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Effect of Zn deficiency and excessive bicarbonate on the allocation and exudation of organic acids in two Moraceae plants

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Abstract The effect of zinc (Zn) deficiency and excessive bicarbonate on the allocation and exudation of organic acids in plant organs (root, stem, and leaf) and root exudates of two Moraceae plants (Broussonetia papyrifera and Morus alba) were investigated. Two Moraceae plants were hydroponically grown and cultured in nutrient solution in four different treatments with 0.02 mM Zn or no Zn, combined with no or 10 mM bicarbonate. The variations of organic acids in different plant organs were similar to those of root exudates in the four treatments except B. papyrifera, which was in a treatment that was a combination of 0.02 mM Zn and no bicarbonate. The response characteristics in the production, translocation, and allocation of organic acids in the plant organs and root exudates varied with species and treatments. Organic acids in plant organs and root exudates increased under Zn-deficient conditions, excessive bicarbonate, or both. An increase of organic acids in the leaves resulted in an increase of root-exuded organic acids. B. papyrifera translocated more oxalate and citrate from the roots to the rhizosphere than M. alba under

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the dual influence of 10 mM bicarbonate and Zn deficiency. Organic acids of leaves may be derived from dark respiration and photorespiration. By comparison, organic acids in stems, roots, and root exudates may be derived from dark respiration and organic acid translocation from the leaves. These results provide evidence for the selective adaptation of plants to environments with low Zn levels or high bicarbonate levels such as a karst ecosystem.

Keywords Adaptation · Excessive bicarbonate · Organic acids · Organs · Root exudates · Zn deficiency

1 Introduction

Zinc (Zn) deficiency is one of the most common micronutrient conditions adversely affecting plant growth in calcareous soils (Hajiboland et al. 2005; Hoffland et al. 2006; Broadley et al. 2010). It influences numerous biological processes, such as carbohydrate metabolism, reactive oxygen species, carbonic anhydrase activity, and phosphorus (P)–Zn interaction (Dickinson and Chang 2011; Gianquinto et al. 2000; Ohki 1976; Rehman et al. 2012). However, bicarbonate (HCO₃⁻) toxicity is frequently associated with Zn deficiency in calcareous soils because excessive HCO_3^- inhibits the growth and stimulates physiological processes such as root-exuded organic acids in rice and wheat (Romera et al. 1992; Wissuwa et al. 2006).

Low-molecular weight organic acids, such as oxalic acid, malic acid, and citric acid, are the most abundant and widely distributed acids in plant organs, such as roots, stems, and leaves (Wang et al. 2010). Derived from glycolysis, tricarboxylic acid cycle (TCA cycle), and anaplerotic pathway, these organic acids combine with ammonium to form amino acids and then influence photosynthesis, photorespiration, and other plant physiological processes (Araújo et al. 2012; Sweetlove et al. 2010). The metabolism of organic acids varies with plant species and genotypes, plant age, and nutritional status (López-Bucio et al. 2000). Organic acids accumulate in plant organs and are exuded by the roots into the rhizosphere; they also function in plant nutrition and environmental stresses (Fan et al. 2007; Liu et al. 2010a; Zeng et al. 2008). Rootexuded organic acids, which are necessary in nutrient acquisition, metal detoxification, alleviation of anaerobic stress in roots, and pedogenesis, are mainly formed in the TCA cycle (Ryan et al. 2001). Studies have been conducted to identify and determine the quantity of organic acids in root exudates with low concentrations of nutrients, such as P, iron (Fe), and Zn (Abadía et al. 2002; Neumann and Römheld 1999; Nguyen et al. 2003; Qin et al. 2007). Variations in root-exuded organic acids are important mechanisms that function in response to environmental stresses (Bais et al. 2006).

Under the dual effect of Zn deficiency and excessive HCO₃⁻, organic acids highly accumulate in plant organs, and the release of root organic acids is influenced in different Zn genotypes of rice (Lee and Woolhouse 1971; Rose et al. 2011; Yang et al. 1994). Low-molecular weight organic acids (predominantly malic and citric acids) excessively accumulate in roots of Zn-deficient rice because of excessive HCO_3^{-} , whereas Zn-sufficient genotypes can exude low-molecular weight organic acids from the roots (Hajiboland et al. 2003, 2005). The amount of root-exuded citrate and malate increases after a long-term exposure to high HCO₃⁻ and low Zn concentrations; furthermore, the amount of root exudates of Zn-deficient rice is higher than that of Zn-sufficient genotypes (Hoffland et al. 2006; Rose et al. 2011; Broadley et al. 2010). Ollat et al. (2003) demonstrated that the concentrations of malic and citric acids differ in the roots of tolerant grapevine (Vitis) rootstock and in those of susceptible genotypes after 5 mM HCO₃⁻ is added under limited iron (Fe) conditions. However, few studies have established the association of organic acid levels with plant organs and root exudates. Furthermore, only a few studies have explored the effect of Zn deficiency, or excessive HCO₃⁻, or both, on the sensitivity of organic acid allocation and exudation. Thus, allocation and exudation of organic acids were influenced as plant species-specific and nutrition-specific.

