Different mechanisms of organic and inorganic phosphorus release from Mollisols induced by low molecular weight organic acids

Journal:	Canadian Journal of Soil Science
Manuscript ID	CJSS-2017-0002.R2
Manuscript Type:	Special Issue Paper (Please select below)
Date Submitted by the Author:	16-Jul-2017
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Keywords:	Inorganic P fraction, Organic P fraction, Sequential extraction, kinetic experiments, Mechanisms

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Different mechanisms of organic and inorganic phosphorus release from Mollisols induced by low molecular weight organic acids

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Running Title: Phosphorus release mechanisms

Abbreviations: P: phosphorus; LMWOA: low molecular weight organic acids;

ABSTRACT

Enhancement of inorganic phosphorus (Pi) release by low molecular weight organic acids (LMWOA) increases soil phosphorus (P) availability to plant, but the release of organic phosphorus (Po) and the associated kinetics of both Pi and Po release by LMWOA have not been well considered. The aim of this study was therefore to investigate the impacts of three common LMWOA (citric acid, oxalic acid and malic acid) on the kinetic release of Pi and Po in a black clay soil in Northeast China. All kinetic data were well described by Elovich and power functions models ($P < 0.01$). LMWOA at 10 mmol kg⁻¹ soil increased the rate of the kinetic release of both Pi and Po. Po released by LMWOA derived from the soil labile Po (NaHCO₃-Po) fraction in the order of oxalic acid (3.58 mg kg⁻¹) > citric acid (2.67 mg kg⁻¹) > malic acid (1.76 mg kg⁻¹). In contrast, Pi release by LMWOA resulted from mobilization of the moderately labile NaOH-Pi (Fe/Al-P) and HCl-Pi (Ca-Pi) fractions in the order of citric acid (4.83 mg kg⁻¹) > oxalic acid (2.40 mg kg⁻¹) > malic acid (2.04 mg kg⁻¹). Therefore, the release of Po by LMWOA is mainly via the dissolution of soil labile Po (NaHCO₃-Po) rather than via chelation of organic acid ligands.

Key words: Inorganic P fraction; Organic P fraction; Sequential extraction; kinetic experiments; Mechanisms

Black clay soil (often known as “black soil” in Chinese soil taxonomy, corresponding to Mollisols in US soil taxonomy) covers more than 3 million hectares in northeast China. The high content of the organic matter (3-10%) makes this fertile black clay soil ideal for growing corn and soybean, which provides 30% of total soybean production and 15% of total corn production in China (Yu 2013). However, due to the strong sorption of phosphorus (P) by Fe and Al oxides/hydroxides and carbonates of this soil (Zhou and Zhang 2005), P is a major limiting nutrient for the food production of this region. P fertilizer and/or manure must be applied into soil annually to guarantee soil P fertility and crop yield. To overcome P deficiencies, plants have also evolved adaptive strategies, such as exudation of low molecular weight organic acids (LMWOA), to enhance P nutrient acquisition from soils (Raghothama 1999). Previous studies have suggested that LMWOA were effective in enhancing the release of inorganic P (Pi), thus increasing the availability of soil P (Strom et al. 2002; Wei et al. 2010). Additionally, the understanding of Pi released by LMWOA was relatively sound, which involved in occupying ligands exchange surface and chelating with cations (Fe^{3+} , Al^{3+} and Ca^{2+}) bound to Pi (Hocking 2001; Lan et al. 1995; Strom et al. 2002). Moreover, LMWOA may also enhance the release of organic P (Po), but the mechanisms involved are not clear yet. Though Po needs to be mineralized prior to plants uptake, it accounts for a substantial proportion of total P and plays an important role in supporting P nutrient (Turner 2008; Xiao et al. 2006). Knowing that Po is associated with organic-mineral complexes, the release of Po by LMWOA could be due to chelating effects of organic acid ligands cations (e.g. Fe and Al on clay surfaces) that bind to Po, but this hypothesis needs to be tested.

While varieties of studies have been performed to investigate the impacts of LMWOA on the quantity of Pi release (Jones 1998), little work has been done to see if

the kinetics of Pi and Po release changes with LMWOA addition. P fertility of soil is related to both the concentration and rate of P release from solid to solution (Shariatmadari et al. 2006). To fully assess the changes in P availability induced by LMWOA, kinetics of P release must be considered. Thus, the overarching aim of this study was to investigate both the concentration and rate of the kinetic release of Pi and Po induced by LMWOA from a black clay soil in northeast China. Since LMWOA may chelate the cations bound to Po, as well as Pi, thereby facilitating the release of Pi and Po, we hypothesize that (1) LMWOA will increase the rate of the kinetic release of both Pi and Po.

