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Path analysis of phosphorus activation capacity as induced by low-molecular-weight organic acids in a black soil of Northeast China

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Abstract

Purpose The present study was designed to assess the potential effects of low-molecular-weight organic acids on the activation of inorganic phosphorus, obtain exact information on the acidity effect of proton and complex effect of organic anion in P availability, and determine the components among phosphorus fractions that contributed the most to inorganic phosphorus activation in black soil.

Materials and methods Both oxalic and citric acids treated with different concentrations and pH values were carefully selected. The activating amounts of total inorganic phosphorus and individual phosphorus fractions were estimated. Path analysis was used to analyze the direct and indirect effects on inorganic phosphorus activation.

Results and discussion The amount of total activating-P_i increased as the concentrations of oxalic and citric acids increased. When the concentrations were ≤ 1.0 mmol L^{-1}, oxalic acid exhibited a lower capability than citric acid in total activating-P_i, but when the concentrations were ≥ 1.5 mmol L^{-1}, oxalic acid exhibited a higher capability. The amount of total activating-P_i decreased as pH of LMWOAs increased. LMWOAs-induced P_i activation might be attributed to combine acidity and complex effects.

Conclusions Correlation analysis showed that the activation of total P_i was significantly correlated with the activation of H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i ($p < 0.05$). Path analysis revealed that soil activating-H_2O-P_i mainly affected P_i activation through an indirect path. The contribution of activating-$NaHCO_3$-P_i alone was maximal on the total activating-P_i by both the direct and indirect effects, followed by activating-NaOH-P_i and activating-HCl-P_i.

Keywords Acidity effect · Complex effect · Path analysis · Phosphorus activation · Phosphorus fractions

1 Introduction

The macronutrient phosphorus (P) is essential for plant growth. As a result of low P contents and the strong binding of phosphate to iron (Fe) and aluminum (Al) oxide forms that are largely unavailable for plant uptake in the short term, most soils are unable to supply P to plants at sufficient rates for sustaining optimal growth; consequently, P availability is often a growth-limiting factor (Wang et al. 2017; Bauke et al. 2017). P fertilizers are often applied to avoid P deficiency in plants (Duminda et al. 2017), but these applications have increased the accompanying risk of P losses to ground and surface waters, contributing to eutrophication (Fischer et al. 2017). Moreover, P in soil exists predominantly in inorganic fractions that are either adsorbed into soil mineral surfaces or occasionally appear as available precipitates (Cao et al. 2015). There is therefore an increasing need for alternative, sustainable methods that facilitate plant acquisition of P from legacy P pools. In studies focusing on P availability, the low-molecular-weight organic acids (LMWOAs) have been suggested to be the most important molecules exuded by plants

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and mycorrhizae (Clarholm et al. 2015). However, there are also researchers who believe that organic acids exert an inhibitory effect or no effect on the availability of P. For example, Iyamuremye and Dick (1996) found that only citric acid showed a significant effect. In addition, the effects of various organic acids on P activation were also different significantly, and different researchers had drawn different conclusions (Strom et al. 2005; Gong et al. 2010; Khademi et al. 2010; Lv et al. 2015; Menezes-Blackburn et al. 2016). Furthermore, the solubility of P-bearing minerals/precipitates is highly pH dependent (Gong et al. 2010; Oburger et al. 2011). Therefore, the activation effect of organic acids needs to be further verified.

The activation mechanisms of organic acids on P also depend on the chemical bonding forms of P in soils (Khademi et al. 2010). These organic acid anions can mobilize and then release inorganic P through forming complexes with Fe, Al, or Ca, blocking P absorption sites on soil particles (Wei et al. 2010). However, different P fractions are often correlated with each other. Such correlations existing among P fractions often make it difficult to determine the true variable that contributes most to P activation in soils. In addition, correlation of a P fraction with the P activation may not necessarily imply a direct influence of the soil P fraction on the P activation. Path analysis, a statistical technique that differentiates between correlation and causation, has been increasingly utilized to define the best criteria for selection in biological and agronomic studies. Path analysis is a standardized partial regression analysis which appears to be helpful in partitioning the correlation coefficients into direct and indirect effects. The direct effect represents the direct contribution of a predictor variable to a response variable. The indirect effect represents the contribution of a predictor variable to a response variable via another predictor variable (Hashimoto et al. 2012). Path analysis has been applied in soil and environmental studies (Hashimoto et al. 2012; He et al. 2018). However, previous studies only reported the activation of P fractions by organic acids (Wang et al. 2009; Gong et al. 2010; Waithaisong et al. 2015; Clarholm et al. 2015), but it remained unclear how the soil P fractions interact with each other and what soil P fraction was the most critical variable in characterizing the total activating P.

