Kinetics of inorganic and organic phosphorus release influenced by low molecular weight organic acids in calcareous, neutral and acidic soils

Yongzhuang Wang^{1,2}, Xin Chen¹, Joann K. Whalen³, Yanhong Cao^{1,2}, Zhi Quan^{1,2}, Caiyan Lu¹, and Yi Shi^{1*}

¹ State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang, 110164, China

² University of Chinese Academy of Sciences, Beijing 100049, China

³ Department of Natural Resource Sciences, Macdonald Campus, McGill University, 21, 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC, Canada H9X 3V9

Abstract

Low molecular weight organic acids exuded by plants roots enhance inorganic P, release into soil solution and thereby increases plant-available Pi in soils. Low molecular weight organic acids may also induce organic P (P_{α}) release into soil solution, but kinetics of both Pi and Po displacement from the soil matrix into soil solution of agricultural soils is poorly understood, and the mechanism for Po release is not well explained. This study used kinetic experiments to determine the concentrations and release rates of P_i and P_o induced by oxalic acid, citric acid, and malic acid in calcareous, neutral and acidic soils. Kinetic data were well described by Elovich $(r^2 = 0.801 - 0.993, P < 0.001)$ and power functions models $(r^2 = 0.721 - 0.977, P < 0.001)$. Low molecular weight organic acids at 10 mmol kg⁻¹ soil induced the exponential release of both P_i and P_a, which reached a plateau approx. 480-2,880 min after the start of the experiment. Cumulative Po release induced by low molecular weight organic acids was ranked as oxalic acid $(0.63-3.17 \text{ mg kg}^{-1})$ > citric acid $(0.61-2.82 \text{ mg kg}^{-1})$ > malic acid $(0.52-1.76 \text{ mg kg}^{-1})$ and mainly resulted from the release of labile P_o (NaHCO₃- P_o) regardless of soil type. By contrast, oxalic acid was most effective in enhancing P_i release from the HCI- P_i (Ca- P_i) fraction of the calcareous soil, and citric acid was most effective in releasing Pi from the NaOH-Pi (Fe/AI-Pi) fraction of the neutral and acidic soils. Therefore, the mechanism for the kinetics of Po release induced by low molecular weight organic acids is ascribed to their ability to mobilize the labile Po (NaHCO₃-P_o) rather than their ability to chelate cations (*i.e.*, Fe³⁺, Al³⁺) bound to P_o in soil.

Key words: citric acid / oxalic acid / malic acid / organic acid ligands / soil phosphorus fractions

Accepted April 30, 2015

1 Introduction

Phosphorus (P) deficiency limits crop production in most agricultural areas in China, mainly due to tight adsorption of P by Fe-, Al-oxides/hydroxides or Ca compounds in soil (Zhang et al., 2004; Verma et al., 2005; Xavier et al., 2009; Jalali and Tabar, 2011). Therefore, P fertilizer and/or manure are commonly added to soils to increase the availability of soil P to sustain crop P requirements and meet vield targets (Aulakh et al., 2003: Kuo et al., 2005). However, the P input from fertilizers and manures tends to accumulate, changing the concentration of P in soil solution and its association with soil minerals and organic matter (OM), due to the low P use efficiency (around 10-15% of applied fertilizer P) of most crops and the high P fixing capacity of soils (Boschetti et al., 2008; Zamuner et al., 2008; Chakraborty et al., 2011; Kang et al., 2011). Despite the fact that a large quantity of P is continously supplied as soluble fertilizer, plant-available P content in soil solution is often very low (< 5 umol L^{-1}). To overcome P deficiencies, plants also have evolved adaptive chemical process (e.g., root-induced depletion/accumulation of P ions, acidification/alkalinization of rhizosphere, and exudation of organic acids/anions) and



alter their root structure to increase the mobility and bioavailability of P in the rhizosphere (*Raghothama*, 1999; *Hinsinger*, 2001; *Zhang* et al., 2004; *Hinsinger* et al., 2009; *Makhani* and *Isaac*, 2014). Among these strategies, exudation of low molecular weight organic acids from plant roots is considered to be one of the common and effective mechanisms mobilizing unavailable P in soil (*Palomo* et al., 2006; *Chen* et al., 2008).

It has been widely speculated that low molecular weight organic acids enhance the mobilization of soil inorganic P (P_i) (*Ström* et al., 2002; *Harrold* and *Tabatabai*, 2006; *Taghipour* and *Jalali*, 2013). Low molecular weight organic acids have the ability to: (1) directly promote the dissolution of sparingly soluble minerals containing P_i, (2) shift pH and chemical equilibria in soil solution, (3) alter the surface characteristics of mineral particles or occupy ligand exchange surfaces, (4) form complexes with ions of Fe, Al and Ca, thus blocking P adsorption sites on soil particles (*Lan* et al., 1995; *Hocking*, 2001; *Ström* et al., 2002; *Chen* et al., 2008). Moreover, low molecular weight organic acids may enhance the release of organic P (P_o) bound to soil minerals (*Fox*, et al., 1990; *Fox*

^{*} Correspondence: Y. Shi; e-mail: shiyi@iae.ac.cn

and *Comerford*, 1992; *Wei* et al., 2010), but the mechanism for P_o release induced by low molecular weight organic acids is not well explained, especially for soils with contrasting physic-chemical properties (e.g., texture, pH, Fe-/Al-oxides, and Ca-carbonate), which may affects P_o release from soil. Though P_o needs to be mineralized prior to plant uptake, this process is facilitated once the P_o is in soil solution where it can be acted upon by phosphatases and the mineralized P_i is subsequently assimilated by plants (*Xiao* et al., 2006; *Turner*, 2008).