Among various LMWOA exuded from plant roots, certain organic acids were more effective than others in enhancing Pi release from solid to solution such as oxalic, citric and malic acids (Harrold and Tabatabai 2006; Nwoke et al. 2008). In addition, oxalic acid is most effective in releasing Pi from calcareous soils while citric acid is most effective in acidic soils, due to the stronger chelating ability of oxalic acid with Ca in calcareous soils, but citric acid with Fe/Al in acidic soils (Bais et al. 2006). Wei et al. (2010) reported that the release of Po may share a similar mechanism with the release of Pi, which involves in chelating the cations bound to Po, as well as Pi, thereby facilitating the release of Pi and Po into soil solution. Based on these research findings, we hypothesize that (2) citric acid will be more effective than oxalic and malic acids in increasing the rate and concentration of both Pi and Po release from soils with greater Fe and Al oxide content than Ca carbonate content.

Enhancement of Pi and Po release induced by LMWOA may cause the redistribution of soil P fractions, especially for the Fe/Al-P and/or Ca-P fractions since they have the ability to chelate cations (Fe^{3+} , Al^{3+} and Ca^{2+}) in soils (Hinsinger 2001; Jones 1998). Thus, the determination of P fractionation in soils after kinetic release of Pi and Po is

helpful for understanding the role of LMWOA in the kinetics of P release. The kinetic release of Pi from soils is usually rapid at first, then slows down until an apparent equilibrium is approached (Hansen and Strawn 2003; Jalali and Ahmadi Mohammad Zinli 2011), and the two phases of P release are respectively related to the labile P and less mobile P (Toor and Bahl 1999). Taghipour and Jalali (2013) also suggested that the release of P induced by LMWOA at 10 mmol L⁻¹ was mainly from soil Ca-P (HCl extracted P) fraction, due to the greater chelation of Ca²⁺ by organic acids in some calcareous soils of Iran. Further, we hypothesize that (3) citric acid will be most effective in changing soil Fe/Al-Pi and Po fractions while oxalic acid will be most effective in changing Ca-Pi fraction, because of the great affinities of citric acid for Fe/Al and oxalic acid for Ca in soils.

The objective of this study was to study the impact of three common LMWOA (oxalic acid, citric acid and malic acid) on the kinetics of both Pi and Po release in a black clay soil in Northeast China. In addition, the mechanisms of Po release involved in LMWOA were inferred by analyzing soil P fractions after P kinetic release experiments.

MATERIALS AND METHODS

Soils

Samples of the topsoil (0-20 cm) were collected in April before corn (*Zea mays* L. cv Longdan) planting from Hailun (47°26'N, 126°38'E) Experimental Stations of Ecology, Chinese Academy of Sciences. The climate is continental monsoon, characterized by warm-wet summer and dry-cold winter. The mean annual temperature is 2°C, frost-free period 120 days and annual precipitation 500-600 mm. The test soil was a major soil type in the local area. 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.

Kinetic experiments

The experiment used a completely randomized design to evaluate a black clay soil subjected to 4 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 132 experimental units. Soil (2 g) was added into a 50-ml centrifuge tubes, and then 20 ml organic acid solution (oxalic acid, citric acid and malic acid) of 1 mmol L⁻¹ was added to the tube. The LMWOA concentration was 10 mmol kg⁻¹ soil. This concentration was chosen by considering the rhizosphere values of these organic acids in soil extracts reported by Jones (1998) and Li et al. (2010), and the values of carboxylic acids in soil extracts (Strobel, 2001). Wei et al. (2010) and Jalali (2011) used 10 mmol kg⁻¹ soil of organic acids to determine the effects of organic acids on Po release from forest soils and the kinetics of Pi release from calcareous soils, respectively. Additionally, P solubility was not markedly increased with citric acid addition when the concentration is below 10 mmol kg⁻¹ soil (Gerke et al. 2000). Thus, this concentration should be sufficient and suitable to enhance P release and explore related mechanisms based upon abiotic mineral studies. Meanwhile, P kinetic release with water was run as a control group. The release of P from soils was then performed by successive extraction of P from 0 to 2880 min. The suspensions were shaken on an end-to-end shaker (200-cycles min⁻¹ at 25±1°C) at time intervals of 5, 10, 15, 30, 60, 120, 240, 480, 960, 1440 and 2880 min, centrifuged at 12000× g for 10 min and filtered through Whatman filter paper (No.42). Two drops of toluene were added to inhibit microbial activity. Pi concentration of the extracts was determined by the malachite-green method (Guppy et al. 2000) using UV-721 Spectrophotometer at 610