Black soils (Chernozem) located in northeast China, are one of the most important soil resources for crop growth and play a crucial role in ensuring national food security (Yao et al. 2017). However, a large proportion of agricultural black soils underwent fertility deterioration and soil erosion over the past several decades because of excessive plowing and pesticide and chemical fertilizer treatment (Ding et al. 2016; Wang et al. 2017). In addition, the application of P fertilizer in the form of animal manure and mineral P fertilizer had various negative effects such as soil acidification, metal toxicity, lower P use efficiencies, although improved soil fertility and crop yields (Zhan et al. 2015; Zhang et al. 2016). Previous research found both tribasic citric acid and the dibasic oxalic acid had induced higher P mobilization than other organic acids among the most commonly produced root and microbial exudates affecting rhizosphere P availability in soils (Menezes-Blackburn et al. 2016). Simultaneously, inorganic P (P_i) pool from surface soil increased with long-term applications of phosphate fertilizer (Rubaek and Sibbesen 1995). So we hypothesized that applying LMWOAs was an effective practice to improve P_i availability. The objectives of this study were (1) to investigate the potential activating capability mechanism of individual P_i fractions by LMWOAs, (2) to distinguish the acidity effects of protons and complex effects of organic anions via the adjusted pH in solution, (3) to provide information on the relative contribution of individual activating-P_i fraction to soil total activating-P_i.

2 Materials and methods

2.1 Study area and sampling

The study area is located in the Black soil (Mollisol) region of northeast China on Keshan Farm (lat. 48° 12′–48° 23′ N, long. 125° 08′–125° 37′ E). The arable soil has a history of being fertilized annually with inorganic P such as potassium dihydrogen phosphate (KH_2PO_4) or diammonium phosphate $(NH_4)_2HPO_4$. Soil samples were collected at depth of 0–20 cm according to the S-type sampling method under a natural secondary forest of *Populus davidiana* Dode (*Populus davidiana*) due to no known history of using external fertilizers, and then mixed in equal amounts, gently crumbled, and sieved through 2 mm prior to analyses. Soil samples exhibited a silt loam texture with 505, 317, and 178 g kg^{-1} in clay, silt, and sand, respectively; moreover, the samples demonstrated a soil bulk density of 1.17 g cm^{-3}, soil pH of 6.25, organic matter of 83.8 g kg^{-1}, total P of 0.92 g kg^{-1}, available P of 13.42 mg kg^{-1}, and total N of 4.89 g kg^{-1}.

2.2 Experimental design

Soil samples were placed in plastic bags and P was added at the rate of 200 mg kg^{-1} soil as KH_2PO_4 and incubated for 90 days at 25 °C. The appropriate amount of distilled water was added until 70% of field capacity was reached. Distilled water was replenished every other day during the incubation in accordance with the gravimetric method to maintain water at initial levels. Four replicates were performed. After incubation, soil samples were mixed in equal amounts, and then air-dried and passed through a 2-mm sieve before use.