Broussonetia papyrifera and Morus alba, belonging to the family Moraceae, are characterized by high growth rate with different adaptabilities to low nutrient and excessive HCO_3^- environments. Some studies have shown that the two Moraceae plants have a great adaptability to low nutrient and excessive HCO_3^- environments in the Karst area of the Yunnan-Guizhou Plateau in southwest China. They have presented some mechanism of plants adaptability to Karst such as the mechanisms of biodiversity, photosynthesis, carbonic anhydrase (Liu et al. 2010b, 2011; Wu et al. 2009, 2011; Wu and Xing 2012). However, few studies have been concerned with the mechanism of organic acids allocation and exudation.

In the present study, the characteristics of both amounts of organic acids in the organs (root, stem, and leaf) and root exudates of *B. papyrifera* and *M. alba* were examined under the effect of Zn and HCO_3^- treatments. The modes of organic acid allocation and exudation of two mulberry plants in response to Zn and HCO_3^- stress were analyzed. Furthermore, the sensitivity of organic acid allocation and exudation and exudation to Zn deficiency, excessive HCO_3^- , and dual stress, respectively, was analyzed.

2 Methods

2.1 Hydroponics experiment

Seeds of the two Moraceae plants (B. papyrifera, Bp; M. alba, Ma) were surface sterilized for 5 min with 95% ethanol and for 30 min with 10% H₂O₂. These seeds were also washed with sterile water after each treatment. Afterward, the surface-sterilized seeds were sown and grown in 30 cm \times 30 cm plastic pots for 20 days. The seedlings were transferred into a modified Hoagland nutrient solution (pH 8.0) containing (mM): KNO₃, 5.0; $Ca(NO_3)_2 \cdot 4H_2O, 4.0; NH_4NO_3, 1.0; MgSO_4 \cdot 7H_2O, 1.0;$ H₃BO₃, 0.05; MnSO₄·4H₂O, 0.004; CuSO₄·5H₂O, 0.005; $ZnSO_4 \cdot 7H_2O$, 0.01; Fe(Na)EDTA, 0.03; (NH₄)₆₋ Mo₇O₂₄·4H₂O, 0.002. After 30 days, the plants were transferred to modified Hoagland nutrient solution with four different Zn and HCO3⁻ concentrations obtained using ZnSO₄·7H₂O and sodium bicarbonate (NaHCO₃) and were grown in a controlled environment with a photosynthetic photon flux density of 300 μ mol quanta m⁻² s⁻¹ during a 14 h photoperiod, with a temperature of 25 ± 0.5 °C, and a relative humidity of $55\% \pm 2\%$. The four different treatments were prepared using the same composition of the nutrient solution with the following components: Zn-sufficiency conditions (+Zn0, with 0.02 mM ZnSO₄·7H₂O and without HCO₃⁻; and +Zn10, with 0.02 mM ZnSO₄·7H₂O and 10 mM HCO₃⁻); Zn-deficiency conditions (-Zn0, no ZnSO₄·7H₂O and no HCO_3^- ; and -Zn10, without $ZnSO_4 \cdot 7H_2O$ and with 10 mM·HCO₃⁻). Each treatment involved three plant seedlings and was performed in triplicate. The pH of all nutrient solutions was adjusted to 8.0 by using 1 M KOH before HCO₃⁻ addition. After 10 days of treatment time, root exudates were collected from the two plants under sterile conditions. The organic acids of the plant organs (roots, stems, and leaves) were analyzed. The plant organ

samples were dried at 105 °C for 30 min and at 70 °C until a constant weight was reached. Dry matter was then weighed.

2.2 Extraction of organic acids in plant organs (roots, stems, and leaves)

Organic acids were extracted from different plant organs according to a previously described method (Oliveira et al. 2008). In brief, the fresh leaves (3 g) of the two plant species were cut into small pieces, extracted with 80 mL of 80% methanol for 6 h by using a Soxhlet extractor, and filtered. Extraction and filtration were repeated twice to extract the organic acids completely. Methanolic extract was concentrated to dryness under a reduced pressure condition and at 40 °C. The concentrated extract was re-dissolved in a solution containing 0.1 mM HCl and then was stored in a freezer.