nm. The malachite green method is based on the ionic association of malachite green with phosphomolybdate under acidic conditions. Reagents for the malachite green method were prepared as indicated in Ohno and Zibilske (1991). Reagent I was 14.2 mmol L^{-1} ammonium molybdate tetrahydrate prepared in $3.15 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$. Reagent II was 3.5 g L^{-1} polyvinyl alcohol (PVA) prepared in deionized water (80°C), then 0.35 g L^{-1} malachite green was added. P extracts (1ml) was transferred to screw-top tubes, and then 0.2 ml of Reagent I and Reagent II were orderly added to the tube. The malachite green method has higher sensitivity than the molybdate-blue method in determining trace phosphorus. It has been applied to determine trace amount of phosphorus in soil samples, river water and tap water with satisfactory results (Motomizu et al. 1983). Total P contents (10 ml of extracts) were quantified by digesting in autoclave (103.4 K Pa , 121°C) for 60 min with $0.6 \text{ g K}_2\text{S}_2\text{O}_8$ and $10 \text{ ml } 0.9 \text{ mmol L}^{-1} \text{ H}_2\text{SO}_4$ (Wei et al. 2010). The concentration of P_o was calculated as the difference between total P and P_i . The pH values of extractants before and after the kinetic experiment were also monitored by a glass electrode.

The cumulative released P_i and P_o were plotted against time and different kinetic models were fitted to the data. The kinetic equations used to describe the rate of P release from the soil by LMWOA are presented in the Table 2. The goodness of fit of each kinetic equation was evaluated according to its coefficient of determination (r^2) and the standard error of estimate (S.E.) calculated from:

$$\text{S.E.} = [\sum (\text{Q}_t - \text{Q}_t')^2 / (n-2)]^{1/2} \quad (1)$$

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. Various P release rate parameters could be subsequently obtained from fitted equations as follows: the k_1 and k_2 from the first and second order equations, α and $1/\beta$ from the Elovich equation, a and b from

power function model, and A and R from parabolic diffusion equation.

Soil P fractionation

Phosphorus fractionation was carried out after the kinetic release of P from soils. The sequential P fractionation was performed according to Hedley et al. (1982), with later modifications by (Linquist et al. 2010): labile P ($\text{NaHCO}_3\text{-P}$), 0.5 g soil extracted with 30 ml $0.5 \text{ mmol L}^{-1} \text{ NaHCO}_3$ (pH=8.5) for 16 h, (2) Fe- and Al-bound P (NaOH-P), residue from the first fraction extracted with 30 ml $0.1 \text{ mmol L}^{-1} \text{ NaOH}$ for 16 h, (3) Ca-bound P (HCl-P), residue from second fraction extracted with $0.5 \text{ mmol L}^{-1} \text{ HCl}$ for 16 h, (4) residual P, residue from the last fraction extracted with digested with $\text{H}_2\text{SO}_4\text{-HClO}_4$ at 360°C . The extracted P_i collected at each step was analyzed colorimetrically with the molybdate-blue method. Total P_i in the NaHCO_3 and NaOH extracts was determined by digestion in autoclave (103.4 K Pa , 121°C) with acidified potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$). The P_o in the NaHCO_3 and NaOH fractions was calculated as the difference between total and inorganic P.

Statistical analysis of data

The linear forms of the kinetic equations (Table 2) were fitted to the kinetic experimental data. Least-square-regression analysis was used to ascertain the model that best described P-release kinetics in the studied soils. All data on soil P fractions were subject to the normality and homogeneity tests before analysis of variance (ANOVA) using SAS statistical package (SAS 9.13). One-way analysis of variance (ANOVA) was used to detect significant differences in each fractionated P among the control, and oxalic, citric and malic acids treatments. Least significant difference (LSD) was conducted only when the analysis of variance was significant at $P < 0.05$.