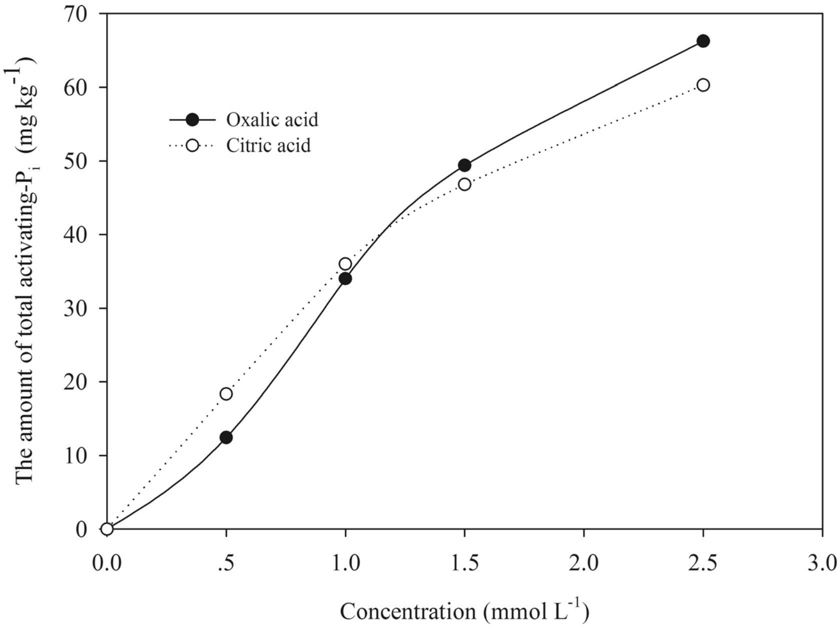
To determine the concentration-dependent extraction efficiency of the organic acids on P_i and individual P_i fractions from the black soil, 20 mL of organic acid solution was added to 1.0 g of spare soil. Both oxalic and citric acids which were the most common organic acids involved in the release of soil P (Khademi et al. 2010; Menezes-Blackburn et al. 2016), was chosen by considering the rhizosphere value at concentrations of 0, 0.5, 1.0, 1.5, and 2.5 mmol L^{-1}. The samples were orbitally shaken for 30 min at 25 °C, centrifuged (5000 rpm, 10 min), and finally filtered through 0.45-μm membrane filters. The P in solution was analyzed calorimetrically using the ascorbic acid-molybdophosphate blue method. The amount of total activating-P_i was estimated as the difference between the total amount of P_i extracted with and without organic acid. Subsequently, the soil extracted by the organic acids was washed with alcohol to remove residual low-molecular-weight organic acids between soil particles (Liu et al. 2009), and then used to determine the P fractions by sequential extractions as described by Hedley et al. (1982) and modified by Tiessen and Moir (1993) (Sui et al. 1999). Briefly, 1 g of soil was successively extracted using 25 mL of deionized water, 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) (labile P_i adsorbed on the soil surface), 0.1 mol L^{-1} NaOH (moderately labile P_i held more strongly by chemisorption to surfaces of Al and Fe oxides), 1 mol L^{-1} HCl (P associated with calcium and derived from primary mineral apatite) and $H_2SO_4 + HClO_4$ (residual P-non-labile; stable organic P forms and relatively insoluble P_i forms) for 16 h each. The activating amount of the individual P_i fractions was determined as the difference between the P extracted from the individual P_i fractions without and with organic acids (Gong et al. 2010). Four replicates of each treatment were performed and the average values were reported.

To determine the pH effect on the activation of organic acids, both oxalate at pH of 2.6–6.0 and citrate at pH of 2.8–6.0 were selected at concentrations of 2.5 mmol L^{-1}. The pH values were adjusted by either 0.1 mol L^{-1} NaOH or HCl. The remaining steps were carried out as described above. The experiment was carried out as described above except for the addition of organic acids to reach the target pH. Four replicates of each treatment were performed and the average values were reported.

2.3 Statistical analyses

The analysis of one-way ANOVA and multiple comparisons with LSD test at $P < 0.05$ were performed using SPSS 16.0. All the data were homoscedastic and normally distributed. Simple correlation analyses as well as path analyses among soil P indices were also performed using SPSS 16.0. The correlation analysis was used to analyze the relationship between the total activating-P_i and P_i fractions. The path diagram was used to examine the causal path of P_i fractions to the total activating-P_i. Direct and indirect effects in the path analysis were derived from (i) multiple linear regressions of P_i fractions on the total activating-P_i and (ii) simple correlation coefficients between P_i fractions. The direct effects of P_i fractions on the total activating-P_i were termed path coefficients and were standardized partial regression coefficients for each of the soil P_i fractions in the multiple linear regression against the total activating-P_i. Indirect effects of P_i fractions on the total activating-P_i were determined from the product of the simple correlation coefficient between soil P_i fractions and the path coefficient. The correlation between the total activating-P_i and individual P_i fraction was the sum of the entire path connecting two variables (Hashimoto et al. 2012).

