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ORIGINAL ARTICLE

Rhizosphere calcareous soil P-extraction at the expense of organic carbon from root-exuded organic acids induced by phosphorus deficiency in several plant species

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Abstract

The amount of organic acids in root exudates rapidly increases under phosphorus (P) deficiency. Loss of carbon from root-exuded organic acids, which are derived from plant net photosynthetic products, is generally considered negligible. The present study aimed to study the characteristics of root-exuded organic acids, extraction of phosphorus (P extraction) in calcareous soil and the expression of organic carbon from root-exuded organic acids in two woody Moraceae plants (*Broussonetia papyrifera* L. Vent and *Morus alba* L.) and two herbaceous cruciferous plants (*Orychophragmus violaceus* L. Schulz and *Brassica napus* L.) under two P levels (P-normal and P-deficient). P extraction and the amount of root-exuded organic acids simultaneously and disproportionately increased in the four plant species tested under P deficiency. The maximum P-extracting capability of the four plant species was observed after 40 days of treatment. Additionally, the response of root-exuded organic acids induced by P deficiency was species-specific. *B. papyrifera* extracted more P in calcareous soil, and expended less organic acid for the same P-extraction than *M. alba*. Similarly, *O. violaceus* extracted more P in calcareous soil, and consumed less organic acid for the same level of P-extraction than *B. napus*. Root-exuded oxalic and malic acids accounted for most of the increment of P extraction in woody Moraceae plants, while root-exuded citric acid accounted for most of the increment in P extraction in herbaceous cruciferous plants. *B. papyrifera* and *O. violaceus* exhibited the strongest P-extracting capability at lower expense of organic carbon over the treatment duration in the four plant species. *O. violaceus* had the most rapid response of root-exuded organic acids to P deficiency, while *B. napus* had the slowest response. Thus, rapid response with low organic carbon cost and high efficiency of extraction on P in calcareous soil may underlie the strong adaptability of *B. papyrifera* and *O. violaceus* to a Karst environment.

Key words: *Broussonetia papyrifera*, calcareous soil, adaptability to Karst environment, *Orychophragmus violaceus*, root exudates.

INTRODUCTION

Root exudation is by far the biggest component of rhizodeposition (Nguyen 2003). A great majority of plants have been proven to release root exudates

consisting of inorganic anions/cations, organic acids, amino acids, sugars, phenolics, enzymes, vitamins and other substances (Jones 1998). Root-exuded organic acids such as malic, citric and oxalic acids have been proposed to play pivotal roles in many rhizosphere processes (Walker *et al.* 2003). Numerous studies have indicated that the amount of organic acids in root exudates rapidly increases under conditions of low nutrient availability, such as phosphorus (P) and zinc (Zn) deficiency (Shahbaz *et al.* 2006; Broadley *et al.* 2010; Carvalhais *et al.* 2011). P is one of the major limiting factors

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affecting plant growth in many soils, especially in both acidic and calcareous soils where P retention is maximal, given its ability to form insoluble complexes (Marschner 1995; Dakora and Phillips 2002; Li *et al.* 2008). Root-exuded organic acids can form organometallic complexes with insoluble phosphoric compounds in soil for releasing available P via complexation and other processes (Jones and Darrah 1994; Yamamura *et al.* 2004).

Plants can expend considerable organic carbon (C) in the form of root-exuded organic acids, which are derived from the transformation of carbon dioxide (CO₂) into carbohydrates during photosynthesis, and waste organic C from plant net photosynthetic products (Jones *et al.* 2009). Krafczyk *et al.* (1984) have shown that root-exuded organic acids account for 30–90% of the total C loss from maize (*Zea mays* L.) roots under nutrient stress. Jones (1998) concluded that organic acid exudation drains 5–25% of the net photosynthetic fixed C of a plant, depending on the species, under P deficiency. Thus, plants function in rhizosphere processes at great expense of organic C in the form of root-exuded organic acids.

The composition and amount of root-exuded organic acids, particularly under environmental stress, can vary across plant species. For instance, the composition and amount of organic acids in root exudates have been reported to differ significantly between Norway spruce (*Picea abies* L. Karst) and silver birch (*Betula pendula* Roth) (Sandnes *et al.* 2005). In seven lowland rice (*Oryza sativa* L.) genotypes, the amount of root-exuded oxalate was quantitatively the most important, but citrate is considered more effective in mobilizing Zn. Citrate exudation rates correlated with tolerance to low soil levels of Zn (Hoffland *et al.* 2006). It has also been reported that the amounts of malate and citrate in root exudations are significantly different among three dicotyledonous plants (rape (*Brassica napus* L.), lupin (*Lupinus albus* L.) and alfalfa (*Medicago sativa* L.)) under P deficiency (Jones 1998). Gaume *et al.* (2001) reported that the total amount of organic acids in root exudations of maize (*Zea mays* L.) seedlings varies significantly across genotypes of maize (*Zea mays* L.) seedlings, and malic, citric and trans-aconitic acids account for more than 80% of total root-exuded organic acids in all genotypes. Thus, the composition and amount of root-exuded organic acid varies with plant species, cultivars and nutritional status.

The ability of root-exuded organic acids to extract P in rhizosphere soil is difficult to determine directly. Therefore, indirect methods can alternatively be used to solve the problem. Soil P extraction increased with the organic acid concentration (Gerke *et al.* 2000a, 2000b; Ström *et al.* 2005). Establishing the relationship between soil P-extraction and organic acid concentration may help

to calculate the P extraction according to the amount of root-exuded organic acids by plants. Therefore, the study attempted to establish the quantitative relationship functions between P-extraction efficiency in calcareous soil and concentration of organic acids, and calculated the P extraction of root-exuded organic acids by plants.

Exudation of organic acids via their roots underlies the response of plants to low nutrients (Dakora and Phillips 2002). Compared to *Morus alba* L. (*Brassica napus* L.), *Broussonetia papyrifera* L. Vent (*Orychophragmus violaceus* L. Schulz) is more tolerant to low P nutrients, respectively (Liu *et al.* 2010, 2011). However, few studies have investigated their root-exuded organic acids and C flux in root exudates under P deficiency. The present study examined dynamic variation in root-exuded organic acids of four plant species ((*Broussonetia papyrifera* L. Vent and *Morus alba* L.), both of which are woody Moraceae plants, and (*Orychophragmus violaceus* L. Schulz and *Brassica napus* L.), both of which are herbaceous cruciferous plants) in response to P deficiency in sterile solution culture. P extraction in calcareous soil and the expenditure of organic C from root-exuded organic acids were investigated. Furthermore, the response modes of root-exuded organic acids induced by P deficiency in different plant species were analyzed.