RESULTS

Kinetics of P release

The kinetic release of both P_i and P_o was initially rapid and continued with a slower reaction until 2880 min (Fig. 1 a and Fig. 2 a). Different kinetic models were used to describe P release from the studied soil. Based on the coefficients of determination (r^2) and the standard errors of estimate (S.E.) for kinetic models (Table 3), kinetic P_i and P_o release by LMWOA were better fitted by the Elovich ($r^2=0.848-0.993$, $P < 0.01$ and S.E.=0.09-0.26), power function ($r^2=0.803-0.949$, $P < 0.01$ and S.E.=0.07-0.22) and Parabolic diffusion models ($r^2=0.599-0.773$, $P < 0.05$ and S.E.=0.16-0.65) than the first- ($r^2=0.316-0.561$, and S.E.=0.16-0.43) and second-order models ($r^2=0.197-0.388$ and S.E.=0.09-0.48). A single straight line nearly covered the entire course of reaction time was only obtained when the kinetic data of P_i and P_o plotted according to the Elovich and power function equations (Fig. 1 d, e and Fig. 2 d, e). These results indicated that Elovich and power function models are more suitable to describe the kinetic release of P_i and P_o of the studied soil.

Overall, the P_i and P_o kinetic release parameters obtained from Elovich (α , β) and power function (a, b) models were higher with LMWOA addition than that in control (Tab. 4). This suggested that LMWOA (oxalic, malic and citric acids) increased the initial release concentration/rate in the process of kinetic P_i and P_o release. Cumulative P_i released by water and LMWOA was of the order: citric acid (4.83 mg kg^{-1}) > oxalic acid (2.40 mg kg^{-1}) > malic acid (2.04 mg kg^{-1}) > water (1.63 mg kg^{-1}). In contrast, the impacts of water and organic acids on P_o release declined in the order of oxalic acid (3.58 mg kg^{-1}) > citric acid (2.67 mg kg^{-1}) > malic acid (1.76 mg kg^{-1}) > water (1.49 mg kg^{-1}). The pH values of the soil-LMWOA mixed solution also declined in the order of malic acid > citric acid > oxalic acid before and after kinetic P release experiments (Tab. 5)

Soil phosphorus fractions

The NaHCO_3 -Pi fraction was average 8.1% ($P > 0.05$) higher after P kinetic release by the three organic acids (citric acid, oxalic acid and malic acid) while the NaHCO_3 -Po fraction was average 9.7% ($P < 0.05$) lower than control. Oxalic acid had the greatest effect on NaHCO_3 -Po fraction, followed by citric acid and then malic acid. Compared with the moderately labile Pi in control, the NaOH-Pi (Fe/Al-bound Pi) decreased by 10.9%, 15.4% ($P < 0.05$) and 8.5%, and the HCl-Pi (Ca-bound Pi) slightly decreased by 20.4 %, 8.6% and 5.6% when soil was treated with oxalic acid, citric acid and malic acid, respectively.

DISCUSSION

Our first hypothesis was that LMWOA will increase the rate of the kinetic release of both Pi and Po, which was supported by the higher Pi and Po kinetic release parameters with LMWOA than with water. Kinetic release of Pi and Po from soil in present study was rapid during the initial 5 to 240 minute and more slowly continued more slowly until an apparent equilibrium was approached. Because of the difficulty in separating the two phases, the combination of dissolution and desorption is used to describe the kinetic release of Pi (McDowell and Sharpley 2003). Toor and Bahl (1999) suggested that the two phases of Pi release were related to two Pi fractions in soil: labile Pi and less mobile Pi. However, the two forms of Pi were released simultaneously during the initial 6 to 12 h, while the less mobile Pi release occurred until equilibrium was established (Elrashidi et al. 1975). The gradual decrease in Pi release rate with time may result from decreased surface charge and subsequent decrease in the interaction between the adsorbed Pi ions as desorption reaction progressed (Kuo and Lotse 1974). In the current study, the kinetic release of Pi seems

to link with the moderately labile NaOH-Pi (Fe/Al-P) or HCl-Pi (Ca-Pi) fractions only on the face of the redistribution pattern of soil Pi fractions. However, it may be masked due to the readsorption of Pi in NaHCO₃-Pi fraction in the process of Pi release (Freese et al. 1995). Therefore, the two phases of Pi release could be separately related to P release from the labile NaHCO₃-Pi (soil soluble and exchangeable P) and moderately labile NaOH-P (Fe/Al-P) or HCl-Pi (Ca-Pi) fractions of the studied soil. Due to the greater affinity of citric acid for Fe³⁺/Al³⁺, citric acid was the most effective acid in increasing Pi release rate in the studied soil with greater Fe and Al oxide content than Ca carbonate content (Fig. 1 a) . This confirmed our second hypothesis of the greater effectiveness of citric acid for increasing the rate of Pi release. Still, we could not confirm that citric acid was the most effective in inducing the Po release, since there were no statistically significant differences in NaHCO₃-Po reductions induced by the three organic acids (citric acid, oxalic acid and malic acid). In addition, by the end of the slower Po release phase, there was no significant difference in the Po concentration in NaOH-Po fraction, so it is unlikely that chelation by organic acid ligands occurred during the kinetic release of Po from soils.