Fig. 1 The amount of total activating-P_i induced by LMWOAs at different concentrations



3 Results

3.1 Total P_i activation

The activating capability of both oxalic and citric acids increased significantly with increasing their concentrations ($p < 0.05$), whereas lower concentration of organic acids at 0.5 mmol L^{-1} could induce the activation of total P_i (Fig. 1). When their concentrations reached 2.5 mmol L^{-1}, the amounts of total activating-P_i by oxalic acid was 5.32 times of that at 0.5 mmol L^{-1} concentration.

The amounts of total activating-P_i were significantly correlated with the type of organic acids (Fig. 1). When the concentrations of the organic acids were ≤ 1 mmol L^{-1}, oxalic acid exhibited a lower activating capability than citric acid, while oxalic acid was higher in total activating-P_i when the concentrations were ≧ 1.5 mmol L^{-1}.

3.2 Activation capacity of individual soil P_i fractions

The activating amounts of individual P_i fractions depended on their individual characteristics and the type and concentration of organic acids (Table 1). Both oxalic and citric acids promoted the activation of soil $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i except activated by 0.5 mmol L^{-1} organic acids, and the activating capacity tended to increase significantly as their concentrations increased ($p < 0.05$). Table 1 also showed that the amounts of activating-$NaHCO_3$-P_i and activating-NaOH-P_i were higher than other P_i fractions. In addition, when the same concentrations of organic acids were prepared, oxalic acid was more effective than citric acid in enhancing P_i activation from the $NaHCO_3$-P_i fraction, while citric acid was more effective in enhancing P_i activation from the NaOH-P_i and HCl-P_i fractions. For Res-P, no significant difference in the amounts of activating-Res-P was found between with and without the treatments of organic acids (Table 1). This could imply that despite concentrations effects, Res-P was generally not activated easily by organic acids.

3.3 Activation of total P_i by protons and organic anions

The amounts of total activating-P_i induced by 2.5 mmol L^{-1} organic acids at different pH levels were studied to distinguish the relative effects of hydrogen protons and organic anions, both of which were components of LMWOAs (Fig. 2). The amount of total activating-P_i decreased significantly with increasing pH ($p < 0.05$), particularly induced by oxalate with the greater relative effect of pH on the activating capability.

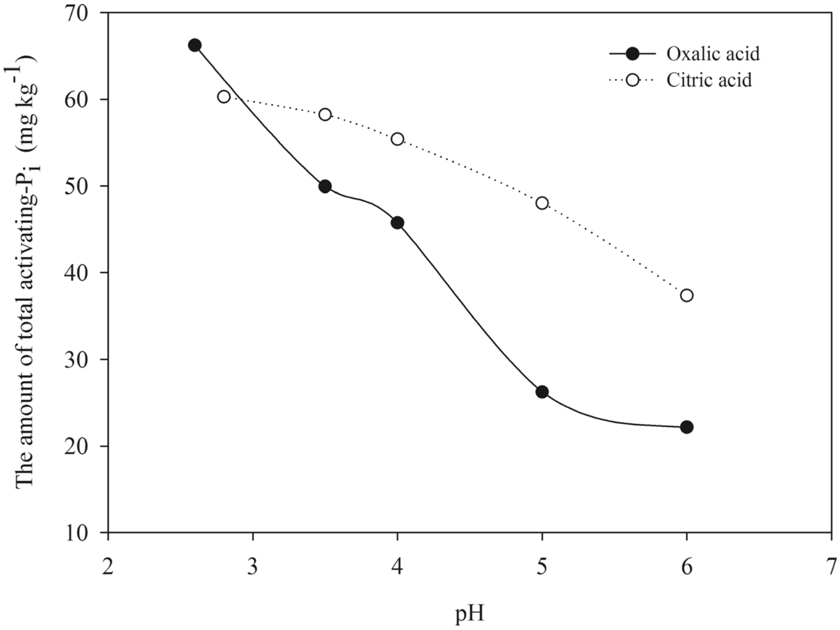
Figure 2 also showed that the pH-dependent effectiveness of organic acids to activate total P_i was strongly influenced by cations. The oxalate was more effective than citrate at pH < 3.0, whereas citrate was more effective in terms of total