Both the concentration and rate of P release from solid to solution controls the lability of soil P, thus influencing P fertility of soils and P uptake by crops (McDowell and Sharpley, 2003; Shariatmadari et al., 2006). To fully assess the changes in P availability, the rate of P release must be considered. Kinetic experiment is an appropriate method to quantify the rate of P release from soil solid into solution phases (Sparks, 1995). Fox et al. (1990) reported that the kinetics of Pi release from Spodosols in the presence of low molecular weight organic acids (e.g., oxalic acid or formic acid) occurs in two phases: rapid release in the initial phase followed by a slower release, due to the ligand exchange or dissolution of metal-P compounds, respectively. The mode of organic acids loading (single vs. repeated sequential) affected the kinetic process but not the cumulative amount of P, release (Fox and Comerford, 1992). The fast and slower rates of P release are related to the rapid desorption of surface labile P and slow dissolution of the crystalline P compounds (Toor and Bahl, 1999). Gerke (1994) also showed that the rate of P_i desorption was related to the release rate of Fe/AI, which is associated with P. However, Jalali and Zinli (2011) stated that the two phases of P_i release were characteristic of a diffusion-controlled process. McDowell and Sharpley (2003) and Nafiu (2009) also believed that the slow diffusion of P from inside of soil particles was possibly the rate limiting step in the kinetics of P, release. Both diffusion and dissolution reactions are probably involved in the slower second phase of P, release (Smet et al., 1998). While much research has been done on the kinetics of P release (Smet et al., 1998; Toor and Bahl, 1999; Nafiu, 2009; Jalali and Zinli, 2011), limited information is available on the impacts of low molecular weight organic acids on the kinetics of Po release from soil solid phase (e.g., Po compounds bound to Fe/Al components). Low molecular weight organic acids enhance P release from labile P and crystalline P compounds (Ström et al., 2002; Harrold and Tabatabai, 2006; Wei et al., 2010), leading us to hypothesize that (1) low molecular weight organic acids will increase the rate of the kinetics of both P_i and P_o release. Of the low molecular weight organic acids, oxalic acid is generally the most effective reagent for enhancing P, release from calcareous soils due to its ability to strongly chelate Ca in calcareous soils, while citric acid is the most effective in acidic soils where Fe and AI are the dominant cations generating surface charge on clays (Harrold and Tabatabai, 2006; Nwoke et al., 2008; Bais et al., 2006; Wei et al., 2010; Moradi et al., 2012). Since the release of Po may share a similar mechanism with the release of Pi, which involves chelating the cations bound to Po as well as P_i, thereby facilitating the release of P_i and P_o into soil solution, we further hypothesized that (2) oxalic acid will be the most effective reagent for increasing the rate of P_i and P_o release into soil solution of calcareous soils, while citric acid will be the most effective in acidic soils.

The ability of low molecular weight organic acids to chelate with Ca. Fe, and AI can be inferred from the decrease in levels of soluble forms of these cations in soil solutions (Ström et al., 2005; Wang et al., 2012), but a direct way to measure how chelation of Ca, Fe, and Al by low molecular weight organic acid affects P_i and P_o release is to measure soil P fractions. Soil contains various P_i and P_o fractions including soil soluble and exchangeable P (NaHCO₃ extracted P_i and P_o), Fe/Albound P (NaOH extracted Pi and Po), Ca-bound P (HCI extracted Pi), and residual P (Hedley et al., 1982; Jalali and Ranjbar, 2010). As chelating agents, low molecular weight organic acids can displace P from Fe/AI-P and/or Ca-P, compounds in soils (Hinsinger, 2001; Jones, 1998), which changes the proportion of the Ca-bound P_i (HCl extracted P_i) and the Fe/Al-bound P (NaOH extracted P) fractions. Additionally, other possible mechanisms for ${\rm P_o}$ release from soil (e.g., desorption or dissolution of soil labile Po) can also be inferred from the changes in labile $\rm P_o$ fraction. Therefore, measuring the concentrations of the soil $\rm P_o$ fractions after kinetic experiment is a direct way to determine the Po pools acted upon by low molecular weight organic acids, and to deduce mechanisms. McDowell and Sharplev (2003) used P fractionation to determine the presences of P forms relevant to the kinetics of P, release. Taghipour and Jalali (2013) also suggested that the release of P, induced by low molecular weight organic acids at 10 mmol L-1 was mainly from the Ca-P, (HCI extracted P_i) fraction due to chelation of Ca²⁺ by organic acids in their calcareous soils. Because Po is associated with organo-mineral complexes, the release of Po by low molecular weight organic acids could be due to chelating effects of organic acid ligand cations (e.g., Fe and Al on clay surfaces) that bind Po, we hypothesized that (3) the release of Po will result from mobilizing the Fe/Al- Po (NaOH extracted Po).