Time-dependent Pi and Po 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 several models fitted to the study data. But graphical test for the parabolic diffusion model suggested that both Pi and Po released from the studied soil was not a diffusion-controlled process. Hansen and Strawn (2003) reported that the dissolution may be a possible mechanism for Pi release from the well-fitted Elovich model. The power function equation has also been successfully used to describe the kinetics of P released from some calcareous soils induced by LMWOA (Taghipour and Jalali 2013) and P adsorption and desorption

from soils (Jalali and Ahmadi Mohammad Zinli 2011; McDowell and Sharpley 2003; Shariatmadari et al. 2006). Thus, the increases of Pi and Po release concentration and rate induced by LMWOA (oxalic, citric and malic acids) may be due to the enhancement of P mobilization from the dissolution of soil labile $\text{NaHCO}_3\text{-Pi}$ and Po compounds or precipitation of $\text{Fe}^{3+}/\text{Al}^{3+}$ - or Ca^{2+} -organic acids (Taghipour and Jalali 2013; Wei et al. 2010).

Cumulative Po released by LMWOA was greater than control, which was related to the lower amounts of labile $\text{NaHCO}_3\text{-Po}$ with LMWOA addition. Yang et al. (2012) also reported that LMWOA increased the availability of soil labile Po by reducing the availability of high stable Po in a calcareous soil. Unexpectedly, the effect of oxalic acid on cumulative Po release was greater than the effect produced by citric and malic acids. This was related to the order of the $\text{NaHCO}_3\text{-Po}$ contents after P release with these organic acids: malic acid > citric acid > oxalic acid. In addition, the lower suspension pH values with oxalic acid addition than the other organic acids before and after P release might also help explain the greater effect of oxalic acids on Po release. Wei et al. (2010) reported that citric acid (at 10 mmol L^{-1} organic acid kg^{-1} soil) compared with oxalic and malic acid was more effective in enhancing the release of Po in some acid soils. This result is inconsistent with the observation from this study that oxalic acid is most effective in enhancing Po release. The differences may be ascribed to the differences of soil use types and P forms it contains. Organic P accounted for about 70% of total P in the forest soils but lower than 20% in the three agricultural soils of this study (Wei et al. 2010). On the other hand, cumulative Pi released by LMWOA of the studied soil is related to the decreases in NaOH-Pi (Fe/Al-Pi) and HCl-Pi (Ca-Pi) fractions after P release, although not all reductions are always significant at $P < 0.05$. In addition, oxalic acid was most effective in decreasing HCl-Pi

(Ca-bound P) while citric acid was most effective in decreasing NaOH-Pi (Fe/Al-bound P), due to the preferential formation and precipitation of Ca-oxalate and Fe/Al-citrate (Bais et al. 2006; Strom et al. 2002; Wei et al. 2009). Results in our study demonstrated that the release of Po was likely to via mechanisms dissimilar to Pi released by LMWOA.

CONCLUSIONS

In a black clay soil with greater Fe and Al oxide content than Ca carbonate content, adding three common LMWOA (oxalic acid, citric acid and malic acid) increased both concentration and rate of the kinetic release of Pi and Po. Although the amounts of Pi and Po released by LMWOA were small, their labile nature made the solubilized Pi and Po contribute more to soil P fertility. The Po released upon treatment with LMWOA represented 21.3-39.7% of the total P released, and Po release from NaHCO₃-Po fraction was greatest when soil was treated with oxalic acid than other organic acid or water. In this study, chelation by organic acid ligands did not contribute to the kinetic release of Po, which promotes the understanding of the mechanisms of Po release with the involvement of LMWOA.

ACKNOWLEDGMENTS

This work was financially supported by National Nature Science Foundation of China (No. 41601322, 41271317 and 31470624), Guangxi Natural Science Foundation (2016GXNSFBA380042), Young Teachers ' Basic Ability Promotion Program of the Education Department of Guangxi (grant No. KY2016YB279), and System Fund of Key Laboratory of Environment Change and Resources Use in Beibu Gulf (grant No. 2015GXESPXT01).