Table 1 Activating amount of individual P_i fractions induced by LMWOAs at different concentrations (means ± standard deviation) (mg kg^{-1})

Addition (mmol L^{-1})	H_2O-P_i		$NaHCO_3$-P_i		NaOH-P_i		HCl-P_i		Res-P	
	Oxalic acid	Citric acid	Oxalic acid	Citric acid	Oxalic acid	Citric acid	Oxalic acid	Citric acid	Oxalic acid	Citric acid
0.5	−1.71 ± 1.59Aa	−5.04 ± 1.75Ba	11.85 ± 1.74Ba	2.99 ± 2.11Aa	2.06 ± 2.55Aa	1.97 ± 3.20Aa	−3.58 ± 1.24Aa	−0.36 ± 2.87Aa	6.99 ± 16.13Aa	9.70 ± 17.59Aa
1.0	−4.47 ± 1.31Ab	−11.12 ± 1.11Bb	20.83 ± 1.28Bb	17.57 ± 2.24Ab	9.98 ± 3.47Ab	19.29 ± 2.51Bb	1.07 ± 3.49Ab	1.94 ± 1.71Aa	6.35 ± 17.26Aa	3.67 ± 14.13Aa
1.5	−8.43 ± 0.80Ac	−14.25 ± 1.34Bc	24.73 ± 1.29Ac	23.63 ± 2.86Ac	17.09 ± 1.88Ac	24.38 ± 4.00Bc	2.80 ± 2.19Abc	5.81 ± 1.96Bb	5.19 ± 17.77Aa	5.38 ± 23.42Aa
2.5	−13.41 ± 2.70Ad	−14.97 ± 1.37Ac	37.18 ± 2.66 Bd	32.94 ± 1.73Ad	25.38 ± 3.02Ad	30.41 ± 1.24 Bd	5.59 ± 2.39Ac	9.25 ± 1.13Bc	3.13 ± 17.44Aa	4.67 ± 19.71Aa

Note: lowercase letters indicate significant differences regarding the activating capability of individual P_i by different concentrations of organic acids ($p < 0.05$); capital letters indicate significant differences regarding the activating capability of individual P_i by different types of organic acids ($p < 0.05$)

Fig. 2 The amount of total activating-P_i induced by LMWOAs at different pH levels



activating-P_i at pH ≥3.5, with 1.21, 1.83, and 1.69 times higher than oxalate at pH 4.0, 5.0, and 6.0, respectively.

3.4 Activation capacity of individual P_i fractions by protons and organic anions

In accordance with the modified Hedley sequential extractions (Table 2), both oxalate and citrate at all tested pH levels inhibited the activation of H_2O-P_i, and this degree of inhibition tended to decrease for oxalate but increase for citrate as the pH increased, while promoted the activation of $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i with the exception of oxalate at pH 6.0. Simultaneously, Table 2 also showed that the activating amounts of $NaHCO_3$-P_i and NaOH-P_i were higher than other P_i fractions, and tended to decrease significantly as pH of LMWOAs increased ($p < 0.05$). In addition, the type of organic acid affected the activating amount of individual P_i fractions. When the same organic acid concentrations were prepared, citrate was more effective than oxalate in activating-$NaHCO_3$-P_i except at pH < 3.0, activating-NaOH-P_i, and activating-HCl-P_i at all tested pH levels (Table 2). For Res-P, both oxalate and citrate at all tested pH levels neither promoted nor inhibited the activation of Res-P, although the amounts of activating-Res-P were positive. In contrast, no significant differences were observed in the presence or absence of organic acids at any tested pH level.