The fresh roots and stems (1 g) were ground in an ice bath with 5 mL of 0.2% meta-phosphoric acid and centrifuged for 15 min at 12500 r·min⁻¹. The residues were extracted using 4 mL of 0.2% meta-phosphoric acid and centrifuged for another 15 min at 12500 r·min⁻¹. The resulting supernatants were mixed, transferred to 10 mL polypropylene tubes, and stored in a freezer (Nisperos-Carriedo et al. 1992).

All of the obtained samples were filtered using a 0.22 μ m membrane before ion chromatography (IC) analysis was conducted.

2.3 Collection, separation, and purification of root exudates

After treatment, the plants were transferred to a 100 mL 10 mM calcium chloride (CaCl₂) solution (pH 7.0) and cultured for 6 h in sterile conditions. The solution containing root exudates was collected and passed through a cation exchange column (12 mm × 15 mm) filled with 5 g of Amberlite IR-120B resin (H⁺ form, Alfa Co.). Afterward, these exudates were passed through an anion exchange column (12 mm × 15 mm) filled with 3 g of Dowex 1 × 8 resin (100 to 200 mesh; OH⁻ form; Acros Co.). The organic acids retained on the anion exchange resin were eluted by 1 MHCl, dried using a rotary evaporator (40 °C), and stored in a refrigerator at -20 °C until analysis.

2.4 Analysis of organic acids in plant organs and root exudates

The contents and compositions of organic acids involved in citric acid (CA), malic acid (MA), oxalic acid (OA), succinic acid (SA) and tartaric acid (TA) in plant organs and root exudates were analyzed by IC (883 Basic IC plus, Metrohm, Swiss) coupled with a conductivity detector. The standards CA, MA, OA, SA and TA were obtained from SigmaAldrich-Fluka (ref. 27488, 02288, 75688, S7501, and 251380, respectively). The five kinds of organic acids were determined without requiring a regeneration solution for Metrohm suppression module (MSM). In this procedure, the following parameters were used: IC column, a Transgenomic Coregel COR-64H (7.8 mm i.d. \times 300 mm); eluent solution, 0.4 mM·H₂SO₄; flow rate, 0.6 mL·min⁻¹; and injection volume, 20 µL. The eluent solution and analyte were filtered using a 0.22 µm membrane and then ultrason-ically degassed before use.

2.5 Statistical analysis

All experiments were performed in triplicate with the same treatment independently replicated. Statistical analyses of data were carried out by t-tests, one-way 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).

3 Results

3.1 Plant organs (roots, stems, leaves) biomass

The biomass of two Moraceae plants organs varied with the plant species and the stress of Zn and HCO_3^- (Table 1). Zn deficiency decreased the biomass of roots, stems and leaves when the nutrition contained HCO_3^- while HCO_3^- treatment increased the organs growth under Zn-sufficiency conditions and decreased their growth in *Bp* and *Ma* under Zn-deficiency conditions. Under the dual treatment of Zn deficiency and excessive HCO_3^- (-Zn10), the organs' growth of two plant species was remarkably decreased.

3.2 Effect of excessive HCO₃⁻ and Zn deficiency on organic acid accumulation in plant organs

Figure 1 shows that the total amount of organic acids in the organs of *Bp* and *Ma* increased when HCO_3^- concentration increased. Under Zn-sufficiency conditions, the total amount of organic acids in the organs (roots, stems, and leaves) of *Bp* was higher than that of *Ma* exposed to +Zn10 treatment (*P* < 0.05), particularly in the leaves of the two Moraceae plants (Fig. 1). By contrast, the total amounts of organic acids in the roots, stems, and leaves of *Bp* exposed to +Zn10 treatment increased by 34.48%, 39.87%, and 96.24%, respectively (Fig. 1a); the total amounts of organic acids in the roots, stems, and leaves of *Ma* were 55.15%, 44.36%, and 47.14%, respectively (Fig. 1b). Under Zn-deficiency conditions, the total amount of organic acids in plant organs under -Zn10 treatment was higher than that under -Zn0 treatment, particularly in the

Table 1 Biomass of the two Moraceae plant organs (roots, stems, leaves) as affected by Zn and bicarbonate stress treatments

