

Full Length Research Paper

The role of root-released organic acids and anions in phosphorus transformations in a sandy loam soil from Yantai, China

Xu Gang^{1,3}, Shao Hongbo^{1,2*}, XuRongfu³, Yuanyuan Nie⁴, Yue Pei⁴, Zhijun Sun⁴, and M. S. A. Blackwell⁵

¹The CAS/Shandong Provincial Key Laboratory of Coastal Environmental Process, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, P. R. China.

²Institute of Life Sciences, Qingdao University of Science and Technology, Qingdao 266042, P. R. China.

³State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry (LAPC), Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing 100039, P. R. China.

⁴College of Sciences, Yantai Campus, China Agricultural University, Yantai 264670, P.R.China
⁵Rothamsted Research, North Wyke, Okehampton, Devon, EX20 2SB, United Kingdom

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One mechanism by which plants can mobilize organic and inorganic forms of phosphorus (P) in soils is by exudation of low-molecular-weight organic acids and anions. However, little is known about their effects on the transformation of P fractions in soils. We used a modified Hedley fraction method to examine the changes in P fractions in a sandy loam soil induced by a range of low-molecular-weight organic acids and anions. Generally, the effectiveness of organic acids to mobilize P occurred in the order of tricarboxylic (tricarboxylate) > dicarboxylic (dicarboxylate) > monocarboxylic acid (monocarboxylate). Addition of organic acids and anions, especially citric acid and citrate significantly increased resin-P content. As the stability of P fractions increased, the effectiveness of organic acids and anions to mobilize the P fractions decreased, with no significant effects on Conc. HCl-P and Residual-P fractions. Both organic acids and H₂SO₄ appeared to be less effective on the solubilization of organic-P because of the relatively low organic-P content of the soil (30% of total-P). The pattern of P mobilization induced by organic acids was highly controlled by the intrinsic P status such as amounts and distributions of P fractions. Exudation of organic acids and organic anions increased the resin-P content which suggests that organic anions rather than rhizosphere acidification are more important for P solubility and availability for plant growth. These results indicate that root released organic acids and anions can increase the solubility of some of the more stable pools of soil P, which can be important for soil P availability and P losses from soil to water.

Key words: Low-molecular-weight organic acids, organic anions, phosphorus (P) transformation, Hedley fractionation, sandy loam soil, microbiological interactions.

INTRODUCTION

Phosphorus (P) is an essential element for plant growth. Although, the total P content of many soils appears more

than adequate, plants can suffer from P deficiency because much of the P is bound to soil minerals and charged sites or complexed into organic forms that are inaccessible to plants (Ryan et al., 2001). In order to enhance the availability of P in soils, plants have developed different strategies to overcome restricted P supplies that either increase P use efficiency or help to

*Corresponding author. E-mail: shaohongbochu@126.com. Tel: +86-535-2109191. Fax: +86-535-2109100.

extract more P from the soil (Gerke, 1993; Raghothama and Karthikeyan, 2005). These strategies include formation of mycorrhizal associations; acidification of the rhizosphere which can promote the release of nutrients from soil minerals; and the exudation of phosphatases and organic acids (Dinkelaker et al., 1989; Ryan et al., 2001; Raghothama and Karthikeyan, 2005).

Recent studies have indicated that root exuded organic acids and anions may be a significant P acquisition mechanism operating in soils (Jones et al., 2003; Ström et al., 2005; Khademi et al., 2009). Organic acids in soils are produced by plant roots, microorganisms or during the degradation of soil organic matter, although small amounts can derive from atmospheric deposition. Formic, acetic, citric, oxalic and malic acids are simple organic acids produced by plants and exuded by plant roots and commonly found in the soils (Strobel, 2001). The concentrations of organic acids in the rhizosphere or in soil solutions vary greatly and range from 10^{-2} μM to over 80 mM (Strom et al., 2005). Veneklaas et al. (2003) estimated that organic acids concentration in the soil solution around chickpea and lupin roots was 84 and 58 mM, respectively. They also indicated that even higher concentrations of these organic acids occur in rhizosphere soils or under plant litter (Jones et al., 2003). Organic acids are hypothesized to play a vital role in the sorption/desorption, precipitation/dissolution of the poorly soluble P in the soils. Gardner et al. (1982) first reported the solubilization of poorly available soil P and enhanced P uptake resulting from root released organic acids. They found that citrate exuded by *Lupinus albus*, improved the P acquisition of the plant by freeing up fixed forms of soil P (Gardner et al., 1982). Subsequently, there have been several reports of evidence for the importance of organic acids exuded from roots in the acquisition of soil and fertilizer P by plants (Ryan et al., 2001; George et al., 2002).