MATERIALS AND METHODS

Solution culture experiment

Seeds of *B. papyrifera* (Bp), *O. violaceus* (Ov), *M. alba* (Ma) and *B. napus* (Bn) were surface sterilized [5 min in 95% ethanol, and 30 min in 10% hydrogen peroxide (H₂O₂), washing with sterile water after each treatment], then sown and grown in plastic pots for 20 d. The seedlings were transferred into modified Hoagland nutrient solution containing (mg L⁻¹): Potassium nitrate (KNO₃), 506; Calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O), 945; Ammonium nitrate (NH₄NO₃), 80; Magnesium sulfate heptahydrate (MgSO₄·7H₂O), 493; Boric acid (H₃BO₃), 3.0; Manganese sulfate tetrahydrate (MnSO₄·4H₂O), 0.5; Zinc sulfate heptahydrate (ZnSO₄·7H₂O), 1.0; Copper sulfate pentahydrate (CuSO₄·5H₂O), 0.05; Ethylenediamine tetraacetic acid ferric sodium salt (Fe(Na)EDTA), 11.1; and Ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O), 0.02, with pH adjusted to 8.0 using 1 M potassium hydroxide (KOH). After 30 d, the plants were transferred to modified Hoagland nutrient solution with two different P concentrations obtained using monopotassium phosphate (KH₂PO₄) and were grown in a controlled environment with a photosynthetic photon flux density of 300 μmol quanta m⁻² s⁻¹ during a 14-h photoperiod, at a temperature of 25 ± 0.5°C

and a relative humidity of $50 \pm 2\%$. The P-normal treatment consisted of modified Hoagland nutrient solution with 0.5 mM KH_2PO_4 , while the P-deficient treatment consisted of the modified Hoagland nutrient solution without KH_2PO_4 . Each treatment involved three plant seedlings, and was performed in triplicate. The pH of all nutrient solutions was adjusted to 8.0 using 1 M KOH. Root exudates of each treatment were then collected from the four plant species at treatment times of 10, 20, 30, 40 and 50 d, respectively. During the experiments, the solution was changed every other day.

Collection, separation and purification of root exudates

After treatment, the plants were transferred to a 100 mL 10 mM calcium chloride (CaCl_2) solution (pH = 7.0) and cultured for 6 h in sterile conditions. The solution containing the exudates was collected, passed through a cation exchange column (12 mm \times 15 mm) filled with 5 g of Amberlite IR-120B resin (H^+ form, Alfa Co.), and then passed through an anion exchange column (12 mm \times 15 mm) filled with 3 g of Dowex 1 \times 8 resin (100 mesh to 200 mesh; OH^- form; Acros Co.). The organic acids retained on the anion exchange resin were eluted by 1 M hydrochloric acid (HCl), dried using a rotary evaporator (40°C), and stored in a refrigerator at -20°C until analysis (Wang *et al.* 2007).

Analysis of organic acids in the root exudates

The contents and compositions of organic acids were analyzed by reversed-phase high-performance liquid chromatography (LA-20, Shimadzu, Japan) (Dinkci *et al.* 2007). The chromatographic column was a Kromasil C_{18} (4 mm i.d. \times 250 mm). The detection wavelength was 214 nm. The mobile phase was 0.01 mol L^{-1} KH_2PO_4 (pH 2.7, controlled by strong Orthophosphoric acid (H_3PO_4)). The flow rate was 0.6 mL min^{-1} . The recording speed was 1 cm min^{-1} . The injection volume was 10 μL . The eluents were methyl alcohol and Milli-Q water. The mobile phase

and analyte were filtered with a 0.22- μm membrane, and then ultrasonically degassed prior to use.

Soil sampling

Soils were collected from typical sites where the experimental plant species, *Bp*, *Ov*, *Ma* and *Bn*, grew in the Karst region in Guiyang, Guizhou Province, People's Republic of China. Soil was collected following the method described by Gollany *et al.* (1997), sieved to pass 2 mm and kept at 4°C until required. The soil properties are summarized in Table 1. The pH was measured at a 1:1 [weight/volume (w/v)] soil-to-deionized water ratio extracting with standard electrodes; available P was extracted by 0.5 mM Sodium bicarbonate (NaHCO_3) solution using a soil-to-solution ratio of 1:20 (w/v) with P determined colorimetrically (Olsen and Sommers 1982). Organic C was determined by loss on ignition at 550°C , while calcium carbonate (CaCO_3) equivalent was determined by neutralizing the soil with HCl and back-titration with sodium hydroxide (NaOH) (Lu 1999), and total nitrogen (N) was determined by extracting the soil with Sulfuric acid-sodium hydroxide (H_2SO_4 -NaOH) and back-titration with Sulfuric acid (H_2SO_4) according to Lu (1999).

Model of rhizosphere soil P extraction and exogenous organic acid concentration

To determine the concentration-dependent P-extraction efficiency of exogenous organic acids (oxalic, malic and citric acid), the extracting solution of individual organic acids, citric acid (CA), malic acid (MA) and oxalic acid (OA) were prepared using distilled water. The concentration of organic acids in the extracting solution ranged from 0 to 10 mM (0, 0.5, 1, 2, 4, 5, 8 and 10 mM, respectively). 30 mL of individual organic acid extracting solution was added to 3.0 g of air-dried rhizosphere soil in 50-mL polypropylene tubes in a sterile environment; 0.01 g L^{-1} thymol was added to reduce microbial consumption of organic acids. Then the samples were orbital shaken for 2 h and filtered. The filtrate was extracted and analyzed following the method described by Olsen

Table 1 Characteristics of rhizosphere soil on which different plant species grew in Karst soil. Note: values are means \pm standard error (SE) ($n = 5$)

	<i>Broussonetia papyrifera</i> L. Vent (<i>B. papyrifera</i>)	<i>Morus alba</i> L. (<i>M. alba</i>)	<i>Orychophragmus violaceus</i> L. Schulz (<i>O. violaceus</i>)	<i>Brassica napus</i> L. (<i>B. napus</i>)
Original pH (1:1 H_2O)	7.81 \pm 0.09	7.55 \pm 0.06	7.75 \pm 0.13	7.59 \pm 0.09
Available phosphorus (mg kg^{-1})	4.90 \pm 0.09	3.43 \pm 0.11	4.21 \pm 0.03	3.54 \pm 0.04
Organic carbon (g kg^{-1})	35.9 \pm 0.11	47.4 \pm 0.23	43.7 \pm 0.28	46.5 \pm 0.19
CaCO_3 (g kg^{-1})	47.6 \pm 0.16	46.9 \pm 0.14	48.3 \pm 0.13	49.4 \pm 0.20
Total nitrogen (g kg^{-1})	2.88 \pm 0.06	2.79 \pm 0.09	3.14 \pm 0.11	2.47 \pm 0.07

and Sommers (1982), and P in the extracting solution was measured using a spectrophotometer (Watanabe and Olsen 1965). Then, models of rhizosphere soil P-extraction and exogenous organic acid concentration were established.