The objective of this study was to study the impact of three common low molecular weight organic acids (oxalic acid, citric acid, and malic acid) on the kinetics of P_i and P_o release in calcareous, neutral, and acidic soils. In addition, the mechanism for P_o release were inferred by analyzing the difference in soil P_o fractions after kinetic experiments.

2 Material and methods

2.1 Soils and organic acids

Surface layers (0–20 cm) of three soils were collected from three Experimental Stations of Ecology (Chinese Academy of Sciences): Luancheng (37°53'N, 114°40'E), Shenyang (41°31'N, 123°24'E), and Taoyuan (28°56'N, 111°26'E), respectively. The mean annual precipitation and temperature for the three climatic regions are 530 mm and 12.2°C, 650 mm and 7.5°C, and 1440 mm and 16.5°C, respectively. Each of the tested soils was a major soil type for agricultural production in their respective regions, where P fertilizer was applied every year to prevent P deficiency of the wheat (Luancheng), corn (Shenyang), and rice (Taoyuan). All soil samples were air-dried and ground to pass through a 2-mm

sieve before use. Selected chemical and physical properties are shown in Table 1.

The organic acids selected for this study were oxalic acid, citric acid, and malic acid because of their widespread natural occurrence in soils. Each organic acid was applied at a concentration of 10 mmol kg⁻¹ soil. This concentration was chosen by considering the rhizosphere values of these organic acids reported by Jones (1998) and Li et al. (2010), and the values of carboxylic acids in soil extracts (Strobel, 2001). Wei et al. (2010), Jalali and Zinli (2011) and Jalali and Tabar (2011) used 10 mmol kg⁻¹ soil of organic acids to determine the impacts of organic acids on the release of Po from forest soils and the kinetics of P_i release from calcareous soils, respectively. In addition, Gerke et al. (2000) reported that below a concentration of around 10 mmol kg⁻¹ soil the P solubility was not markedly increased by the addition of citric acid. Thus, this concentration should be sufficient and suitable to enhance P mobilization and explore related mechanisms based upon abiotic mineral studies.

2.2 Kinetic experiments

The experiment used a completely randomized design to evaluate the three soils (calcareous, neutral, and acidic) subjected to four treatments (water, oxalic acid, citric acid, and malic acid) and kinetic evaluation at 11 time points, with three replicates for each time point giving a total of 396 experimental units. Soil (2 g) was placed in 50 mL centrifuge tubes, and then 20 mL of deionized water (control) or 20 mL of organic acid solution, containing 10 mmol kg⁻¹ soil of oxalic acid, citric acid or malic acid, were added to each tube. Two drops of toluene were added to inhibit microbial activity prior to cap the tubes. It should be noted that this sterilization method may have the potential disadvantage of altering soil physical and

chemical properties (i.e., increasing pH and cation exchange capacity) for the duration of the equilibration phase (Lotrario et al., 1995). The suspensions were shaken on an end-to-end shaker (200-cycles min⁻¹ at 25 \pm 1°C) for 5, 10, 15, 30, 60, 120, 240, 480, 960, 1,440 or 2,880 min. centrifuged at 12,000 \times g for 10 min and filtered through Whatman filter paper (No. 42). The P_i concentration in a 10 mL aliquot of the extract was determined by the malachitegreen method (Ohno and Zibilske, 1991; Guppy et al., 2000). Total P content in a second 10 mL of extract was quantified by digestion with 0.6 g $K_2S_2O_8$ and 10 ml 0.9 M H₂SO₄ in autoclave (103.4 K Pa, 121°C) for 60 min. The concentration of organic P in the solution was the difference between total P and inorganic P. Soil was collected from centrifuge tubes that were shaken for 2,880 min, rinsed and dried (40°C for 72 h) for subsequent P fractionation analysis. We prepared 72 extra centrifuge tubes (triplicates) for each soil type and treatment to evaluate the pH values of extracts before the kinetic experiment and at 2,880 min with a glass electrode.

The cumulative P_i and P_o released into soil solution was plotted against time for each soil and different kinetic models were fitted to the data. The P_i and P_o release with time was fitted using the following equations:

First order
$$\ln Q_t = B - k_1 t$$
, (1)

Second order $1/Q_t = B + k_2 t$, (2)

Elovich equation
$$Q_t = (1/\beta) \ln (\alpha\beta) + (1/\beta) \ln t$$
, (3)

Power function equation $\ln Q_t = \ln a + b \ln t$, (4)

Parabolic diffusion model $Q_t = A + R t^{1/2}$, (5)

 Table 1: Some physical and chemical properties of the three soils studied from Experimental Stations of Ecology, Chinese Academy of Sciences.