Although, a number of results show that addition of organic acids, especially citric and oxalic acids to soils can solubilize significant quantities of fixed P and reduce the sorption of newly applied fertilizer P (Jones et al., 1994; Hocking, 2001). However, there are few studies on the transformations of P fractions induced by organic acids or organic anions, which are important for understanding the mobilization mechanisms of P and for exploring better ways of using different forms of P in soils (Wang et al., 2008, 2010). The objective of this study is to examine the effects of a range of organic acids and anions on the transformation, solubilization plant uptake of soil P fractions in a sandy clay loam soil with microbiological interactions.

MATERIALS AND METHODS

Soil samples

Soil samples were collected to 0-10 cm depth on the east side of the Yantai City (121°26'E, 37°28'N), Shandong Province, China.

The soil at this location is a sandy loam Alfisols (Wang et al., 2008). This region has warm temperate continental monsoon climate with distinctive seasons and rainy summer. The annual average temperature is 12.1°C and the annual average rainfall is 627 mm. The major species grown in the sampling site included *Pinusthunbergii parlatore* and *P. ussuriensis*.

Grain size distributions in the soil samples were determined using a Mastersizer2000 Laser Grain size (Manufactured by Malvern Instruments Ltd. UK; measuring range: 0.02–2000 μm). Carbon and nitrogen content was determined by combustion on a LECO CNS-2000 auto-analyzer. Soil pH was determined with a glass electrode in 1:2.5 soil : water ratio. The soil contained 2% clay, 28% silt, 70% sand, total C-8 mg kg^{-1} , total N-435 mg kg^{-1} , and total P-581 mg kg^{-1} (organic P-168 mg kg^{-1}). Soil pH was 4.67. After sampling, the soils were air-dried and sieved through a 2 mm sieve for experiment (Table 1).

Soil incubation experiment

Table 1 describes the soil with 11 soil treatments. They included one tricarboxylic (citric), two dicarboxylic (Malic and oxalic), two monocarboxylic (formic and acetic) and their corresponding sodium salts (Table 1). A mineral acid (H_2SO_4) was used to study the difference between organic acids and mineral acids. Deionized water treatment was used as a control.

For each treatment to be investigated, 200 g dry soil was weighed into a 250 mL polyethylene plastic bottle with 100 mM organic acid kg^{-1} soil or deionized water. Chloroform (CHCl_3) was added to prevent microbial activity and the potential biodegradation of organic acids along the sample incubation. The bottles were kept at $25 \pm 1^\circ\text{C}$ and 60% soil moisture content based on soil mass for an incubation period of 2 weeks. The bottles were periodically weighed and brought back to their initial weight to maintain soil moisture at 60% by adding deionised water during the experiment. At the end of the incubation the samples were air dried prior to analysis. All treatments were performed in triplicate (Wang et al., 2008, 2010).

Chemical analysis

A modified sequential Hedley fractionation was used to extract phosphorus from the incubated soils (Hedley et al., 1982; Tiessen et al., 1993). Generally, soils were extracted step by step using 1) anion exchange membrane (BDH Chemicals Ltd., England), 2) 0.5 M NaHCO_3 , 3) 0.1 M NaOH , 4) 1 M HCl and 5) concentrated HCl (Con. HCl) after shaking for 16h following each addition. The final step was to measure residual-P with $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ digestion at 360°C (Xu et al., 2011). The NaHCO_3 , NaOH and Con. HCl extracts were divided into two aliquots in order to measure the total P and inorganic P. Organic P was calculated as the difference between total and inorganic P (Haygarth et al., 1998). On the whole, 9 fractions of P followed decreasing availability were extracted and the significance of the P fractions was described in detail in Table 2.

Phosphorus in the extracts and digests was determined by Tu-1810 Spectrophotometer (Manufactured by PERSEE, China), using the ascorbic acid molybdenum blue method (Murphy and Riley 1962). The precision of the methods was approximately 5% at the 95% confidence level.