Measurement of root biomass of four plant species under P treatments

After 50 d of treatment, root samples of four plant species from two P treatments (P-normal and P-deficient treatments) were dried at 105°C for 30 min, then the dry weight (DW) of each plant species under the two P treatments was determined at 70°C. Root biomass is indicated by its DW. Before P treatments, the root biomass of four plant species was also determined by randomly selecting three other plants' seedlings (0 d).

Rhizosphere soil P extraction from root-exuded organic acids (PEX)

The models of rhizosphere soil P extraction and exogenous organic acid concentration are shown in Eq. 1:

$$Y = f(X) \quad (1)$$

where Y is the amount of rhizosphere soil P-extraction (mg kg^{-1} dried-weight soil) and X is the exogenous organic acid concentration ($\leq 10 \text{ mM}$).

According to Eq. 1 and the composition and concentration of root-exuded organic acids, the rhizosphere soil P extraction from root-exuded organic acids (PEX) was calculated as follows (Eq. 2):

$$\Sigma Y_i = \Sigma f(X_i) \quad (2)$$

where X_i is the amount of root-exuded OA, CA or MA; Y_i is the corresponding rhizosphere soil P extraction from root-exuded OA, CA or MA; ΣY_i is the sum of rhizosphere soil P extraction from root-exuded OA, CA and MA, and is named for PEX.

Increment of P extraction, loss of organic carbon and P-extraction cost

The increment proportion of phosphorus extraction (IP_{PEX}) was calculated using the relative difference between rhizosphere soil P extraction under the P-deficient treatment and that under the P-normal treatment, as shown in Eq. 3:

$$IP_{\text{PEX}} (\%) = (\text{PEX}_1 - \text{PEX}_0) / \text{PEX}_0 \times 100 \quad (3)$$

where PEX_1 is rhizosphere soil P extraction from the root-exuded organic acids under the P-deficient treatment and PEX_0 is P extraction from the root-exuded organic acids under the P-normal treatment.

Loss of organic carbon (LOC) from root-exuded organic acid was determined by the moles of carbon from the root-exuded organic acid at the same treatment time under P-normal and P-deficient treatments, as indicated in Eq. 4:

$$\text{LOC} (\%) = (\text{OC}_1 - \text{OC}_0) / \text{OC}_0 \times 100 \quad (4)$$

where OC_1 is the moles of carbon from the root-exuded organic acid under the P-deficient treatment and OC_0 is the moles of carbon from the root-exuded organic acid under the P-normal treatment (OC unit: mol).

P-extraction cost is the amount of organic carbon consumed by a plant when it extracts a unit of phosphorus, and can be expressed as $\text{LOC}/IP_{\text{PEX}}$.

Statistical analysis

All experiments were performed in triplicate with the same treatment independently replicated on different days. Statistical analyses of data were carried out by t-tests, one-way analysis of variance (ANOVA) and bivariate correlations. Significance was assigned at the $p < 0.05$ level with Duncan's test. All analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Plant root growth

The increase in root dry weight varied with plant species and P treatment (Table 2). Sufficient P increased root growth, while P deficiency decreased it in four plant species with treatment of 50 d. The decrease in root DW in *Bp* was the least and that in *Bn* the greatest among the four plant species under P deficiency.

Relationship between rhizosphere soil P extraction and exogenous organic acid concentration

Table 3 shows the relationship between rhizosphere soil P extraction and exogenous organic acid concentration. Available P content had a linear correlation with the extracting organic acid concentration. The coefficient of determination (R) ranged from 0.942 to 0.999. A t-test on the data showed that the differences were significant at the 0.001 alpha levels, except for that of MA for *B. napus*, which was significant at the 0.005 alpha levels. Therefore, a linear equation (Eq. 5) can be used to express the

Table 2 Root dry weight and dry weight increase of four plant species as affected by phosphorus (P) treatments. Note: values are means \pm standard error (SE) ($n = 3$). The different small letters indicate the significant difference in four plant species under two P treatments at $p < 0.05$

Plant species	P treatment	Root dry weight (g per plant)		Increase in root dry weight during 50 days' treatment (g per plant)	The percentage inhibition of root dry weight (%)
		0 days	50 days		
<i>Broussonetia papyrifera</i>	P-normal	0.732 \pm 0.094	0.892 \pm 0.036	0.156 \pm 0.023	-21.86 \pm 2.83 a
L. Vent (<i>B. papyrifera</i>)	P-deficient	0.807 \pm 0.023	0.725 \pm 0.010	-0.082 \pm 0.016	8.92 \pm 1.06 d
<i>Morus alba</i> L.	P-normal	0.653 \pm 0.047	0.706 \pm 0.033	0.050 \pm 0.009	-8.12 \pm 1.09 c
(<i>M. alba</i>)	P-deficient	0.685 \pm 0.051	0.599 \pm 0.029	-0.086 \pm 0.002	12.55 \pm 1.66 d
<i>Orychophragmus violaceus</i>	P-normal	0.764 \pm 0.044	0.873 \pm 0.012	0.109 \pm 0.011	-14.27 \pm 2.08 b
L. Schulz (<i>O. violaceus</i>)	P-deficient	0.778 \pm 0.038	0.694 \pm 0.033	-0.084 \pm 0.006	10.80 \pm 1.77 d
<i>Brassica napus</i> L. (<i>B. napus</i>)	P-normal	0.598 \pm 0.063	0.681 \pm 0.041	0.083 \pm 0.004	-13.88 \pm 1.91 b
	P-deficient	0.675 \pm 0.067	0.487 \pm 0.024	-0.188 \pm 0.013	27.85 \pm 3.49 e

Table 3 Relationships between rhizosphere soil phosphorus (P)-extraction and exogenous organic acid concentration (≤ 10 mM) for different plant species and organic acids. Note: Y is the amount of P extraction from rhizosphere soil (mg kg^{-1} DW soil); X is the organic acid concentration (mM). ** indicates significant difference at $p < 0.001$; * indicates significant difference at $p < 0.005$. Per carbon P-extraction expresses the amount of rhizosphere soil P extraction by a mole weight of carbon from CA, or OA, or MA