Site	Soil	Total N	Total P	Organic C	Available P	pH (H ₂ O)	Soil texture / %		Bulk density		
		/ g kg ⁻¹	/ g kg ⁻¹	/ g kg ⁻¹	/ mg kg ⁻¹	1:2.5	sand	silt	clay	g cm ⁻³	
Luancheng	Calcareous	1.17	0.89	13.92	4.5	8.24	32.5	48.8	18.7	1.32	
Shenyang	Neutral	1.11	0.37	10.65	7.2	7.02	14.7	60.4	24.9	1.11	
Taoyuan	Acidic	0.89	0.49	4.37	3.9	4.72	12.1	44.6	44.3	1.19	
Site	P fractions / mg kg ⁻¹						Fe _d e	Al _d e	Fe _o e	Al _o e	CaCO ₃
	NaHCO ₃ -P _i ^a	NaHCO ₃ -P _o ^a	NaOH-P _i ^b	NaOH-P _o ^b	HCI-P _i c	Residual P ^d	/ g kg ^{_1}				
Luancheng	9.1	10.8	8.3	48.1	482.6	291.4	6.56	1.21	1.27	-	98.24
Shenyang	12.2	23.6	58.5	49.7	103.6	188.8	13.56	9.21	3.27	0.21	11.18
Taoyuan	8.2	14.3	105.1	26.7	6.5	306.1	50.08	9.61	3.41	5.56	-

^aNaHCO₃-P_i was soil soluble and exchangeable inorganic P_i, and NaHCO₃-P_o was comprised of P_o in the soil solution and P_o that sorbed on the soil surface.

^bNaOH-P_i and Po were P_i and Po compounds held more strongly by chemisorption to Fe and Al components of soil surface.

^cHCI-P_i , soil Ca bound inorganic P.

dResidual P, soil resistant inorganic and organic P.

 ${}^{e}Fe_{d}$ and AI_{d} were dithionite-citrate-bicarbonate-extractable Fe and AI, respectively, and Fe_{o} and AI_{o} were oxalate-extractable Fe and AI, respectively.

where Q_t (mg P kg⁻¹) is the amount of P released at time t, A (mg P kg⁻¹), α (mg P kg⁻¹ min⁻¹), a [(mg P kg⁻¹ min⁻¹) ^b], and A (mg P kg⁻¹) are initial P release (rate), k_1 (min⁻¹) and k_2 [(mg P kg⁻¹)⁻¹] are the first- and second-order rate constant, β [(mg P kg⁻¹)⁻¹], b [(mg P kg⁻¹)⁻¹] and R [(mg P kg⁻¹)^{-0.5}] are P-release rate constants. Each kinetic model was tested by coefficient of determination (r^2) and the standard error of estimate (S.E.) was calculated from:

SE=
$$[\Sigma (Q_t - Q_t')^2 / (n-2)]^{1/2}$$
, (6)

where Q_t and Q'_t are the measured and predicted amounts of released P at time *t*, respectively, and *n* is the number of measurements.

2.3 Soil P fractions

For each soil, bulk soil that represented the original P chemistry and soil that was subjected to treatments (water, oxalic acid, citric acid, and malic acid) by shaking 2,880 min in the kinetic experiment was subjected to sequential P fractionation using the Hedley et al. (1982) procedure modified by Kang et al. (2011). A representative soil sample (0.5 g) was weighed into a 50 mL centrifuge tube and P fractions were determined as follows: (1) labile P (NaHCO3-P), soil extracted with 30 mL 0.5 M NaHCO₃ (pH = 8.5) for 16 h; (2) Fe- and Al-bound P (NaOH-P), residue from the first fraction extracted with 30 mL 0.1 M NaOH for 16 h; (3) Ca-bound P (HCI-P), residue from second fraction extracted with 1.0 M HCl for 16 h; (4) residual P (Res-P), residue from the last fraction digested with H₂SO₄-HClO₄ at 360°C. Extracted P_i collected at each step was analyzed colorimetrically with the molybdate-blue method. Total P of NaHCO₂ and NaOH extracts was determined by digesting in autoclave (103.4 kPa, 121°C) with acidified K-persulfate $(K_2S_2O_8)$. The P_o in NaHCO₃ and NaOH fractions was calculated as the difference between total P and P_i.

2.4 Statistical analysis

Linear forms of the kinetic equations (1–5) were fitted to the kinetic experimental data, separately for each soil. Least-square-regression analysis was used to ascertain the model that best described P-release kinetics for each soil studied. Soil P fraction data were subject to the normality and homogeneity tests before analysis of variance (ANOVA) using SAS statistical package (SAS 9.13). Significant differences (P < 0.05) in the concentration of each P fraction before and after shaking with each treatment solution (water, oxalic acid, citric acid, and malic acid) was evaluated with one-way analysis of variance (ANOVA) for each soil studied. Least significant difference (LSD) was conducted only when the analysis of variance was significant at P < 0.05.

3 Results

3.1 Soil properties

The three soils were calcareous (pH 8.24), neutral (pH 7.02), and acidic soil (pH 4.72), having a low available P status (3.9 to 7.2 mg kg⁻¹; Table 1). The HCl-P_i (Ca-P_i) concentration

and Ca-carbonate equivalent were greatest in the calcareous soil, and lowest in the acidic soil, while the NaOH-P_i (Fe, AI-P_i) fraction and Fe-/Al-oxides concentrations were the greatest in the acidic soil and the lowest in the calcareous soil. Soil textures were sandy loam, silty loam. and silty clay for the calcareous, neutral and acidic soils, respectively.