3.5 Correlation analysis

The activating amount of total P_i was significantly correlated with that of individual P_i fractions except Res-P (Table 3), and showed the highest correlation with the activating-$NaHCO_3$-P_i ($r = 0.917$), followed by activating-NaOH-P_i ($r = 0.891$). This indicated that the activation of total P_i was associated more with the activation of $NaHCO_3$-P_i and NaOH-P_i than that of other P_i fractions. Table 3 also showed that different correlations existed between the activation of individual P_i fractions, and a stronger correlation was found between the activation of $NaHCO_3$-P_i and NaOH-P_i ($r = 0.852$). These correlations among the individual P_i fractions could be indicative of collinearity among predictor variables for the total activating-P_i. Despite this, such significant collinearity could mask the true causal factors related to the activation of total P_i.

3.6 Path analysis

Path analysis was used to analyze the relationship among the activation of individual P_i fractions, and determine the relative importance of direct and indirect effects on the total activating-P_i. Soil activating-H_2O-P_i had a small direct-path coefficient and a large indirect-path coefficient which implied that it mainly affected P_i activation through an indirect path (Table 4). The activation of $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i directly significantly affected the activation of total P_i ($p <$ 0.05), and the contribution of activating-$NaHCO_3$-P_i alone (direct effects) was maximal, followed by NaOH-P_i and HCl-P_i. Moreover, examination of the indirect effects revealed that the indirect-path coefficients of other activating-P_i fractions on the total activating-P_i via activating-$NaHCO_3$-P_i were larger than those via other activating-P_i fractions, with the exception of activating-Res-P. For NaOH-P_i, soil activating-NaOH-P_i had a large direct and indirect-path coefficient which implied that had direct and indirect effects on P_i activation (Table 4). In addition, the indirect-path coefficient of activating-NaOH-P_i via activating-$NaHCO_3$-P_i was larger than that via other activating-P_i fractions. With respect to HCl-P_i, the indirect-path coefficient of activating-HCl-P_i via activating-$NaHCO_3$-P_i was larger than that via other activating-P_i fractions. For Res-P, Table 4 showed that soil activating-Res-P had a small direct-path coefficient and indirect-path coefficient which implied that its contribution was small to P_i activation.

Table 2 Activating amount of individual P_i fractions induced by LMWOAs at different pH levels (means ± standard deviation) (mg kg^{-1})

pH	H_2O-P_i		$NaHCO_3$-P_i		NaOH-P_i		HCl-P_i		Res-P	
	Oxalate	Citrate	Oxalate	Citrate	Oxalate	Citrate	Oxalate	Citrate	Oxalate	Citrate
pH < 3.0	−13.41 ± 2.69Ac	−14.97 ± 1.37Aa	37.18 ± 2.66Be	32.94 ± 1.73Ad	25.38 ± 3.02Ad	30.41 ± 1.24Bb	5.59 ± 2.39Ac	9.25 ± 1.13Bb	3.13 ± 17.44Aa	4.67 ± 19.71Aa
pH = 3.5	−9.06 ± 1.69Ab	−16.38 ± 1.69Ba	28.40 ± 3.65Ad	29.31 ± 1.92Ac	24.78 ± 1.43Ad	29.90 ± 3.01Bb	2.53 ± 2.12Ab	4.70 ± 1.88Aa	3.37 ± 21.49Aa	15.38 ± 18.00Aa
pH = 4.0	−8.17 ± 1.44Ab	−16.59 ± 1.23Bab	22.59 ± 2.45Ac	30.46 ± 1.14Bbc	19.00 ± 2.72Ac	28.25 ± 3.02Bb	1.29 ± 1.60Aab	4.82 ± 2.55Ba	6.95 ± 18.48Aa	5.42 ± 17.88Aa
pH = 5.0	−4.35 ± 1.26Aa	−18.42 ± 1.15Bb	16.97 ± 1.23Ab	28.62 ± 2.39Bb	13.75 ± 1.97Ab	26.49 ± 2.44Bb	0.46 ± 1.92Aab	4.60 ± 2.51Ba	3.27 ± 22.75Aa	13.22 ± 31.27Aa
pH = 6.0	−2.73 ± 1.33Aa	−22.09 ± 0.89Bc	11.27 ± 1.99Aa	24.78 ± 1.40Ba	9.86 ± 2.25Aa	18.08 ± 2.95Ba	−0.74 ± 1.58Aa	5.01 ± 1.19Ba	5.41 ± 21.05Aa	8.84 ± 18.98Aa