Plant species	Organic acid	Regression equation ^a	Determination coefficient (R^2)	Per carbon P-extraction
<i>Broussonetia papyrifera</i> L. Vent (<i>B. papyrifera</i>)	Citric acid	$Y = 5.397X + 2.040$	0.998**	0.900
	Oxalic acid	$Y = 4.545X + 2.166$	0.996**	2.273
	Malic acid	$Y = 2.970X + 1.850$	0.986**	0.743
<i>Morus alba</i> L. (<i>M. alba</i>)	Citric acid	$Y = 2.510X + 1.544$	0.997**	0.418
	Oxalic acid	$Y = 2.171X + 1.341$	0.996**	1.086
	Malic acid	$Y = 1.222X + 1.649$	0.992**	0.306
<i>Orychophragmus violaceus</i> L. Schulz (<i>O. violaceus</i>)	Citric acid	$Y = 2.178X + 3.827$	0.972**	0.363
	Oxalic acid	$Y = 1.941X + 3.540$	0.976**	0.971
	Malic acid	$Y = 1.526X + 3.356$	0.999**	0.382
<i>Brassica napus</i> L. (<i>B. napus</i>)	Citric acid	$Y = 2.078X + 2.463$	0.991**	0.346
	Oxalic acid	$Y = 0.743X + 2.501$	0.975**	0.372
	Malic acid	$Y = 0.457X + 2.229$	0.942*	0.114

relationship between P extraction from rhizosphere soil and the organic acid concentration (≤ 10 mM):

$$Y = aX + b \quad (5)$$

where Y is the amount of P-extraction from rhizosphere soil, X is the organic acid concentration in 30 mL of extractant, "a" is the extraction coefficient indicating P-extraction ability and "b" is a constant indicating P extraction by water.

The rhizosphere soil P-extraction ability varied with the plant and organic acid species. Based on a comparison of extraction coefficients, citric acid was found to have the greatest P-extraction ability among the three organic acids, whereas MA had the lowest P-extraction ability. *Bp* can release the most P, and *Bn* can release the least P in rhizosphere soil extracted by CA, OA or MA (Table 3).

The amount of P extraction by a unit of carbon from root-exuded organic acids can be expressed as per carbon P extraction. The differences in per carbon P

extraction among organic acid species on the four species of plants' rhizosphere soil are shown in Table 3. Organic carbon from oxalate acid had the highest per carbon P extraction among the three organic acids.

Variations in root-exuded organic acids

P deficiency increased the sum of root-exuded CA, MA and OA in four plants from the root during 6 h in 10, 20, 30, 40 and 50 treatment days (Fig. 1). The sum of OA, MA and CA in *Bp* root exudation was remarkably higher than that in *Ma*, except of CA under P-deficiency treatment ($p < 0.05$). The root-exuded OA, MA and CA increased sharply in response to P deficiency in woody Moraceae plants, except of OA in *Ma*. The amount of OA and MA in *Bp* root exudation was two-fold higher than that in *Ma* under the same P level (Fig. 1A). The sum of root-exuded OA in *Ov* was also higher than that in *Bn* under the P-deficient condition, but the sum of MA was not significantly different in herbaceous cruciferous plants

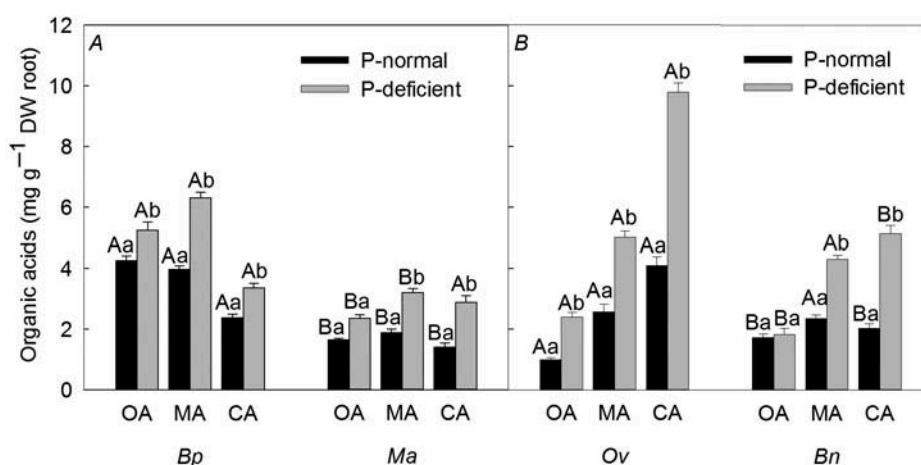


Figure 1 The sum of root-exuded oxalic, malic, and citric acids in (A) woody Moraceae plants [*B. papyrifera* (*Bp*) and *M. alba* (*Ma*)] and (B) herbaceous cruciferous plants [*O. violaceus* (*Ov*) and *B. napus* (*Bn*)] from the root during 6 h in 10, 20, 30, 40 and 50 treatment days. Note: blocks with bars indicate mean \pm standard error (SE). The mean \pm SE with the same letter are not statistically different at $p = 0.05$. Small letters refer to differences between phosphorus (P) treatments in the same plant species. Capital letters refer to differences between plant species under the same organic acid. DW, dry weight.

at either P level. Compared with OA and MA, the root-exuded CA showed a sharp increase in response to P deficiency in herbaceous cruciferous plants (Fig. 1B).

Dynamic variation of the increment proportion of phosphorus extraction (IP_{PEX})

IP_{PEX} reached the maximum level at the treatment time of 40 d in four plant species, indicating the greatest P extraction occurred at the treatment time of 40 d under the P-deficient condition (Fig. 2). The dynamic variation of IP_{PEX} showed the same trend under P-deficient treatment

in woody Moraceae plants. IP_{PEX} in *Bp* was higher than that in *Ma* at the same treatment time; IP_{PEX} increased rapidly from 30 to 40 d of treatment, and the increased values in *Bp* and *Ma* were 7.1 and 5.9%, respectively (Fig. 2A). The IP_{PEX} in *Ov* was significantly higher than that in *Bn* at each treatment time. The average increased value of IP_{PEX} in *Ov* was 7.1% greater than that in *Bn* during 50 d of treatment time. The IP_{PEX} in herbaceous cruciferous plants changed only slightly with treatment time: the greatest increased values were 2.7 and 2.5% in *Ov* and *Bn* from 30 to 40 d of treatment, respectively, which is significantly less than that in woody Moraceae plants ($p < 0.05$) (Fig. 2B).