3.2 Kinetics of P_i and P_o release

Comparison of coefficients of determination (r^2) and the standard errors of estimate (S.E.) for kinetic models indicated that kinetics of Pi and Po release by low molecular weight organic acids in the three soils were better fitted with the Elovich ($r^2 = 0.801 - 0.993$; P < 0.001; S.E. = 0.05-0.37), power function ($r^2 = 0.721 - 0.977$; P < 0.001; S.E. = 0.03-0.20), and Parabolic diffusion models ($r^2 = 0.427 - 0.831$; P < 0.05; S.E. = 0.05–1.17) than the first- (r^2 = 0.325–0.572; S.E. = 0.06–0.52) and second-order models ($r^2 = 0.226-0.487$; S.E. = 0.04–0.59) (Table 2). The kinetics of both P_i and P_o release was initially rapid, followed by a slower reaction (Fig. 1: CS-a, NS-a, AS-a; Fig. 2: CS-a, NS-a, AS-a). A single straight line nearly covering the entire course of reaction time was only obtained when the soil P release data for each soil were plotted according to the Elovich and power function equations (Fig. 1: CS-b/c, NS-b/c, AS-b/c; Fig. 2: CS-b/c, NS-b/c, AS-b/c). These results indicated that Elovich and power function models were the most suitable to describe and compare the kinetics of P release of the three different soils. Overall, the kinetic parameters, obtained from Elovich (α , β) and power function (a, b) models were higher when soils were treated with low molecular weight organic acids than with water (Table 3).

Organic acids generated more Po release than water in the three soils, and there was a consistent trend of greater P release in soil-solution mixtures induced by oxalic acid $(0.63-3.17 \text{ mg P}_{o} \text{ kg}^{-1})$ than by citric acid $(0.61-2.82 \text{ mg P}_{o})$ kg⁻¹) or malic acid (0.52–1.76 mg P_o kg⁻¹). Cumulative P_i release from soil induced by water ranged from 0.32 to 1.75 mg P_i kg⁻¹ for the three soils, while P_i release from soil induced by oxalic acid, citric acid, and malic acid ranged from 0.64 to 7.25 mg kg⁻¹, 0.64 to 6.67 mg kg⁻¹ and 0.50 to 2.18 mg P_i kg⁻¹, respectively. The type of organic acid affects P_i release and the impact on P_i release in soil-solution mixtures was ranked as oxalic acid > citric acid > malic acid for the calcareous soil, and citric acid > oxalic acid > malic acid for the neutral and acidic soils. The pH values of the soil-low molecular weight organic acids mixed solution could be ranked as malic acid > citric acid > oxalic acid at the beginning and at the end of the kinetic experiments (Table 4).

3.3 Soil P fractions

In the calcareous soil, treatment with low molecular weight organic acids resulted in a mean increase of the NaOH-P_i (Fe/Al-bound P_i) fraction by 34.7% (P < 0.05), and the NaH-CO₃-P_i (soil labile P_i) fraction by 12.8% (P > 0.05). The increase in the NaHCO₃-P_i and NaOH-P_i fractions stems from the decrease of the HCl-P_i fraction (2.2–26.3%; P > 0.05).



Figure 1: The kinetics of inorganic P (P_i) release by water, oxalic acid, citric acid, and malic acid at 10 mmol kg⁻¹ soil from the three studied soils (CS: calcareous; NS: neutral, AS: acidic) as a function of time (a) and similar data described by selected kinetic equations: Elovich (b), power function (c), and parabolic diffusion (d).

Following treatment with low molecular weight organic acids, the NaHCO₃-P_i fraction on average increased by 9.6% and 24.1% while the NaOH-P_i fraction decreased by 9.2% and 7.7% (P < 0.05) for the neutral and acidic soils, respectively (Fig. 3). In general, oxalic acid was the most effective treatment leading to changes in the concentration of P_i fractions (NaOH- and HCI-P_i) for the calcareous soil. Citric acid was more effective in reducing the NaOH-P_i fraction in the acidic soil than the other organic acids or water.

The concentration of labile P_o (NaHCO₃-P_o) fractions significantly decreased (P < 0.05) after the kinetic experiment, whereas the Fe/Al-P_o (NaOH-extracted P_o) fractions were not significantly affected by treatments (Fig. 4). Low molecular weight organic acids reduced the concentration of the NaHCO₃-P_o fraction, relative to water, by as much as 22.5% (P < 0.05) in the calcareous soil, 12.1% in the neutral soil,

and 16.2% (P < 0.05) in the acidic soil. The reduction in the concentration of NaHCO₃-P_o fraction was influenced by oxalic acid > citric acid > malic acid for all three soils.