References

- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. and Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**: 233-266.
- Elrashidi, M., Diest, A. and El-Damaty, A. 1975. Phosphorus determination in highly calcareous soils by the use of an anion exchange resin. *Plant Soil* **42**: 273-286.
- Freese, D., Lookman, R., Merckx, R. and Vanriemsdijk, W.H. 1995. New method for assessment of long-term phosphate desorption from soils. *Soil Sci. Soc. Am. J.* **59**: 1295-1300.
- Gerke, J., Beibner, L., and Romer, W. 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. I. The basic concept and determination of soil parameters. *J. Plant Nutr. Soil Sci.* **163**: 207 – 212.
- Guppy, C.N., Menzies, N.W., Moody, P.W., Compton, B.L. and Blamey, F.P.C. 2000. A simplified, sequential, phosphorus fractionation method. *Commun. Soil Sci. Plan.* **31**: 1981-1991.
- Hansen, J.C. and Strawn, D.G. 2003. Kinetics of phosphorus release from manure-amended alkaline soil. *Soil Sci.* **168**: 869-879.
- Harrold, S.A. and Tabatabai, M.A. 2006. Release of inorganic phosphorus from soils by low-molecular-weight organic acids. *Commun. Soil Sci. Plan.* **37**: 1233-1245.
- Hedley, M., Stewart, J. and Chauhan, B. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* **46**: 970-976.
- Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* **237**: 173-195.
- Hocking, P.J. 2001. Organic acids exuded from roots in phosphorus uptake and

- aluminum tolerance of plants in acid soils. *Adv. Agron.* **74**: 63-97.
- Jalali, M. and Ahmadi Mohammad Zinli, N. 2011. Kinetics of phosphorus release from calcareous soils under different land use in Iran. *J. Plant Nutr. Soil Sci.* **174**: 38-46.
- Jones, D.L. 1998. Organic acids in the rhizosphere - a critical review. *Plant Soil* **205**: 25-44.
- Kpomblekou-A, K. and Tabatabai, M.A. 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils. *Agr. Ecosyst. Environ.* **100**: 275-284.
- Kuo, S. and Lotse, E. 1974. Kinetics of phosphate adsorption and desorption by lake sediments. *Soil Sci. Soc. Am. J.* **38**: 50-54.
- Lan, M., Comerford, N.B. and Fox, T.R. 1995. Organic-anions effect on phosphorus release from spodic horizons. *Soil Sci. Soc. Am. J.* **59**: 1745-1749.
- Li, H.G., Shen, J.B., Zhang, F.S., and Lamber, H. 2010. Localized application of soil organic matter shifts distribution of cluster roots of white lupin in the soil profile due to localized release of phosphorus. *Ann. Bot.* **105**: 585 – 593.
- Linquist, B.A., Ruark, M.D. and Hill, J.E. 2010. Soil order and management practices control soil phosphorus fractions in managed wetland ecosystems. *Nutr. Cycl. Agroecosys.* **90**: 51-62.
- McDowell, R. and Sharpley, A. 2003. Phosphorus solubility and release kinetics as a function of soil test P concentration. *Geoderma* **112**: 143-154.
- Motomizu, S., Wakimoto, T. and Tōei, K. 1983. Spectrophotometric determination of phosphate in river waters with molybdate blue and malachite green. *Analyst* **108**: 3227.
- Nwoke, O.C., Diels, J., Abaidoo, R., Nziguheba, G. and Merckx, R. 2008. Organic acids in the rhizosphere and root characteristics of soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) in relation to phosphorus uptake in poor savanna soils. *Afr. J.*

Biotechnol. **7**: 3617-3624.

Ohno, T. and Zibilske, L.M. 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Sci. Soc. Am. J.* **55**: 892-895.

Raghothama, K.G. 1999. Phosphate acquisition. *Annu. Rev. Plant Phys.* **50**: 665-693.

Shariatmadari, H., Shirvani, M. and Jafari, A. 2006. Phosphorus release kinetics and availability in calcareous soils of selected arid and semiarid toposequences. *Geoderma* **132**: 261-272.

Strobel, B.W. 2001. Influence of vegetation on low-molecular-weight carboxylic acids in soil solution - a review. *Geoderma* **99**: 169-198.

Strom, L., Owen, A.G., Godbold, D.L. and Jones, D.L. 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. *Soil Biol. Biochem.* **34**: 703-710.

Taghipour, M. and Jalali, M. 2013. Effect of low-molecular-weight organic acids on kinetics release and fractionation of phosphorus in some calcareous soils of western Iran. *Environ. Monit. Assess.* **185**: 5471-5482.

Toor, G. and Bahl, G. 1999. Kinetics of phosphate desorption from different soils as influenced by application of poultry manure and fertilizer phosphorus and its uptake by soybean. *Bioresource Technol.* **69**: 117-121.

