSMP treatments were detected by XANES (Fig. 4). In the study control, phosphate precipitation includes $FePO_4 \cdot 2H_2O$, $AlPO_4$ and $MgHPO_4$, excluding hydroxyapatite (HAP). The sum of $FePO_4 \cdot 2H_2O$ and $AlPO_4$ proportion exceeded 50% (Fig. 4a). Compared with the study control, it was found that the CSMP amendment decreased the proportion of $FePO_4 \cdot 2H_2O$ and $AlPO_4$ proportion and increased the proportion of hydroxyapatite and $MgHPO_4$. The sum of hydroxyapatite and $MgHPO_4$ proportion exceeded 70%, while the sum of $FePO_4 \cdot 2H_2O$ and $AlPO_4$ proportion was < 30% (Fig. 4b).

3.4. Relationships between the P fractions, P adsorption characteristics and microbial nutrient limitation

The relationship among the soil P fractions, sorption parameters fitted by *Langmuir* and *Freundlich*, phosphatase and microbial nutrient limitation was investigated using a Spearman correlation heatmap analysis (Fig. 5). The $CaCl_2$-P and citric acid-P contents showed negative relationship with DPS, Q_{max}, K_L, PBC, K_F and vector length (microbial C limitation), while these two P fractions contents were positively related to the phosphatase activity ($P < 0.01$). The positive relationship between soil enzyme-P and the phosphatase activity was also shown ($P < 0.05$). Notably, the present result revealed that phosphatase activity is negatively linked to vector length ($P < 0.001$).

4. Discussion

4.1. CSMP addition improves soil P mobilization

The results of this study show, that the content of $CaCl_2$-P was increased in soil amended with CSMP ($P < 0.05$, Fig. 1a), suggesting that CSMP addition mobilized P which can be directly lost to adjacent waterbodies. Phosphate extracted by citric acid (citric acid-P) represents the components that could be activated and released by organic acids. A considerable content of citric acid-P appeared in treatments with CSMP amendment, which indicated that CSMP amendment could increase potentially available P (Fig. 1b). It has been shown that organic acids can both compete for soil P sorption sites and promote the dissolution of insoluble phosphate (Zhu et al., 2018). The enhanced citric acid-P content was attributed to the enhanced desorption of P adsorbed on the soil matrix and the accumulation of unstable phosphate precipitates in the soil amended with CSMP, because some of the phosphate precipitates can be dissolved in organic acid conditions. The citric acid-P content was reduced slightly in the U + CSMP treatment compared with CSMP treatment. This may be due to the nitrification of NH_4^+ radicals hydrolyzed from urea causing an acidifying effect, which in turn dissolves some phosphate precipitates. Phosphate extracted by enzyme (enzyme-P) commonly simulates an organophosphorus pool that can be hydrolyzed by phosphatase. The enzyme-P content also increased after the CSMP amendment (Fig. 1c). Significant microbial P solubilization