4 Discussion

4.1 Kinetics of P release from soils: pattern and processes

Our first hypothesis was that low molecular weight organic acids will increase the rate of the kinetics of both P_i and P_o release, which was supported by the higher P_i and P_o kinetic parameters with low molecular weight organic acids than water. Release of P_i and P_o from soils was rapid during the first 5 to 240 min of the kinetic study and then plateaued as



Figure 2: The kinetics of organic P (P_o) release by water, oxalic acid, citric acid, and malic acid at 10 mmol kg⁻¹ soil from the three studied soils (CS: calcareous; NS: neutral, AS: acidic) as a function of time (a) and similar data described by selected kinetic equations: Elovich (b), power function (c), and parabolic diffusion (d).

an apparent equilibrium was reached. This two-phase pattern of initially rapid P release followed by slower release was reported by *Smet* et al. (1998), *Jalali* and *Zinli* (2011) and *Jalali* and *Tabar* (2011), and is attributed to a combination of dissolution and desorption process. In the current study, soil NaHCO₃-P_i fraction tends to increase after releasing P_i into soil solution with the addition of low molecular weight organic acids. The kinetics of P_i release seems to be linked with the moderately labile NaOH-P_i (Fe/AI-P) or HCI-P_i (Ca-P_i) fractions. However, it may be masked due to the re-adsorption of P_i in NaHCO₃-P_i fraction in the process of P_i release (*Freese* et al., 1995). In the initial rapid phase, the dissolution of labile surface-adsorbed P is likely to be the dominate process releasing P from soil into solution while the dissolution of the crystalline P compounds will be the rate-limiting step in the slower P release phase (*McDowell* and *Sharpley*, 2003). As we did not monitor soil P fractions throughout the study, it is not possible to confirm the source of P_i and P_o during the initial rapid phase, but the significant reduction in HCl-P_i (Ca-P_i) or NaOH-P_i (Fe/Al-P_i) concentration by the end of the kinetic experiment indicates dissolution of crystalline P compounds occurred, particularly when soil was treated with low molecular weight organic acids. In addition, due to the greater affinities of oxalate for Ca²⁺ and citrate for Fe³⁺/Al³⁺, oxalic acid was the most effective in increasing P_i release rate in calcareous soil while citric acid was the most effective in acidic soil. This confirmed our second hypothesis that oxalic acid is most effective in P_i release in calcareous soils, while citric acid is the most effective solution for P_i release in acidic soils.

Table 2: Coefficients of determination (r^2) and standard errors of estimates (S.E.) for selected kinetic equations used to describe the kinetic release of inorganic P (P_i) and organic P (P_o) during 2,880 min from calcareous, neutral and acidic soils shaken on an end-over-end shaker with water, oxalic acid, citric acid, and malic acid solutions (1:10 soil: solution ration with organic acid concentrations of 10 mmol kg⁻¹ soil).^a

	Soil type Treatmen		Elovich		Power func	Power function		liffusion
			r ²	S.E.	r ²	S.E.	r ²	S.E.
P _i release	Calcareous soil	Water	0.955***	0.11	0.860***	0.20	0.674**	0.29
		Oxalic acid	0.972***	0.37	0.891***	0.19	0.722***	1.17
		Citric acid	0.987***	0.16	0.941***	0.17	0.801***	0.63
		Malic acid	0.969***	0.14	0.941***	0.13	0.788***	0.37
	Neutral soil	Water	0.965***	0.09	0.889***	0.19	0.768**	0.22
		Oxalic acid	0.954***	0.24	0.968***	0.09	0.831***	0.46
		Citric acid	0.991***	0.14	0.977***	0.07	0.821***	0.64
		Malic acid	0.986***	0.08	0.973***	0.08	0.841***	0.26
	Acidic soil	Water	0.869***	0.08	0.853***	0.09	0.816***	0.10
		Oxalic acid	0.946***	0.17	0.939***	0.09	0.735***	0.37
		Citric acid	0.993***	0.15	0.929***	0.14	0.775***	0.89
		Malic acid	0.987***	0.07	0.962***	0.11	0.818***	0.27
P_{o} release	Calcareous soil	Water	0.908***	0.06	0.878***	0.07	0.694**	0.11
		Oxalic acid	0.913***	0.11	0.873***	0.06	0.686**	0.21
		Citric acid	0.898***	0.06	0.852***	0.03	0.427*	0.05
		Malic acid	0.838***	0.10	0.823***	0.10	0.657**	0.14
	Neutral soil	Water	0.986***	0.08	0.963***	0.06	0.773***	0.15
		Oxalic acid	0.979***	0.05	0.971***	0.04	0.802***	0.25
		Citric acid	0.957***	0.09	0.929***	0.06	0.789***	0.19
		Malic acid	0.802***	0.15	0.721***	0.18	0.615**	0.21
	Acidic soil	Water	0.920***	0.11	0.850***	0.18	0.636**	0.24
		Oxalic acid	0.962***	0.10	0.929***	0.11	0.780***	0.24
		Citric acid	0.801***	0.12	0.791***	0.13	0.543**	0.19
		Malic acid	0.856***	0.06	0.803***	0.09	0.714**	0.09

^ans: not significant at p < 0.05, *significant at p < 0.05, *significant at p < 0.01, *significant at p < 0.001.