Turner, B.L. 2008. Resource partitioning for soil phosphorus: a hypothesis. *J. Ecol.* **96**: 698-702.

Wei, L.L., Chen, C.R. and Xu, Z.H. 2009. The effect of low-molecular-weight organic acids and inorganic phosphorus concentration on the determination of soil phosphorus by the molybdenum blue reaction. *Biol. Fert. Soils* **45**: 775-779.

Wei, L.L., Chen, C.R. and Xu, Z.H. 2010. Citric acid enhances the mobilization of organic phosphorus in subtropical and tropical forest soils. *Biol. Fert. Soils* **46**:

765-769.

Xiao, K., Katagi, H., Harrison, M. and Wang, Z.Y. 2006. Improved phosphorus acquisition and biomass production in *Arabidopsis* by transgenic expression of a purple acid phosphatase gene from *M. truncatula*. *Plant Sci.* **170**: 191-202.

Yang, S.Q., Dang, T.H., Qi, R.S. and Ma, R.P. 2012. Effect of low molecular weight organic acid on organic phosphorus fraction and availability in calcareous soil. *J. Soil Water Conserv.* **26**: 167-171.

Yu, L. 2013. Correlation and path analysis of yield of different types of maize in the east of Heilongjiang province. *Modern Agriculture* 19-23. (In Chinese)

Zhou, B.K. and Zhang, X.L. 2005. Effect of long-term phosphorus fertilization on the phosphorus accumulation and distribution in black soil and its availability. *Plant Nutr. Fert. Sci.* **11**: 143-147. (In Chinese)

Table 1. Selected physical and chemical properties of a black clay soil at the Hailun Experiment Station of Ecology, Northeast China

Total N	Total P	Organic C	Available P	pH	Soil texture (%)			Bulk density
$\text{g} \cdot \text{kg}^{-1}$	$\text{g} \cdot \text{kg}^{-1}$	$\text{g} \cdot \text{kg}^{-1}$	$\text{mg} \cdot \text{kg}^{-1}$	1:2.5H ₂ O	sand	silt	clay	$\text{g} \cdot \text{cm}^{-3}$
1.71	0.41	17.52	9.2	6.91	39.7	24.8	35.5	1.02

Soil P fractions ($\text{mg} \cdot \text{kg}^{-1}$)						Fe _d ^e	Al _d ^e	Fe _o ^e	Al _o ^e	CaCO ₃
NaHCO ₃ -Pi ^a	NaHCO ₃ -Po ^a	NaOH-Pi ^b	NaOH-Po ^b	HCl-Pi ^c	Residual P ^d	-----g kg ⁻¹ -----				
12.1	18.8	36.5	69.9	35.6	221.4	10.08	6.16	1.97	0.32	9.24

Note: ^aNaHCO₃-Pi and Po, soil soluble and exchangeable inorganic and organic phosphorus.

^bNaOH-Pi and Po, soil Fe/Al oxides bound inorganic and organic phosphorus.

^cHCl-Pi, soil Ca bound inorganic phosphorus.

^dResidual P, soil resistant inorganic and organic phosphorus.

^eFe_d and Al_d were dithionite-citrate-bicarbonate-extractable Fe and Al, respectively, and Fe_o and Al_o were oxalate-extractable Fe and Al, respectively.

Table 2. Kinetic models tested to describe P release kinetic data

Kinetic equation	Parameters
First order:	
$\ln Q_t^a = B^b - k_1 t$	k_1 , first-order rate constant (min^{-1})
Second order:	
$1/Q_t = B + k_2 t$	k_2 , second-order rate constant $[(\text{mg P kg}^{-1})^{-1}]$
Elovich:	
$Q_t = (1/\beta) \ln (\alpha\beta) + (1/\beta) \ln t$	α , initial P release rate ($\text{mg P kg}^{-1} \text{min}^{-1}$) β , P release constant $[(\text{mg P kg}^{-1})^{-1}]$
Power function:	
$\ln Q_t = \ln a + b \ln t$	a , initial P release rate constant ($\text{mg P kg}^{-1} \text{min}^{-1}$) ^b b , P release rate constant $[(\text{mg P kg}^{-1})^{-1}]$
Parabolic diffusion:	
$Q_t = A + R t^{1/2}$	R , diffusion rate constant $[(\text{mg P kg}^{-1})^{-0.5}]$

Note: ^a Q_t , the amount of P released after t minute (mg P kg^{-1}).

^b B , the amount of P release at equilibrium (mg P kg^{-1}).