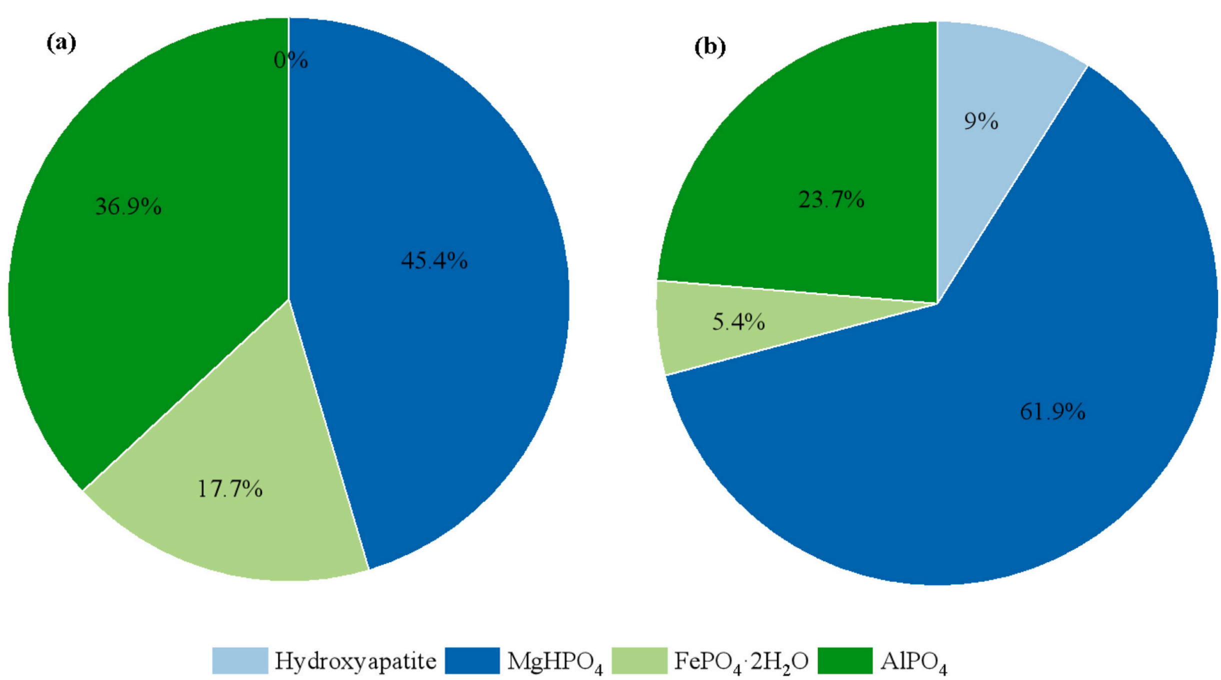

Fig. 4. The soil P speciation detected by P K edge X-ray absorption near-edge structure in Control (a) and CSMP treatments (b).