Still, we could not confirm that oxalic acid and citric acid were more effective in inducing the P_o release in calcareous and acidic soils, respectively, since oxalic acid reduced the concentration of the NaHCO₃-P_o fraction more than the other organic acids regardless of soil type. This was taken as circumstantial evidence that P_o entered the soil solution during the initial rapid P release phase through the rapid desorption and dissolution of the labile P_o (NaHCO₃-P_o) fraction. The NaHCO₃-P_o fraction, comprised of P_o in the soil solution and P_o that adsorbed on the soil surface, is thought to be rapidly dissolved and easily desorbed from soil surface. (*Tiessen* and *Moir*, 1993; *Guppy* et al., 2000; *Vu* et al., 2008). However, at the end of the slower P_o release phase, there was no significant difference in the P_o concentration within the NaOH-P_o fraction, so it is unlikely that chelation by organic acid ligands occurred during the kinetics of P_o release from the studies soils. The NaOH-P_o (Fe/Al-P_o) fraction was characterized as less labile P_o that held more strongly by chemisorption to Fe and Al components of soil surface (*Hedley* et al., 1982). These results were inconsistent with our third hypothesis, indicating that the mechanism for the kinetics of P_o release involved in low molecular weight organic acids was dissimilar to P_i release (mainly due to chelating effects of organic acid ligand cations that bind P_i).

Time-dependent P-release data were better modeled by Elovich, power function, and parabolic diffusion equations, based on the coefficients of determination (r^2) and the standard errors of estimate (S.E.) of the five chosen models fitted to the study data. However, graphical test for the parabolic diffusion model did not present a requisite straight line covering the whole time scale and data led to curved plots for short **Table 3**: Kinetic parameters of the selected equations describing the kinetic release of inorganic P (P_i) and organic P (P_o) during 2,880 min from calcareous, neutral, and acidic soils shaken on an end-over-end shaker with water, oxalic acid, citric acid, and malic acid solutions (1:10 soil : solution ration with organic acid concentrations of 10 mmol kg⁻¹ soil).

	Soil	Treatment	Elovich		Power funct	lion
			α	1/ β	a	b
P _i release	Calcareous soil	Water	0.441	0.221	0.382	0.220
		Oxalic acid	1.112	0.964	1.324	0.242
		Citric acid	0.293	0.623	0.506	0.299
		Malic acid	0.359	0.352	0.493	0.235
	Neutral soil	Water	23.461	0.081	0.734	0.078
		Oxalic acid	133.018	0.157	1.770	0.066
		Citric acid	201.471	0.017	1.899	0.008
		Malic acid	111.039	0.099	0.747	0.090
	Acidic soil	Water	0.272	0.205	0.303	0.235
		Oxalic acid	0.489	0.484	0.719	0.224
		Citric acid	1.037	0.670	1.175	0.210
		Malic acid	0.497	0.292	0.537	0.204
P _o release	Calcareous soil	Water	2.652	0.141	0.544	0.137
		Oxalic acid	43.023	0.249	1.482	0.103
		Citric acid	166.871	0.188	1.391	0.089
		Malic acid	6.836	0.138	0.612	0.132
	Neutral soil	Water	18.551	0.097	0.579	0.104
		Oxalic acid	2.301	0.310	0.945	0.157
		Citric acid	1.282	0.836	1.377	0.221
		Malic acid	0.234	0.286	0.970	0.247
	Acidic soil	Water	0.713	0.173	0.409	0.186
		Oxalic acid	0.975	0.227	0.572	0.176
		Citric acid	16.726	0.109	0.615	0.111
		Malic acid	143.552	0.069	0.561	0.085

Table 4: The pH value of treatment solutions and the change in pH of soil suspensions before shaking and after 2,880 min of end-over-end shaking in centrifuge tubes with each treatment (1:10 soil : solution ration with organic acid concentrations of 10 mmol kg⁻¹ soil).^a

Treatment	Solution pH	Calcareous soil		Neutral soil		Acidic soil	
		before pH	after pH	before pH	after pH	before pH	after pH
Water	7.13	7.47 A	7.93 B	7.04 A	7.51 B	5.35 A	5.97 B
Citric acid	3.11	6.62 A	7.75 B	4.11 A	5.11 B	3.66 A	4.58 B
Oxalic acid	2.84	6.37 A	7.34 B	3.92 A	5.03 B	3.25 A	4.23 B
Malic acid	3.25	6.89 A	7.92 B	4.26 A	5.22 B	3.58 A	4.45 B

^aWithin each soil, pairwise comparison of the treatment effect on the pH in soil suspension was made before and after 2,880 min of end-overend shaking, where different capital letters indicate significant differences at P < 0.05.





Figure 3: Inorganic P (P_i) fractions in the three studied soils (CS: calcareous; NS: neutral, AS: acidic) following 2,880 min shaking with water, oxalic acid (OA), citric acid (CA), and malic acid (MA) solutions in a kinetic release study. Columns are the mean and standard deviation bars of three replicates, and those with different letters had significantly different (p < 0.05) concentrations of P_i after the kinetic release study. NaHCO₃-P_i, soil soluble and exchangeable P_i ; NaOH-P_i, P_i compounds held more strongly by chemisorption to Fe and AI components of soil surface; HCI-P_i, soil Ca bound P_i.

contact times (Fig. 1: CS-d, NS-d, AS-d; Fig. 2: CS-d, NS-d, AS-d). This suggests that P_i and P_o were not released from soils by a diffusion-controlled process similar to the observations by Shariatmadari et al. (2006). The dissolution of the Fe³⁺/Al³⁺ or Ca²⁺ compounds that bind most of P in soils by low molecular weight organic acids may be a possible mechanism for P release according to the well-fitted Elovich model (Hansen and Strawn, 2003), although most of these compounds were the major source of P_i rather than P_o. Taghipour and Jalali (2013) also used the power-type equation to describe the kinetics of P_i release from some calcareous soils by oxalic acid, citric acid, and malic acid at 10 mmol L^{-1} . While the power type equation may describe P_i release by organic acids, involving both dissolution and chelation of mineral-bound P, by organic acid ligands (Taghipour and Jalali, 2013), the slope and intercept of the curve suggest that the

Elovich and power type equations provided similar descriptions of the measured phenomena.