Note: lowercase letters indicate significant differences regarding the activating capability of individual P_i by organic acids at different pH levels ($p < 0.05$); capital letters indicate significant differences regarding the activating capability of individual P_i by different types of organic acids ($p < 0.05$)

4 Discussion

Both oxalic and citric acids could solubilize significant amounts of soil P_i, making the nutrient more accessible to plants, and higher concentrations of organic acids always resulted in greater amounts of total activating-P_i than lower concentrations did. These results agreed with previous studies (Harrold and Tabatabai 2006; Gong et al. 2010; Lv et al. 2015). The concentrations of protons and organic acid anions increased as the organic acid concentrations increased, and the acidification effects of the protons and the complex effects of the organic anions were also subsequently enhanced. This eventually led the improvement of activating capability to P_i. However, Wei et al. (2010) reported that organic acids (maleic, oxalic, and citric acids) at concentrations of 10 mmol kg^{-1} soil did not enhance the release of P_i but were effective at mobilizing P from the soil. In addition, oxalic acid impacted the activating capability less than citric acid did at lower concentrations (≤ 1.0 mmol L^{-1}) from black soil. Our research about the adsorption capacity of low-molecular-weight organic acids from black soil showed that the adsorption amount of oxalic acid was lower than citric acid when the concentration was ≤ 1.0 mmol L^{-1} (Supplementary material). This indicated that oxalic acid at lower concentrations had a lower ability to activate P than citric acid. For individual activating-P_i fractions, the activating amounts tended to increase as the concentration of organic acids increased, except Res-P. Thus, increased amounts or the generally high exudation of oxalate and citrate could benefit the uptake of P by plants.

The results of the P_i release induced by organic acids at different pH levels showed that the capability of total activating-P_i tended to decrease as the pH increased. This might be due to the inhibition of LMWOAs on P adsorption weakened with increasing pH, and finally changed to promotion of P adsorption (Wang et al. 2012). Moreover, when the same concentrations of organic acids were added, the concentration of hydrogen protons tended to decline with increasing pH value. This indicated that the acidization effect weakened. By contrast, the chelation ability was enhanced. Only when the weakened degree of acidization effect was greater than the enhancement of the chelation effect, it was possible that the amount of total activating-P_i decreased with increasing organic acid pH. This observation indicated that the acidification effect was higher than the chelation ability of LMWOAs to activate P_i. This finding was supported by Gong et al. (2010). The activation of individual P_i fractions by organic acid anions at different pH levels significantly differed. The

Table 3 Correlation matrix between the activating amounts of total P_i and individual P_i fractions

	A	H_2O-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i	Res-P
A	1.000	− 0.670**	0.917**	0.891**	0.725**	0.034
H_2O-P_i		1.000	− 0.654**	− 0.765**	− 0.690**	− 0.187
$NaHCO_3$-P_i			1.000	0.852**	0.634**	− 0.017
NaOH-P_i				1.000	0.662**	0.116
HCl-P_i					1.000	0.014
Res-P						1.000

A the amount of total activating-P_i, **Significant at $p < 0.01$

activating amounts of NaOH-P_i, $NaHCO_3$-P_i, and HCl-P_i decreased as the pH increased. This finding supported by Oburger et al. (2011), who demonstrated that acidification might enhance Fe/Al mineral solubility, resulting in the liberation of adsorbed P (proton-promoted dissolution).