Table 3. Coefficients of determination (r^2) and standard errors of estimates (S.E.) for kinetic equations used to describe the inorganic and organic P release data

treatments		First order		Second order		Elovich		Power function		Parabolic diffusion	
		r^2	SE	r^2	SE	r^2	SE	r^2	SE	r^2	SE
Inorganic	Water	0.386 ^a	0.36	0.288ns ^b	0.48	0.937 ^{***c}	0.11	0.869 ^{**}	0.17	0.701 ^{**}	0.23
P release	Oxalic acid	0.492 [*]	0.35	0.388 [*]	0.31	0.957 ^{**}	0.14	0.949 ^{**}	0.19	0.773 ^{**}	0.31
	Citric acid	0.417 [*]	0.39	0.280ns	0.19	0.993 ^{**}	0.12	0.928 ^{**}	0.14	0.771 ^{**}	0.65
	Malic acid	0.438 [*]	0.41	0.330ns	0.47	0.977 ^{**}	0.09	0.947 ^{**}	0.13	0.766 ^{**}	0.29
Organic P	Water	0.334ns	0.19	0.259ns	0.20	0.880 ^{**}	0.09	0.803 ^{**}	0.11	0.599 ^{**}	0.16
release	Oxalic acid	0.316ns	0.43	0.197ns	0.31	0.928 ^{**}	0.26	0.816 ^{**}	0.22	0.651 ^{**}	0.58
	Citric acid	0.434 [*]	0.16	0.353ns	0.09	0.937 ^{**}	0.11	0.889 ^{**}	0.07	0.704 ^{**}	0.23
	Malic acid	0.561 [*]	0.22	0.365 [*]	0.22	0.848 ^{**}	0.13	0.830 ^{**}	0.12	0.702 ^{**}	0.19

Note: ^a*, significant at $P < 0.05$.

^bns, not significant at $P < 0.05$.

^c**, significant at $P < 0.01$.

Table 4. Kinetic parameters of the Elovich and power function equations for describing Pi and Po release patterns from the soil studied.

Treatment		Elovich		Power function	
		α	$1/\beta$	a	b
Inorganic	Water	0.584	0.183	0.401	0.193
P release	Oxalic acid	0.431	0.298	0.511	0.211
	Citric acid	0.944	0.606	1.005	0.220
	Malic acid	0.269	0.268	0.367	0.238
Organic P	Water	0.494	0.105	0.753	0.095
release	Oxalic acid	12.201	0.421	1.818	0.211
	Citric acid	15.515	0.184	1.333	0.092
	Malic acid	8.174	0.141	0.678	0.121

Table 5. The pH value of treatment solutions and the change in pH of soil suspensions before shaking and after 2,880 min shaking in centrifuge tubes with each treatment (1:10 soil : solution ration with organic acid concentrations of 10 mmol kg⁻¹ soil).

Treatments	Solution pH	Before pH	After pH
Water	7.13	7.52 <i>a</i>	7.91 <i>b</i>
Citric acid	3.11	5.42 <i>a</i>	6.35 <i>b</i>
Oxalic acid	2.84	5.19 <i>a</i>	6.24 <i>b</i>
Malic acid	3.25	5.71 <i>a</i>	6.52 <i>b</i>

Note: Pairwise comparison of the treatment effect on the pH in soil suspension was made before and after 2,880 min shaking, where different lowercase italic letters indicate significant differences at $P < 0.05$.

Figure captions:

Fig. 1. The kinetics of inorganic phosphorus (Pi) release by water, oxalic acid, citric acid, and malic acid at 10 mmol kg⁻¹ soil from the studied soil as a function of time (a) and similar data described by selected kinetic equations: first order (b), second order (c), Elovich (d), power function (e) and parabolic diffusion (f).

Fig. 2. The kinetics of organic phosphorus (Po) release by water, oxalic acid, citric acid, and malic acid at 10 mmol kg⁻¹ soil from the studied soil as a function of time (a) and similar data described by selected kinetic equations: first order (b), second order (c), Elovich (d), power function (e) and parabolic diffusion (f).

Fig. 3. Fractionation of P of the studied soil after the kinetic study using water, OA (oxalic acid), CA (citric acid) and MA (malic acid). Error bars show the standard deviation of three replicate samples. The capital letter is used to indicate the difference of P fraction after kinetic study using water, oxalic, citric and malic acids. Values with the same letter are not significantly different at $P < 0.05$.

128x153mm (300 x 300 DPI)

133x151mm (300 x 300 DPI)

80x88mm (300 x 300 DPI)