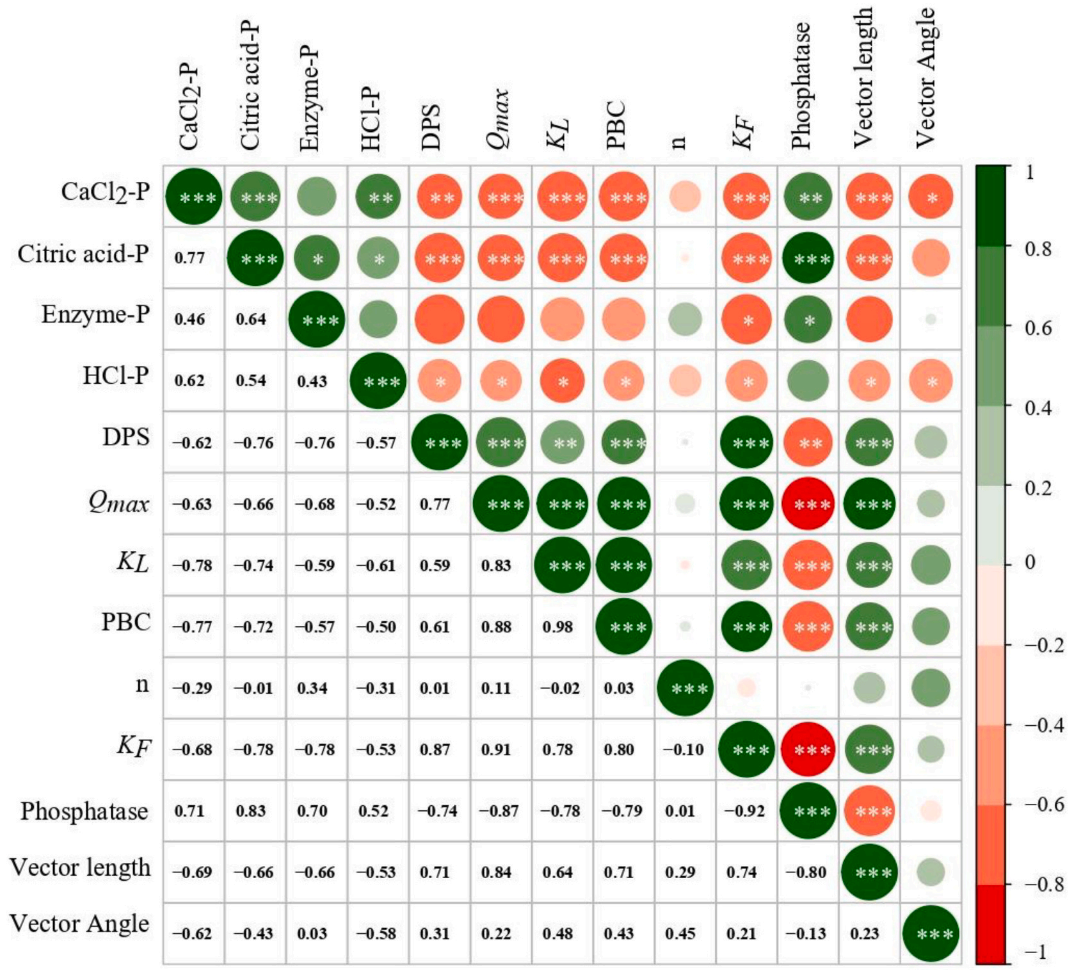

Fig. 5. Heatmap of Spearman's correlation between the P fractions, adsorption parameters fitted by *Langmuir* and *Freundlich* models, phosphatase and microbial nutrient limitation. *KL*, n and *KF* are *Langmuir* and *Freundlich* constants. Negative correlation and positive correlation were represented by red color and green color. *indicates $0.01 < P < 0.05$, ** indicates $0.001 < P < 0.01$, *** indicates $P < 0.001$.

has also been seen in biochar-amended soil (Simarani et al., 2018). It is possible that such results are due to the increased soil phosphatase activity and mineralization of organic P dominated by microbes (Yin et al., 2023). Rui et al. (2009) have found that significantly stimulated microbial growth produces increased phosphatase activity, which would contribute to increasing soil P mobilization. A lower content of enzyme-P was observed in the U + CSMP treatment, as compared with the CSMP treatment. Organic P mineralization driven by microbial activity after urea addition may be responsible for the decrease in enzyme-P content in the U + CSMP treatment (Yan et al., 2016). The HCl-P indicates the maximum potential P pool which can be mobilized by hydrogen protons. The content of HCl-P showed no remarkable difference between the study control and other treatments (Fig. 1d). The sum of the different P fractions quantified by the BBP method represents the maximum capacity of available P pools in the soil. Results showed that after CSMP addition, the contents of $CaCl_2$-P, citric acid-P, and enzyme-P significantly increased, while that of HCl-P remained unchanged. Therefore, the addition of CSMP promoted the transformation of unavailable P into available P, thereby expanding the potential maximum capacity of available P pools.

4.2. Changes of soil P sorption capacity and precipitation species contributed to P mobilization

The CSMP amendments enhanced the soil labile P pools, which were influenced by several processes including adsorption strength of soil minerals and precipitation-dissolution of phosphate associated with Fe, Al and Ca (Adhikari et al., 2017). The present data fitted well with *Langmuir* equations, and they were used to analyze the P sorption characteristics (Yang et al., 2019). Table 1 summarizes that Q_{max} for CSMP amended soils was lower than for the study control, which indicated that the P sorption intensity by soil minerals decreased with CSMP addition. Lower K_L values in soils with CSMP amendment also indicated reduced P sorption affinity. All these findings showed that the P sorption capacity of soils with CSMP amendments decreased when compared with the study control. Spearman correlations presents the negative relationship between $CaCl_2$-P/citric acid-P and sorption parameters fitted by *Langmuir* (Fig. 5). These results have shown that the decreased soil P sorption capacity contributed to enhanced soil $CaCl_2$-P and citric acid-P pools, as the free phosphate becomes less immobilized by the soil. Previous studies have also identified that decreased soil P sorption capacity can be contributed to enhanced soil P mobilization (Zhang et al., 2022), which supports the present results. Conversely, increased soil P sorption capacity has been shown to decrease soil $CaCl_2$-P content (Ding et al., 2023). In acidic soils, poorly crystalline Al/Fe oxides dominate the process of P fixation (Ding et al., 2023). The DPS on weakly crystalline Al/Fe oxides was also examined in the present study and it was found that CSMP amendments successfully reduced soil DPS (Table 1). This indicated the decreased contribution of weakly crystalline Al/Fe oxides to P fixation in soils and it appeared to be the main explanation for the decline of soil P sorption capacity. Furthermore, compared with the control, the soil pH increased by almost two units as a consequence of