According to the correlation matrix and path coefficients, the activation of $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i showed a direct effect and an indirect effect significantly on the activation of total P_i ($p < 0.05$) among five individual P_i fractions. Those strongest path coefficients matched the result of the simple correlation analysis that determined the correlation with the total activating-P_i. Among them, the activating amounts of $NaHCO_3$-P_i and NaOH-P_i were greater than those of other P_i fractions with the different treatments of concentrations and pH values of oxalic and citric acids, and could constitute a potential P source for plants. Firstly, LMWOAs could solubilize the orthophosphate ions adsorbed on minerals (Waithaisong et al. 2015), and this might be the main reason for larger contribution of activating-$NaHCO_3$-P_i which had the largest direct-path coefficient. However, oxalic acid had a stronger ability than citric acid to compete with P for adsorption sties (Wang et al. 2012), which could be explained why oxalic acid was better than citric acid at activating-$NaHCO_3$-P_i; Secondly, citrate and oxalate benefitted for the formation of stable 5- or 6-bond ring structures that had trivalent cations such as Al^{3+} and Fe^{3+} (Shen et al. 2002; Wang et al. 2012), and this explained the contribution of activating-NaOH-P_i which was moderately labile P_i which held more strongly by chemisorption to the surfaces of Al and Fe oxides (Wang et al. 2017) for total activating-P_i. However, citric acid had a stronger affinity for trivalent metal ions such as with Al^{3+} and Fe^{3+}, which were dominant in acidic soils, and might have exhibited more P-solubilizing activity than oxalic acids (Shen et al. 2002). This phenomenon might explain the greater amount of activating-NaOH-P_i by citric acid than that by oxalic acid; For HCl-P_i which was associated with Ca and derived from primary mineral apatite (Wang et al. 2017), higher concentrations (≥ 1.0 mmol L^{-1}) promoted the activation. Many studies also had indicated that P release occurred only at relatively high organic acid concentrations and the critical threshold for P release ranged from 2.5 to 10 μmol carboxylate g^{-1} soil (Oburger et al. 2011). In the case of oxalate, P might also be released from various Ca phosphate minerals via the formation and precipitation of Ca-oxalate. In contrast, citric acid was much more effective than oxalic acid in the present study, and this finding supported the results of Wang et al. (2009). Khademi et al. (2010) reported that the reaction between Ca and oxalate was rapid, and consequently, the oxalate-mediated release of P from the soil was similarly rapid. However, influences on the rate of organic acid reaction time with P-containing minerals and metals in the soil remain unknown and may differ. Therefore, further work is needed to determine whether the reaction time is linked to differences between oxalic and citric acids in activating-HCl-P_i.

5 Conclusions

These laboratory experiments clearly demonstrated that both oxalic and citric acids could potentially increase P activation

Table 4 Direct effects and indirect effects of the activation of individual P_i fractions on the total activating-P_i ($n = 68$)

Variable	Direct-path coefficient	Indirect-path coefficient					
		→H_2O-P_i	→$NaHCO_3$-P_i	→NaOH-P_i	→HCl-P_i	→Res-P	Total
H_2O-P_i	0.134		− 0.346	− 0.301	− 0.152	− 0.004	− 0.803
$NaHCO_3$-P_i	0.530**	− 0.087		0.335	0.140	− 0.000	0.388
NaOH-P_i	0.393**	− 0.102	0.451		0.146	0.002	0.497
HCl-P_i	0.220**	− 0.092	0.336	0.261		0.000	0.505
Res-P	0.019	− 0.025	− 0.009	0.046	0.003		0.015

**Significant at $p < 0.01$

from black soils and the activating capability increased as increasing their concentrations. The activating amounts of $NaHCO_3$-P_i and NaOH-P_i were more than those of other inorganic fractions. As the pH increased, the activating capability of LMWOAs on the total P_i and individual P_i fractions diminished, and the acidification effect was higher than the chelation ability of the LMWOAs. According to the results of the simple correlation and path analyses, activating $NaHCO_3$-P_i was the most directly important causal factor for predicting the activation of total P_i, and became a potential source of P in black soil, followed by NaOH-P_i and HCl-P_i.

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