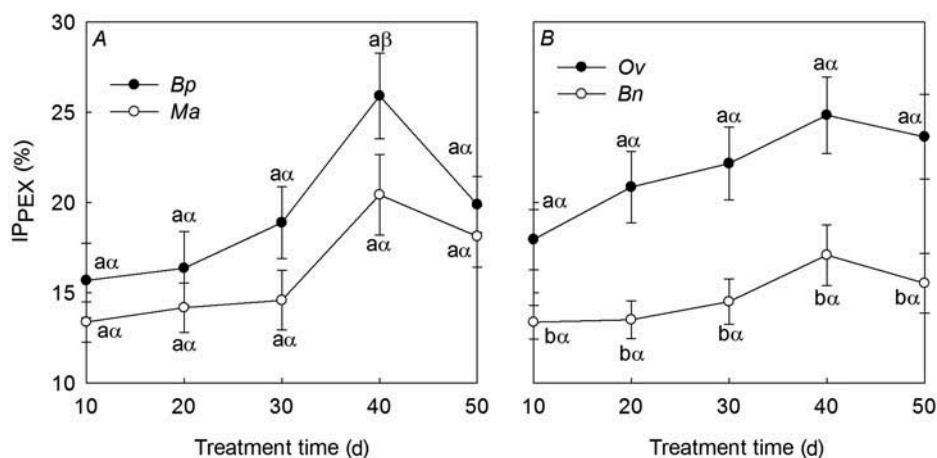


Figure 2 The increment proportion of phosphorus extraction (IP_{PEX}) by the root-exuded organic acids of (A) woody Moraceae plants [*B. papyrifera* (*Bp*) and *M. alba* (*Ma*)] and (B) herbaceous cruciferous plants [*O. violaceus* (*Ov*) and *B. napus* (*Bn*)] in phosphorus (P)-deficient compared with P-normal plants during different treatment times. Note: circles and triangles with bars indicate mean \pm standard error (SE). Means \pm SE with the same letter are not statistically different at $p = 0.05$. Small Latin letters refer to differences between two plant species under the same treatment time. Small Greek letters refer to differences between treatment times under the same plant species.

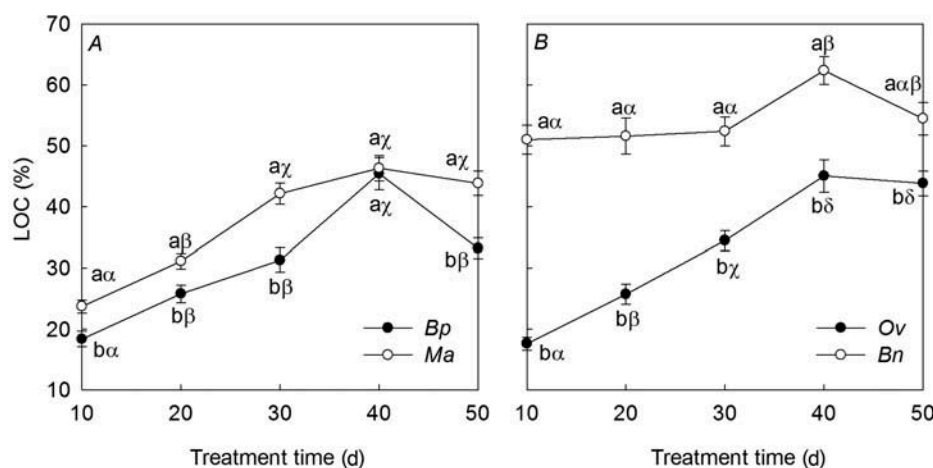


Figure 3 Loss of organic carbon (LOC) caused by root-exuded organic acids of (A) woody Moraceae plants [*B. papyrifera* (*Bp*) and *M. alba* (*Ma*)] and (B) herbaceous cruciferous plants [*O. violaceus* (*Ov*) and *B. napus* (*Bn*)] in phosphorus (P)-deficient compared with P-normal plants during different treatment times. Note: circles and triangles with bars indicate mean \pm standard error (SE). Means \pm SE with the same letter are not statistically different at $p = 0.05$. Small Latin letters refer to differences between two plant species under the same treatment time. Small Greek letters refer to differences between treatment times in the same plant species.

Dynamic variation of the loss of organic carbon (LOC)

Loss of organic C caused by root-exuded organic acids changed under different treatment times and plant species under the P-deficient treatment. The maximum LOC was reached at the treatment time of 40 d in four plant species (Fig. 3). The variation trend in LOC caused by root-exuded organic acids of woody Moraceae plants was similar (Fig. 3A). LOC in *Bp* was lower than that in *Ma* under the P-deficient treatment at the same treatment time. In woody Moraceae plants, *Bp* had greater IP_{PEX} but less LOC than *Ma*. The LOC in *Ma* increased steadily along with treatment times, which emerged first as an increase from 10 to 30 d, then increased from 30 to 40 d (14.2%) and decreased from 40 to 50 d (12.3%) overwhelmingly in *Bp*. In herbaceous cruciferous plants, LOC caused by the root-exuded organic acids of *Ov* was significantly lower than that of *Bn* ($p < 0.05$) (Fig. 3B). LOC in *Ov* was only two-fifths to four-fifths that of *Bn* under P-deficiency depending on the treatment time. From 10 to 40 d, LOC of *Ov* increased rapidly and the increased value was 27.4%, while *Bn* increased slowly from 10 to 30 d (1.4%) and increased rapidly from 30 to 40 d (10.0%).

Dynamic variation of P-extraction cost

P-extraction cost varied with plant species. Among the four species examined, P-extraction cost in *Bn* was the greatest and that in *Ov* was the least (Fig. 4). In woody Moraceae plants, the average value of P-extraction cost in *Ma* during the treatment time was 2.31, and it was higher than that in *Bp* (1.56). From 10 to 30 d of

treatment time, *Bp* and *Ma* increased 41.9% and 62.7%, respectively (Fig. 4A). In herbaceous cruciferous plants, P-extraction cost in *Bn* was significantly higher than that in *Ov*; the average value of P-extraction cost in *Bn* during the treatment time was 3.68, compared to 1.49 in *Ov*. The P-extraction cost in *Bn* decreased smoothly and steadily (about 8%) with treatment. However, from 10 to 40 d of treatment time, P-extraction cost in *Ov* increased up to 86% (Fig. 4B).