Statistical analysis

Paired-Samples T Test was carried out using the SPSS computer package (SPSS Inc. 1999, Chicago, IL, USA) for all sets of data, and the differences were considered statistically significant when $P < 0.05$.

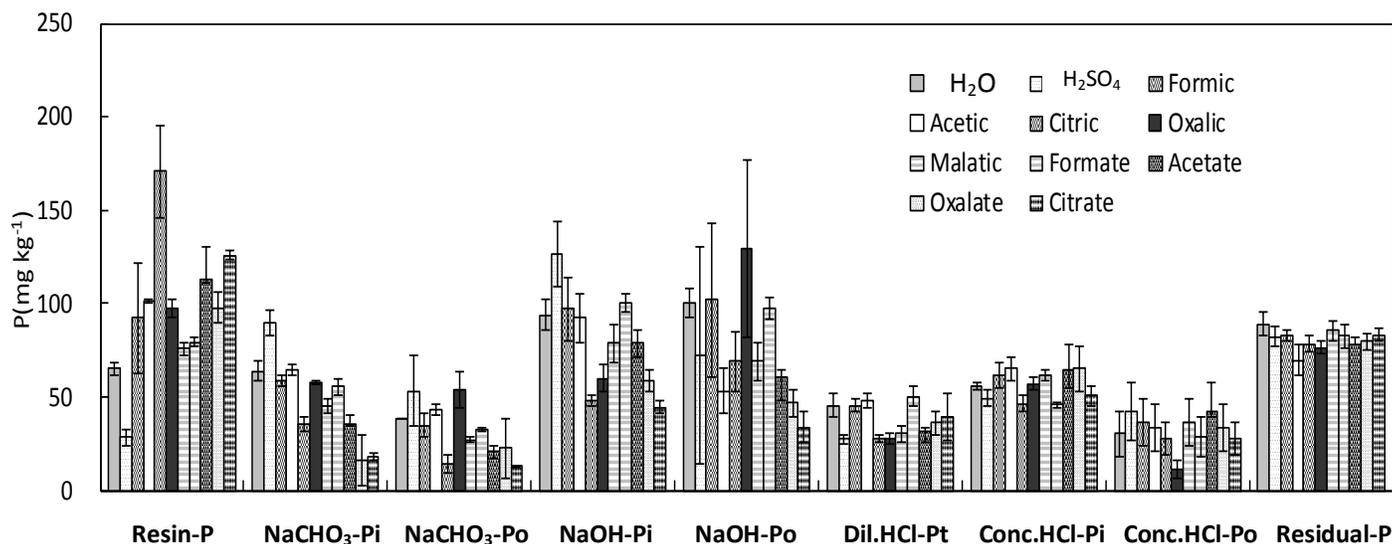
Table 1. Properties of LMW organic acids in our study.

Organic acid	Formula	Molecular weight	pKa ₁ *	pKa ₂ *	pKa ₃ *
Formic acid	HCOOH	46	3.75		
Acetic acid	CH ₃ COOH	60	4.76		
Oxalic acid	HOOC-COOH	90	1.25	4.26	
Malic acid	HOOC-CH(OH)-CH ₂ -COOH	134	3.46	5.1	
Citric acid	HOOC-CH ₂ -C(COOH)(OH)-CH ₂ -COOH	192	3.13	4.76	6.4

*pKa₁, pKa₂, pKa₃ indicated the first, second and third dissolution constant of the acids.

Table 2. Sequential extraction procedure of Cross et al. (1995), showing extraction reagents and their associated phosphorus (P) fractions.

Fractions of P extracted	Significance
Resin-P	Labile Pi, directly exchangeable with soil solution
NaCO ₃ -Pi	Labile Pi, adsorbed on surfaces crystalline compounds
NaCO ₃ -Po	Labile Po, easily mineralized and contributes to plant available P
NaOH-Pi	Moderately labile Pi, associated with exteriors of amorphous A1 and Fe phosphates
NaOH-Po, Con. HCl-Po	Stable Po, involved with long term transformation of P in soils
Dil. HCl	Stable Pi, largely calcium bound
Con. HCl-Pi	Stable Pi, associated with interiors of amorphous A1 and Fe phosphates
Residual-P	Stable Pi, covered with sesquioxides and other Po

**Figure 1.** Changes in soil P fractions after addition of organic acids or mineral acid compared with control (Data in the figure indicate means of three replicates (\pm SD)).