CSMP amendment (Table 1). The considerably enhanced soil pH would be responsible for decreased DPS on weakly crystalline Al/Fe oxides in soils.

Phosphorus precipitation species detected by the XANES displayed that CSMP addition remarkably increased the dissolution of $FePO_4{\cdot}2H_2O$ and $AlPO_4$ and stimulated the precipitation of $MgHPO_4$ and hydroxyapatite, compared with the control treatment ($P < 0.05$, Fig. 4). CSMP is rich in calcium and magnesium, and while it increases soil pH, it also promotes the transfer of phosphate from Al/Fe oxides to calcium and magnesium minerals. Compared with the present findings, previous studies have shown that the incorporation of dolomite in an acidic soil enhanced soil pH led to Al/Fe bound-P release, promoting soil P mobilization (Fan et al., 2019). Correspondingly, the released P tends to accumulate in the soil as dibasic calcium phosphate dihydrate ($CaHPO_4{\cdot}2H_2O$) (Fan et al., 2019). These results support the theory that the dissolution of phosphate precipitation stimulated by CSMP addition also contributes to the mobilization of soil P. The relatively unstable phosphate precipitation, such as $MgHPO_4$ and $CaHPO_4{\cdot}2H_2O$, is also responsible for the increase of citric acid-P since they seem to dissolve more easily in citric acid solution.

4.3. Enzyme stoichiometry mediates P mobilization in acidic soil

The soil enzyme-P content in the CSMP treatment increased remarkably compared to the study control (Fig. 1c). The soil enzyme-P pool simulates the organic P mobilization potential through the release of phosphatases by microorganisms (Gao and DeLuca, 2018). Thus, in addition to the abiotic progress, the metabolic activity of microbes is an important factor in regulating P mobilization during acidic soil amelioration with CSMP amendments (Sun et al., 2021). In the soil ecosystem, the microbial utilization efficiency of nutrients is regulated by extracellular enzymes (Waldrop et al., 2017; Coonan et al., 2020). Soil phosphatase activity reveals its potential ability to release labile P from organic P pool. Acid phosphatase generally contributes more to mineralizing organic P than alkaline phosphatase under acidic conditions (Tazisong et al., 2015; Li et al., 2021). Acid phosphatase activity significantly increased after CSMP addition, which indicated that more organic P was mineralized (Fig. 2a). This result coupled with the positive relationship between phosphatase activity and $CaCl_2$-P, enzyme-P and citric acid-P supported that CSMP addition stimulated microbial turnover to enhancing P mobilization including release more phosphatase enzymes (Fig. 5).

The correlation between the microbial metabolism needs and soil nutrient supplement is generally reflected by ecoenzymatic stoichiometry (Cui et al., 2022; Duan et al., 2022). In this research, the characteristics of extracellular enzymes stoichiometry after incubation varied with the treatments, which indicated the varying C, N or P nutrient limitation of soil microbes (Xu et al., 2022). According to the vector analyses of enzyme activity, the vector angle of $>45^{\circ}$ denoted P limitation of microorganisms in acidic soil (Fig. 2b). Negative correlations between soil $CaCl_2$-P content and microbial P limitation (vector angle) can be identified through Spearman correlation analysis (Fig. 5). This demonstrated that the improvement of labile P content produced the reduced microbial P limitation in the soil after CSMP addition. In line with the results of this study, Wang et al. (2022) found microbial P limitation correlated negatively with labile P fractions. Amendments with CSMP induced higher pH, which would contribute to higher microbial activity and elevated organic matter mineralization, weakening microbial P limitation and enhancing P mobilization in the soils (Richardson and Simpson, 2011; Xu et al., 2020a). In summary, CSMP addition negatively affected microbial P limitation, which supports the hypothesis of this study that soil microbial metabolism demands for P would be limited to a lower extent after CSMP addition because enzyme stoichiometry changes give rise to P mobilization.