DISCUSSION

The rhizosphere soil P extraction versus the root-exuded organic acids

The amount of P extracted from rhizosphere soil increased with the exogenous organic acid concentration. In the study, a linear function can well describe the relationship between rhizosphere soil P extraction and exogenous organic acids. Therefore, the present study can successfully calculate the ability of root-exuded organic acids to extract P in rhizosphere soil according to the composition and concentration of root-exuded organic acids by different plant species under different treatments.

Efficiency of P extraction from root-exuded organic acids

Organic acids have the capacity to complex metals in solutions. The degree of complexation depends on the organic acid (number and proximity of carboxyl group), the concentration and type of metal and the pH of the

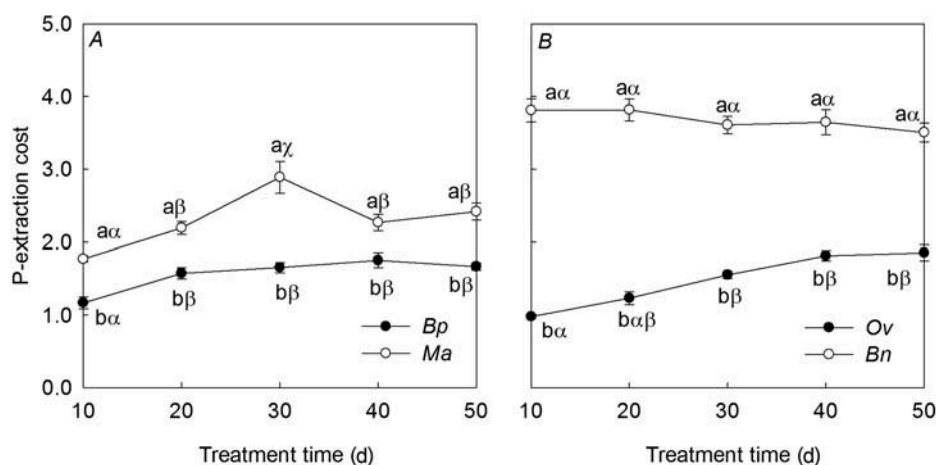


Figure 4 Phosphorus (P)-extraction cost caused by root-exuded organic acids in (A) woody Moraceae plants [*B. papyrifera* (*Bp*) and *M. alba* (*Ma*)] and (B) herbaceous cruciferous plants [*O. violaceus* (*Ov*) and *B. napus* (*Bn*)] in P-deficient compared with P-normal during different treatment times. Note: circles and triangles with bars indicate mean \pm standard error (SE). Means \pm SE with the same letter are not statistically different at $p = 0.05$. Small Latin letters refer to differences between two plant species under the same treatment time. Small Greek letters refer to differences between treatment times in the same plant species.

soil solution (Jones 1998). The complexation capacities of dicarboxylic and tricarboxylic acid have been found to be remarkably higher than that of monocarboxylic acid. Malate, citrate and oxalate all have high complexation with many metallic cations such as calcium ion (Ca^{2+}) and iron ion (Fe^{3+}). These root-exuded organic acids move P from insoluble phosphoric compounds such as calcium phosphate (Ca-P) and iron phosphate (Fe-P) to overcome P deficiency in calcareous soil (Xu *et al.* 2004). The stability constants of Ca^{2+} with citrate, oxalate and malate have been found to be 4.85, 3.19 and 2.72, respectively. Complexing capacity of organic acids determines the ability for P extraction from the root-exuded organic acids (Parker *et al.* 1995). Thus, CA and MA were found to have the greatest and the least P-extraction efficiency, respectively. In our study, we found that in woody Moraceae plants, *Bp* root exuded greater amounts of OA, MA and CA. The percentage of OA, MA and CA in root-exuded organic acids of *Bp* (26.75, 32.14 and 24.74%, respectively) was higher than that of *Ma* (18.29, 27.16 and 17.15%, respectively). Therefore, *Bp* was able to obtain a great amount of available P from rhizosphere soil under P deficiency via complexation with OA, MA and CA. This explains a previous observation that *Bp* has a higher net photosynthetic rate than *Ma* in the Karst environment (Wu *et al.* 2009). In herbaceous cruciferous plants, the amount and percentage of root-exuded organic acids in *Ov* was higher than those in *Bn*. Particularly, the amount and percentage of root-exuded CA in *Ov* (9.78 mg g^{-1} DW root, 44.01%) were distinctly higher than those in *Bn* (4.09 mg g^{-1} DW root, 29.51%) Therefore, *Ov* was able

to obtain a great amount of available P under P deficiency from rhizosphere soil via complexing mainly with root-exuded CA, and secondarily with two other species of organic acids. Similarly, this explains a previous observation that the accumulation of available P in the rhizosphere soil of *Ov* was higher than that of *Bn* in a low-P environment (Wu *et al.* 1997).

Moreover, the efficiency of extraction of P in the rhizosphere soil varied among different plant species (Ström 1997). *Bp* rhizosphere soil maintained a high concentration of available P (Table 1), and a great amount of root exudates including malate, citrate and oxalate. Thus, P extraction by the subsequent root-exuded organic acids in *Bp* was reinforced by the fact that root-exuded organic acids pre-existed in the experimental rhizosphere soil. Similarly, the effect of P extraction by subsequent root-exuded organic acids in *Ov* was magnified by root-exuded organic acids that pre-existed in the experimental rhizosphere soil.

The response of P extraction from root-exuded organic acids on P deficiency

Oxalic, malic and citric acids are derived from the transformation of CO_2 into carbohydrates during photosynthesis; they are the main carboxylic acid components of Krebs cycle (López-Bucio *et al.* 2000). These three species of organic acids do not have the same formation time, rate and sequence, which results in the difference in the composition and amount of root-exuded organic acids at different stages of stress treatments (Vranova *et al.* 2013). The present study demonstrates that the composition and amount of root-exuded

organic acids at the same stage of P deficiency varies with the plant species, and the mode of response of P extraction to P deficiency was species-specific. In woody Moraceae plants, the mode of response of P extraction to P deficiency in *Bp* and *Ma* is similar, and the rate of response of P extraction in *Bp* and *Ma* was slow at earlier stages of P deficiency (10–20 d of treatment time), then increased rapidly at the mid-stage (30–40 d of treatment time) (Fig. 2). The slow response of P extraction to P deficiency in *Bp* and *Ma* at the earlier stage resulted from the sharp increase in root-exuded OA and MA, which formed later than CA in the Krebs cycle. In herbaceous cruciferous plants, the rate of response of P extraction in *Ov* was rapid at the earlier stage of P deficiency (10–20 d of treatment time), then increased steadily at the mid-stage (30–40 d of treatment time), while the rate of response of P extraction in *Bn* was slow at the earlier stage of P deficiency (10–20 d of treatment time) and stayed stable at subsequent stages (20–30 d of treatment time) (Fig. 2). Similarly, the rapid response of P extraction to P deficiency in *Ov* at earlier stages resulted from the sharp increase in root-exuded CA, formed in advance of MA and OA in the Krebs cycle. The time of active response to P deficiency in four plant species was within 40 d. Afterwards, owing to plant senescence and decrease in photosynthesis, plants only grew upon replenishment of their P nutrient supply; P-extraction by root-exuded organic acids was unnecessary and passive (Strobel 2001; Jones *et al.* 2003).