RESULTS

The concentrations of different P fractions in the control soil (incubated with deionized water) are presented in Figure 1. The largest fraction was NaOH-Po (100.4 ± 7.7 mg kg⁻¹), comprising 17% of total P, followed by NaOH-Pi, Residual-P and Conc. HCl-Pi, which accounted for 16% (93.8 ± 8 . mg kg⁻¹), 15% (89.1 ± 6.3 mg kg⁻¹) and 10% P fractions in the soil were compared with those obtained

(55.4 ± 2.1 mg kg⁻¹) of the total P, respectively. At the same time, NaHCO₃-Pi and Resin-P comprised of about 11% to the total P. Dil. HCl-Pt and NaHCO₃-Po concentrations were generally lower in the soil. They comprised approximately 8% (45.4 ± 4.4 mg kg⁻¹) and 7% (30.1 ± 2.5 mg kg⁻¹) of the total P. Conc. HCl-Po was present in the lowest concentration, accounting for 5% of the total P.

The effects of the 9 treatments on the transformation of using deionized water. Generally, the tri- and di-carboxylic

Table 3. Changes in soil P fractions induced by organic acids or mineral acid (\uparrow or \downarrow , $\uparrow\uparrow$ or $\downarrow\downarrow$, $\uparrow\uparrow\uparrow$ or $\downarrow\downarrow\downarrow$ means increase or decrease relative to control at the significance $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively, and - indicates $P > 0.05$).

	Resin-P	NaHCO ₃ -Pi	NaHCO ₃ -Po	NaOH-Pi	NaOH-Po	Dil.HCl	Conc.HCl-Pi	Conc.HCl-Po	Residual-P
Formic	\uparrow	-	-	-	-	-	-	-	-
H ₂ SO ₄	$\downarrow\downarrow$	$\uparrow\uparrow\uparrow$	\uparrow	$\uparrow\uparrow$	-	$\downarrow\downarrow$	-	-	-
Acetic	$\uparrow\uparrow$	-	-	-	-	-	-	-	-
Citric	$\uparrow\uparrow\uparrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	-	$\downarrow\downarrow$	-	-	-
Oxalic	$\uparrow\uparrow$	-	\uparrow	$\downarrow\downarrow$	-	$\downarrow\downarrow$	-	-	-
Malic	$\uparrow\uparrow$	$\downarrow\downarrow\downarrow$	-	-	-	\downarrow	-	-	-
Formate	-	-	-	-	-	-	-	-	-
Acetate	$\uparrow\uparrow\uparrow$	$\downarrow\downarrow\downarrow$	\downarrow	-	-	-	-	-	-
Oxalate	$\uparrow\uparrow\uparrow$	$\downarrow\downarrow\downarrow$	\downarrow	$\downarrow\downarrow$	-	-	-	-	-
Citrate	$\uparrow\uparrow\uparrow$	$\downarrow\downarrow\downarrow$	\downarrow	$\downarrow\downarrow\downarrow$	-	-	-	-	-

acids were more effective in mobilizing P from the soil than the mineral and mono-carboxylic acids, with their effectiveness following the order: citric acid > oxalic acid > malic acid (Figure.1).

The resin-P concentration was significantly increased by the addition of organic acids and anions (Figure.1). Compared with the control, citric acid, citrate, acetate, acetic acid and oxalic acid increased resin-P concentrations by 105.3, 60.6, 48, 35.9, 32.5 mg kg⁻¹, respectively. The solubilized resin-P concentrations decreased in the order: citric acid > citrate > acetate > acetic > oxalic > oxalate > formic acid > formate. Conversely, addition of the mineral acid, H₂SO₄ reduced the resin-P concentration by 37.1 mg kg⁻¹.

The NaHCO₃-Pi fraction was less affected by addition of organic acids and anions. Oxalate, citrate, citric acid, acetate and malic acid reduced the NaHCO₃-Pi fraction by approximately 75, 72, 45, 38 and 29%, respectively. However, H₂SO₄ significantly increased the NaHCO₃-Pi concentration by 41%. The addition of organic acids (for example, citric acid, citrate, acetate, oxalic acid and oxalate) also reduced the NaHCO₃-Po concentration while addition of the mineral acid H₂SO₄ increased it (Table 2).