As can be seen from Fig. 2, CSMP addition effectively mitigated C limitation during soil microbial metabolism. The result of the Spearman correlation analysis indicates that a negative relationship between phosphatase and vector length (i.e. microbial C limitation) exits. The cessation of C limitation in microbial metabolism is supported by the increase in phosphatase activity, which promotes mineralization of organophosphorus thereby enhancing P mobilization. The increase in soil pH after CSMP addition suggests a change in organic matter thereby altering the activity of soil microorganisms, thereby preventing C limitations (Zhang et al., 2020). The decomposition of soil organic matter could also be affected by the P-acquisition strategies of soil microorganisms (Ding et al., 2021; Wang et al., 2022). Such soil microbial P-acquisition strategies are regulated by changes in soil properties (Lambers, 2022). Such findings indicate that P transformation processes induced by microbial metabolism changes could be affected by soil C storage and microbial C acquisition.

While microbial metabolic activity contributes to the mobilization of P, this in turn may regulate microbial metabolic activity. As the available P in the soil increases, the P limitation of crops and microorganisms is also alleviated. This promotes the input of C via the plant root and in turn regulates the balance between labile P immobilization and organophosphorus mineralization caused by microbial metabolism. For example, Xu et al. (2020b) found that C additions in intensive agricultural systems reduced soil available P by enhancing microbial immobilization of P. Cao et al. (2022) found that soil phosphatase activity decreased after applying more phosphate fertilizers in a field experiment. Therefore, in the present study, it is inferred that the contribution of the microbial metabolic activity on the mobilization of P after CSMP addition should be dynamic. The decrease of P sorption capacity, the dissolution of phosphate-precipitation, and microbial turnover processes work together to promote soil P mobilization to cope with insufficient soil P availability. Subsequently, microbial metabolic activities may gradually begin to regulate the balance between labile P immobilization and organophosphorus mineralization based on P availability level to buffer changes in the size of soil available P pools. In the future, research needs to focus on technological advancements to determine the contribution microbial metabolism plays in soil P availability where amendments to mitigate soil acidification are used.

In summary, CSMP addition alleviated P sorption by the soil, accelerated the $FePO_4{\cdot}2H_2O$ and $AlPO_4$ precipitation transfers into $MgHPO_4$ and increased the metabolic activity of microorganisms, thus stimulating P mobilization.

5. Conclusions

The results presented in this study revealed that CSMP amendments remarkably enhanced mobilization of P and brought an increased P availability or P loss risk. The reduced P sorption intensity of the soil, accompanied by the decreased proportion of $FePO_4{\cdot}2H_2O$ and $AlPO_4$, lead to the increase of P mobilization in acidic soil. Simultaneously, the enhanced organic P mineralization (indicated by the phosphatase activity) and mitigated C and P limitations of soil microorganisms indicated that microbial turnover processes would also contribute to P mobilization in amended acidic soil. This study helps to better understand the P transformation process in acidic soil improvement and environmental management. Microbes regulating nutrient mobilization should be considered during soil amendment, and further work is in progress to investigate the potential microbial mechanisms from the perspective of soil enzyme in more detail, including P cycle function genes and microbial communities.

CRediT authorship contribution statement

Jilin Lei: Data curation, Writing-Reviewing & Editing, Conceptualization, Validation
Junhui Yin: Methodology, Software, Writing-Reviewing & Editing
Shuo Chen: Scientific advice, Formal analysis
Owen Fenton: Scientific advice, Review & Editing

Rui Liu: Conceptualization, Writing-Reviewing & Editing
Qing Chen: Funding acquisition, Project administration, Reviewing & Editing
Bingqian Fan: Scientific advice, Reviewing & Editing
Shuai Zhang: Writing-Reviewing & Editing, Conceptualization, Visualization, Supervision

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170294.

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