4.2 Effectiveness of organic acids to induce P_o and P_i release from soils

Cumulative P_o release induced by low molecular weight organic acids was greater than that induced by water, and the source of P_o released was the NaHCO₃-P_o fraction, which was depleted by 12.1–22.5% (P < 0.05) during the kinetic experiment with the three soils studied. Although not all were significantly different, there was greater P_o release in soils treated with oxalic acid than with citric acid or malic acid. This finding might be explained by the lower pH value in solutions with oxalic acid than that with the other organic acids and



Figure 4: Organic P (P_o) fractions in the three studied soils (CS: calcareous; NS: neutral, AS: acidic) following 2,880 min shaking with water, oxalic acid (OA), citric acid (CA), and malic acid (MA) solutions in a kinetic release study. Columns are the mean and standard deviation bars of three replicates, and those with different letters had significantly different (p < 0.05) concentrations of P_o after the kinetic release study. NaHCO₃-P_o comprises of P_o in the soil solution and that adsorbed on the soil surface; NaOH-P_o covers P_o compounds held more strongly by chemisorption to Fe and Al components of soil surface; residual P, soil resistant P_i and P_o.

water, due to the fact that oxalic acid $(C_2H_2O_4)$ is a relatively strong acid with 2 carboxyl groups, and citric acid $(C_6H_8O_7)$ and malic acid $(C_4H_6O_5)$ are weak organic acids, respectively, with three and two carboxyl groups. Although P_o is mainly associated with organo-mineral complexes (e.g., Po compounds strongly adsorbed to Fe and Al components of soil surface), the release of Po by low molecular weight organic acids in this study was not due to chelating effects of organic acid ligand cations (e.g., Fe and Al on clay surfaces) that bind P_o. This implies that acid strength is more important than chelating ability of low molecular weight organic acids in releasing Po. Still, our findings may not be generalizable, as Wei et al. (2010) reported that citric acid release more Po than oxalic and malic acids in some neutral and acid forest soils. Although the mechanism was not well explained, there was no significant change in pH of suspensions containing low molecular weight organic acids in the study of *Wei* et al. (2010), perhaps due to the buffering capacity of their forest soils. Another difference was that P_o accounted for 70% of the total P in those forest soils, but no more than 20% of the total P in the agricultural soils of this study. We deduced that P_o release occurs through different mechanisms than those controlling P_i release in the presence of low molecular weight organic acids. Still, further studies on P_o speciation by means of ³¹P-NMR would provide insight into the strength of the reactions of low molecular weight organic acids affecting kinetics of P_o release.

Cumulative P_i release from soils was greater when treated with low molecular weight organic acids than with water (Fig. 1). Probably the source of P_i released by low molecular weight organic acids in the present study was the HCI- P_i

(Ca-P_i) fraction in the calcareous soil and the NaOH-P_i (Fe/AI-P_i) fraction in the neutral and acidic soils, both of which declined significantly (P < 0.05) by the end of the kinetic experiment. Although the amount of P₁ mobilized by low molecular weight organic acids was small (0.64-7.25 mg kg⁻¹), the dynamic nature of soluble P, may make it contribute significantly to plant-available P in agricultural soils. Soil soluble P_i was thought to be the most available P for immediate biological uptake and keep certain level of equilibrium with other P forms (Schmidt et al., 1997). Oxalic acid was most effective in reducing the Ca-P, fraction in the calcareous soil while citric acid was the most effective in reducing the Fe/Al-P, fraction, which was also reported by others (Ström et al., 2002; 2005; Andrade et al., 2013). Although we did not investigate the mechanism involved, Ström et al. (2001) found similar results due to the preferential formation and precipitation of Ca-oxalate in calcareous soils and Fe-, Al-citrate in non-calcareous soils.

5 Conclusions

In three agricultural soils with contrasting physicochemical properties, the addition of low molecular weight organic acids increased both the concentration and rate of the kinetics of P_i and Po release. The Po released upon treatment with low molecular weight organic acids represented 29.7-44.6% of the total P released, and Po release from the NaHCO3-Po fraction was greater when soils were treated with oxalic acid than with the other organic acids, regardless of soil type. While enhanced P_i release in the presence of low molecular weight organic acids was likely due to accelerated desorption and dissolution process, our findings suggest that the acid strength of solution containing low molecular weight organic acids contributed to the kinetic release of Po. In this shortterm (2 d) kinetic experiment, chelation by organic acid -ligands did not contribute to the kinetic of Po release, which promotes the understanding of the mechanisms of P_a release in the presence of low molecular weight organic acids.

Acknowledgements

This work is financially supported by *National Natural Science Foundation of China* (*Grant No. 41271317, 41101285*, and *31470624*). Finally we thank the SERG writing group (McGill University) for reviewing earlier versions of the manuscript.

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