The moderately labile-P (NaOH-Pi) appears to be less sensitive to the addition of organic acids. Citric acid, citrate, oxalic acid and oxalate significantly reduced the NaOH-Pi concentration compared to the control. Their capacity for decreasing NaOH-Pi followed the order: citrate > citric acid > oxalate > oxalic acid. The NaOH-Pi concentration was reduced by 49.4, 45.9, 35.1, 33 mg kg⁻¹, respectively. Organic acids and mineral acid had no observable effects on the NaOH-Po concentration with an exception of citrate which slightly increased concentrations.

Finally, Dil. HCl P was also affected by addition of organic acids. The amounts of Dil. HCl P solubilized by citric acid and oxalic acid were greater than that solubilized by Malic acid and the other organic acids or water. Quantities of Dil. HCl P solubilized by citric acid ranged from 12.8 to 24.7 mg kg⁻¹ (mean 17.7±6.2 mg kg⁻¹),

those solubilized by oxalic acid ranged from 10.0 to 27.2 mg kg⁻¹ (mean 17.9±8.7 mg kg⁻¹), and those solubilized by maleic acid ranged from 7.1 to 14.2 mg kg⁻¹ (mean 10.2±3.5 mg kg⁻¹). There were no significant differences in the solubilization of Dil. HCl P by the other organic acids and deionized water. Neither organic acids nor H₂SO₄ had observable effects on the solubilization of occluded-P fractions such as Conc. HCl-Pi, Conc. HCl-Po and Residual-P.

DISCUSSIONS

P availability enhanced by organic acids/anions

Our results clearly demonstrate that, addition of organic acids/anions can significantly change the concentrations of P fractions, and the quantity of P released was highly dependent on the type of organic acid/anion (Table 3). Generally, the effectiveness of organic acids/anions to alter the P fractions followed in the order of tricarboxylic (tricarboxylate) > dicarboxylic (dicarboxylate) > monocarboxylic acid (monocarboxylate) which is consistent with previous studies (Hocking, 2001; Lu et al., 2002). Most importantly, addition of organic acids/anions significantly increased Resin-P content which is important for plant growth because resin-P is defined as the plant available P and reasonably defined as freely exchangeable P, since the resin extract does not chemically modify the soil solution (Tiessen and Moir, 1993). There is considerable evidence for the importance of organic acids exuded from roots in the acquisition of soil and fertilizer P by plants (Jones, 1998; Palomo et al., 2006; Khademi et al., 2010). Addition of organic acids, especially citrate and oxalate to soils can solubilize significant quantities of fixed P and reduce the sorption of newly applied fertilizer P (Bolan et al., 1994; Jones and Darrah, 1994). Our results suggested that the solubilized P induced by organic acids/anions was mainly maintained in Resin-P which is biological available for most plants. This is supported by Khademi et al. (2010), who reported that addition of organic acids significantly increased shoot

^{33}P content in a KH_2PO_4 ^{33}P -isotopically labeled patch of calcareous soil. As a result, the secretion of organic acids/anions by plants is an important mechanism for enhancing P availability in the rhizosphere because organic acids/anions significantly increased the resin-P content and thereby increased P availability and uptake by plants.

Effect of P transformation induced by organic acids or mineral acids

The effectiveness of organic acids to change different P fractions significantly decreased with increased stability of P fractions (Table 3). For example, citric acid highly increased the resin-P concentration at the expense of $\text{NaCHO}_3\text{-Pi}$, $\text{NaCHO}_3\text{-Po}$ and NaOH-Pi . To a lesser extent, citric acid also decreased Dil. HCl P content. However, citric acid had almost no observable effects on Conc. HCl-Pi, Conc. HCl-Po and Residual-P concentrations. The results suggest that citric acid at concentration of 100 mM kg^{-1} in soil is capable of driving transformations of P from more stable fractions to more labile forms, although, this capability seem to cease at highly stable forms of P, for example, Conc.HCl-Pi. Organic acids appear to be able to induce a 2 to 4 fold increase in mineral dissolution rates in comparison with rainwater alone; however, this is highly dependent on mineral type, pH, Al content of the mineral and organic acid type (Drever et al., 1997; Oburger et al., 2009). This P transformation pattern is further confirmed by the fact that, formic acid had no observable effects on the P fractions except for a slight increase in resin-P concentration because more P fractions were activated with increasing mobilization capacities of organic acids. Our results suggested that organic acids tend to mobilize the stable P fractions and the mobilization is significantly reduced with increased P stability.