Economy of P extraction from root-exuded organic acids under P deficiency

The concentration of C (40000 $\mu\text{mol g}^{-1}$) in plants is remarkably higher than that of P (60 $\mu\text{mol g}^{-1}$) (Atkinson and Smith 1983). In general, plants contribute more than one atom of organic carbon for one atom of phosphorus, and phosphorus is more valuable than organic carbon for plant life activity (Jones *et al.* 2009; Zhang *et al.* 2011). In this study, P extraction and the amount of root-exuded organic acids simultaneously and disproportionately changed with the treatment time (Figs. 2 and 3). Therefore, an economic transaction of organic carbon for P extraction is very important for the adaptability of plants.

P deficiency stimulated root-exuded organic acids (the main organic acids are OA, CA and MA), and the ratio of the amount of OA, CA or MA accounting for the total amount of organic acids in root exudations was influenced (Farrar *et al.* 2003; Jones *et al.* 2009). When the ratio of OA accounting for the total root-exuded organic acids increased, P extraction increased while P-extraction cost decreased owing to the strong complexation with insoluble phosphate and the least carbon mole of OA

(Farrar and Jones 2000). Therefore, plants with a high ratio of OA accounting for the total root-exuded organic acids had a low P-extraction cost.

P-extraction cost varied with plant species (Fig. 4). In the present study, in two woody Moraceae plants, *Bp* had a lower P-extraction cost than *Ma* (Fig. 4). The low P-extraction cost in *Bp* may come primarily from large per carbon P-extraction (Table 3). Thus, *Bp* was more economic for P extraction, and was more adaptable than *Ma* under P deficiency. Similarly, in two herbaceous cruciferous plants, the amount of OA in *Ov* root-exuded organic acids under P deficiency was 2.3 times that under the P-normal treatment, and the amount of OA in *Bn* root-exuded organic acids under P deficiency was only 1.2 times than that under the P-normal treatment. OA can reduce P-extraction cost to the maximum extent (Table 3). Therefore, *Ov* also had a lower P-extraction cost than *Bn* (Fig. 4). This indicated that *Ov* depleted less organic C than *Bn* to extract the same amount of P, and the adaptability of *Ov* to a low-P environment was better than that of *Bn*.

CONCLUSIONS

The efficiency and economy of P extraction varied with the plant species. In Moraceae plants, *B. papyrifera* secreted more root-exuded oxalate and malate, resulting in stronger P-extracting capability at a lower expense of organic C. In cruciferous plants, *O. violaceus* exuded more citric acid, resulting in stronger P extraction at the lower expense of organic C cost. Root-exuded citrate is responsible for the rapid response of P extraction to P deficiency; oxalate can reduce P-extraction cost. The rapid, low organic-carbon cost and highly efficient P extraction in calcareous soil may involve the adaptability of *B. papyrifera* and *O. violaceus* to Karst environments.

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REFERENCES

- Atkinson MJ, Smith SV 1983: C: N: P ratios of benthic marine plants [carbon: nitrogen: phosphorus]. *Limnol. Oceanogr.*, 28, 568–574. doi:10.4319/lo.1983.28.3.0568

- Broadley MR, Rose T, Frei M, Pariasca-Tanaka J, Yoshihashi T, Thomson M, Hammond JP, Aprile A, Close TJ, Ismail AM, Wissuwa M 2010: Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytol.*, **186**, 400–414.
- Carvalho LC, Dennis PG, Fedoseyenko D, Hajirezaei MR, Borriss R, von Wirén N 2011: Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *J. Plant Nutr. Soil Sci.*, **174**, 3–11. doi:10.1002/jpln.201000085
- Dakora FD, Phillips DA 2002: Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil*, **245**, 35–47. doi:10.1023/A:1020809400075
- Dinkci N, Akalın AS, Gönc S, Ünal G 2007: Isocratic reverse-phase HPLC for determination of organic acids in *Kargı Tulum* cheese. *Chromatographia*, **66**, 45–49. doi:10.1365/s10337-007-0234-6
- Farrar J, Hawes M, Jones DL, Lindow S 2003: How roots control the flux of carbon to the rhizosphere. *Ecology*, **84**, 827–837. doi:10.1890/0012-9658(2003)084[0827:HRCTFO]2.0.CO;2
- Farrar JF, Jones DL 2000: The control of carbon acquisition by roots. *New Phytol.*, **147**, 43–53. doi:10.1046/j.1469-8137.2000.00688.x
- Gaume A, Mächler A, De León C, Narro L, Frossard E 2001: Low-P tolerance by maize (*Zea mays* L.) genotypes: significance of root growth, and organic acids and acid phosphatase root exudation. *Plant Soil*, **228**, 253–264. doi:10.1023/A:1004824019289
- Gerke J, Beißner L, Römer W 2000a: 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. doi:10.1002/(SICI)1522-2624(200004)163:2<207::AID-JPLN207>3.0.CO;2-P
- Gerke J, Römer W, Beißner L 2000b: The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. II. The importance of soil and plant parameters for uptake of mobilized P. *J. Plant Nutr. Soil Sci.*, **163**, 213–219. doi:10.1002/(SICI)1522-2624(200004)163:2<213::AID-JPLN213>3.0.CO;2-0
- Gollany HT, Bloom PR, Schumacher TE 1997: Rhizosphere soil-water collection by immiscible displacement-centrifugation technique. *Plant Soil*, **188**, 59–64. doi:10.1023/A:1004208530887
- Hoffland E, Wei C, Wissuwa M 2006: Organic anion exudation by lowland rice (*Oryza sativa* L.) at zinc and phosphorus deficiency. *Plant Soil*, **283**, 155–162. doi:10.1007/s11104-005-3937-1
- Jones DL 1998: Organic acids in the rhizosphere—a critical review. *Plant Soil*, **205**, 25–44. doi:10.1023/A:1004356007312
- Jones DL, Darrah PR 1994: Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. *Plant Soil*, **166**, 247–257. doi:10.1007/BF00008338
- Jones DL, Dennis PG, Owen AG, van Hees PAW 2003: Organic acid behavior in soils – misconceptions and knowledge gaps. *Plant Soil*, **248**, 31–41. doi:10.1023/A:1022304332313
- Jones DL, Nguyen C, Finlay RD 2009: Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant Soil*, **321**, 5–33. doi:10.1007/s11104-009-9925-0
- Kracczyk I, Trolldenie G, Beringer H 1984: Soluble root exudates of maize: influence of potassium supply and rhizosphere microorganisms. *Soil Biol. Biochem.*, **16**, 315–322. doi:10.1016/0038-0717(84)90025-7
- Li H, Shen J, Zhang F, Clairrotte M, Drevon JJ, Le Cadre E, Hinsinger P 2008: Dynamics of phosphorus fractions in the rhizosphere of common bean (*Phaseolus vulgaris* L.) and durum wheat (*Triticum turgidum durum* L.) grown in monocropping and intercropping systems. *Plant Soil*, **312**, 139–150. doi:10.1007/s11104-007-9512-1
- Liu CC, Liu YG, Guo K, Fan DY, Li GG, Zheng YR, Yu LF, Yang R 2011: Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot.*, **71**, 174–183. doi:10.1016/j.envexpbot.2010.11.012
- Liu CC, Liu YG, Guo K, Zheng YR, Li GQ, Yu LF, Yang R 2010: Influence of drought intensity on the response of six woody karst species subjected to successive cycles of drought and rewatering. *Physiol. Plantarum.*, **139**, 39–54. doi:10.1111/j.1399-3054.2009.01341.x
- López-Bucio J, Nieto-Jacobo MF, Ramírez-Rodríguez V, Herrera-Estrella L 2000: Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Sci.*, **160**, 1–13. doi:10.1016/S0168-9452(00)00347-2
- Lu RS 1999: Methods in Agricultural Chemical Analysis of Soil. China Agricultural Science and Technology Publishing House, Beijing.
- Marschner H 1995: Mineral Nutrition of Higher Plants, 2nd edn. Academic Press, London.
- Nguyen C 2003: Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie*, **23**, 375–396. doi:10.1051/agro:2003011
- Olsen SR, Sommers LE 1982: Phosphorous, Methods of Soil Analysis, Part 2-Chemical and Microbiological Properties, pp. 403–430. Soil Science Society of America, Madison, Wisconsin.
- Parker DR, Chaney RL, Norvell WA 1995: Chemical Equilibria Models, Applications to Plant Research, Chemical Equilibria and Reaction Models, pp. 163–200. Soil Science Society of America, Madison, Wisconsin.
- Sandnes A, Eldhuset TD, Wollebaek G 2005: Organic acids in root exudates and soil solution of Norway spruce and silver birch. *Soil Biol. Biochem.*, **37**, 259–269. doi:10.1016/j.soilbio.2004.07.036
- Shahbaz AM, Oki Y, Adachi T, Murata Y, Khan MHR 2006: Phosphorus starvation induced root-mediated pH changes in solubilization and acquisition of sparingly soluble P sources and organic acids exudation by Brassica cultivars. *Soil Sci. Plant Nutr.*, **52**, 623–633. doi:10.1111/j.1747-0765.2006.00082.x

- Strobel BW 2001: Influence of vegetation on low-molecular-weight carboxylic acids in soil solution—a review. *Geoderma*, **99**, 169–198. doi:10.1016/S0016-7061(00)00102-6
- Ström L, Owen AG, Godbold LG, Jones DL 2005: Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling. *Soil Biol. Biochem.*, **37**, 2046–2054. doi:10.1016/j.soilbio.2005.03.009
- Ström L 1997: Root exudation of organic acids: importance to nutrient availability and the calcifuge and calcicole behaviour of plants. *Oikos*, **80**, 459–466. doi:10.2307/3546618
- Vranova V, Rejsek K, Skene KR, Janous D, Formanek P 2013: Methods of collection of plant root exudates in relation to plant metabolism and purpose: a review. *J. Plant Nutr. Soil Sci.*, **176**, 175–199. doi:10.1002/jpln.201000360
- Walker TS, Bais HP, Grotewold E, Vivanco JM 2003: Root exudation and rhizosphere biology. *Plant Physiol.*, **132**, 44–51. doi:10.1104/pp.102.019661
- Wang P, Zhou R, Cheng JJ, Bi SP 2007: LC determination of trace short-chain organic acids in wheat root exudates under aluminum stress. *Chromatographia*, **66**, 867–872. doi:10.1365/s10337-007-0418-0
- Watanabe FS, Olsen SR 1965: Test of an ascorbic acid method for determining phosphorus in water and NaHCO₃ extracts from soil. *Soil Sci. Soc. Am. J.*, **29**, 677–678. doi:10.2136/sssaj1965.03615995002900060025x
- Wu YY, Jiang JY, Shuai SW, Chen DM 1997: Approach to mechanism of inorganic nutrition about Karst adaptability of *Orychophragmus violaceus*. *J. Oil Crop Sci.-China*, **19**, 47–49.
- Wu YY, Liu CQ, Li PP, Wang JZ, Xing DK, Wang BL 2009: Photosynthetic characteristics involved in adaptability to Karst soil and alien invasion of paper mulberry (*Broussonetia papyrifera* (L.) Vent.) in comparison with mulberry (*Morus alba* L.). *Photosynthetica*, **47**, 155–160. doi:10.1007/s11099-009-0026-3
- Xu RK, Zhu YG, Chittleborough D 2004: Phosphorus release from phosphate rock and iron phosphate by low-molecular-weight organic acids. *J. Environ. Sci.-China*, **16**, 5–8.
- Yamamura T, Dateki H, Wasaki J, Shinano T, Osaki M 2004: Possibility of rhizosphere regulation using acid phosphatase and organic acid for recycling phosphorus in sewage sludge. *Soil Sci. Plant Nutr.*, **50**, 77–83. doi:10.1080/00380768.2004.10408454
- Zhang GG, Kang YM, Han GD, Mei H, Sakurai K 2011: Grassland degradation reduces the carbon sequestration capacity of the vegetation and enhances the soil carbon and nitrogen loss. *Acta Agric. Scand. B-S P*, **61**, 356–364.