According to our studies, organic acids seem to be less effective in the release of organic P than inorganic P because only oxalic acids slightly increased the $\text{NaCHO}_3\text{-Po}$ content ($P < 0.05$). However, Wei et al. (2010) reported that citric acid at a concentration of 10 mM kg^{-1} soil significantly increased organic P concentration but did not show the effective mobilization of inorganic P as compared with water in forest soils in subtropical and tropical Australia. The different results may reflect the different concentrations and forms of organic P in the soil types studied. In Wei's studies, organic P accounted for 16–87% of total soil P (mainly over 62%), whereas in our studies, organic P content is approximately 30% of the total soil P. From the comparisons, we can conclude that the pattern of P mobilization by addition of organic acids was highly soil type dependent, and was controlled by the intrinsic P status such as amounts and distributions of P fractions in the soils.

Compared with organic acids/anions, the mineral acid (H_2SO_4) seems to involve a different mechanism for

mobilizing P. H_2SO_4 significantly reduced the resin-P concentration, but increased the $\text{NaCHO}_3\text{-Pi}$, $\text{NaCHO}_3\text{-Po}$ and NaOH-Pi concentrations at the expense of Dil. HCl P. The results suggest that the H_2SO_4 solubilized Dil. HCl P to moderately labile-P. However, the available P ($\text{Resin-P} + \text{NaCHO}_3\text{-Pi} + \text{NaCHO}_3\text{-Po}$) remains stable compared to the deionized water treatment. The mechanism involved in the reduction of the resin-P concentration by H_2SO_4 is still not clear.

Effects of pH (organic acids or anions) on the P distributions

There were no significant differences between the transformations of P fractions induced by either organic acids or organic anions (Table 3). Both organic acids and organic anions can significantly increase the resin-P concentration at the expense of $\text{NaCHO}_3\text{-Pi}$, $\text{NaCHO}_3\text{-Po}$ and NaOH-Pi , although only some organic acids but no organic anions significantly decreased Dil. HCl-Pt concentration. The results suggest that rhizosphere acidification does not necessarily increase phosphate solubility. However, organic anions, the conjugate bases of organic acids, may play an important role in improving the availability of soil phosphate (Geelhoed et al., 1999). Staunton and, Leprince (1996) also found no evidence of an effect of pH on the release of phosphate into solution for a neutral Calcic Luvisol with a large pH buffering capacity. The pH dependence of phosphate solubilization is complex because studies have reported varying results from no effect, an increase or a decrease in solution phase phosphate with decreasing pH, depending on the soil and pH range investigated (Jones and Darrah, 1994; Staunton and , Leprince 1996; Jones et al., 2003).

Conclusions

Soil samples incubated with low-molecular-weight organic acids and mineral acids were sequentially fractionated using a modified Hedley fraction method. The results suggested that, addition of organic acids significantly enhances resin-P concentration and potentially can improve P uptake by plants. The effectiveness of organic acids to release P occurred in the order of tricarboxylic > dicarboxylic > monocarboxylic acid. As P fraction stability increases, the effectiveness of organic acids to mobilize P significantly decreases. The results suggested that organic acids at the concentration of 100 mM kg^{-1} soil accelerate the transformation of Pi from more stable fractions to more labile forms, although stable conc. HCl-Pi is not affected. Neither organic acids nor mineral H_2SO_4 had observable effects on the solubilization of organic-P, probably because of the relatively low content of organic-P (30% of total-P) in the soil studied. The pattern of P mobilization by addition of organic acids is different from study to study. The

comparison suggested that the mobilization of P was highly soil type dependent, and the soil P status such as amounts and distributions of P fractions may be important for solubilization of P after addition of organic acids. Both organic acids and organic anions increased the resin-P content which means that organic anions rather than rhizosphere acidification was more important for P solubility and availability for plant growth. Our studies indicated that root released organic acids seem to accelerated the weathering of stable P pool, which would be significant for soil P supply and P losses from soil to water.

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