		+Zn0	+Zn10	-Zn0	-Zn10
Вр	Roots (g)	$1.280{\pm}0.092A^{\alpha}$	$1.507{\pm}0.036B^{\alpha}$	$1.171{\pm}0.073A^{\alpha}$	$0.845{\pm}0.074C^{\alpha}$
	Stems (g)	$1.088{\pm}0.044A^{\alpha}$	$1.530{\pm}0.067B^{\alpha}$	$0.948{\pm}0.063C^{\alpha}$	$0.768{\pm}0.081C^{\alpha}$
	Leaves (g)	$1.693{\pm}0.046A^{\alpha}$	$2.278{\pm}0.079B^{\alpha}$	$1.662{\pm}0.047A^{\alpha}$	$1.330{\pm}0.072C^{\alpha}$
Ма	Roots (g)	$1.065{\pm}0.068A^{\alpha}$	$1.491{\pm}0.085B^{\alpha}$	$0.932{\pm}0.094C^{\alpha}$	$0.669{\pm}0.053D^{\beta}$
	Stems (g)	$0.873{\pm}0.070A^{\alpha}$	$1.295{\pm}0.077B^{\alpha}$	$0.792{\pm}0.019A^{\beta}$	$0.480{\pm}0.037C^{\beta}$
	Leaves (g)	$1.471{\pm}0.057A^{\alpha}$	$2.149{\pm}0.006B^{\alpha}$	$1.247{\pm}0.043A^{\beta}$	$0.915{\pm}0.021C^{\beta}$

Note Values are mean \pm standard error (SE). The biomass data was shown by dried matter weight. The different small Greek letters superscript indicate the significant difference in two plant species under the same treatment and organ at *P* < 0.05; the different capital letters indicate the significant difference in four treatments under the same plant organs at *P* < 0.05



Fig. 1 Organic acid content in the organs (roots, stems, and leaves) of the two Moraceae plants exposed to excessive HCO_3^- and Zn deficiency. *Note: Blocks with bars* indicate mean \pm SE. The *different small letters* indicate the significant difference of the total content of organic acids in four treatments under the same plant organs at P < 0.05

leaves of the two Moraceae plants. The increased ratios of organic acids in the leaves of Bp and Ma that were exposed to -Zn10 treatment were 169.71% and 67.24%, respectively (Fig. 1). The total amount of organic acids did not significantly vary in the two Moraceae plants exposed to -Zn0 treatment compared with +Zn0 treatment. The total amount of organic acids significantly increased in the leaves of Bp, whereas no significant increase in the total amount of organic acids was observed in the organs of Ma exposed to -Zn10 treatment.

3.3 Effect of HCO₃⁻ and Zn deficiency on rootexuded organic acids

The amount of root-exuded organic acids increased under Zn deficiency and excessive HCO_3^- conditions; this increase varied with plant species (Fig. 2). The total amount of root-exuded organic acids in the HCO_3^- addition was higher than that in no HCO_3^- under Zn-sufficiency or Zn-deficiency

conditions. Regardless of the involvement of HCO_3^- , the total amounts of root-exuded organic acids in Zn-deficiency conditions were higher than that in Zn-sufficiency conditions; they did not significantly differ between *Bp* and *Ma* in Zn-deficiency conditions except in -Zn10 treatment (P < 0.05). In contrast, only the amount of OA in *Bp* significantly increased in +Zn10, -Zn0, and -Zn10 treatments. The increased values were 91.31%, 194.32%, and 330.14%, respectively. By comparison, the amount of CA in *Bp* increased by 57.70% and 84.84% in -Zn0 and -Zn10 treatments, respectively (Fig. 2a). The amount of organic acids in root exudations of *Ma* did not significantly vary in the treatments except in -Zn10 treatment (Fig. 2b).

3.4 Allocation of organic acids among organs

The percentage of organic acids was decreased from aboveground parts (leaf and stem) to belowground parts (root) in the two Moraceae plants exposed to the four Fig. 2 Organic acid content in the root exudates of the two Moraceae plants exposed to excessive HCO_3^- and Zn deficiency. *Note: Blocks with bars* indicate mean \pm SE. The *different small letters* indicate the significant difference of the total content of root-exuded organic acids in four treatments under the same plant species at P < 0.05



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treatments (Table 2). The allocation of organic acids in the two Moraceae plants did not differ in +Zn0 treatment. The total amount of organic acids increased in the leaves of Bp exposed to +Zn10 treatment. Additionally, OA and CA in the leaves of Bp were accounted for approximately 50% of total amount of organic acids. In -Zn0 treatment, MA and TA were allocated at a greater extent in the leaves of Bpthan in the roots and stems. SA in the roots of Bp was higher than that in the leaves and stems. The amount of organic acids in the organs of Ma did not significantly differ in +Zn10 and +Zn0 treatments. In -Zn10 treatment, OA, CA, and MA in the leaves of two Moraceae plants were higher than that of the stems or roots. The percentage of the total organic acids in the two Moraceae plants increased, particularly in the leaves of Bp exposed to -Zn10 treatment.

3.5 Correlation of organic acids in plant organs and root exudates

The correlation data involved in the Pearson correlation coefficient and P value are presented in Table 3. The allocated organic acids exhibited a good correlation between the organs and root exudates of Ma exposed to the four treatments. By contrast, the allocated organic acids in the organs did not correlate with those in the root exudates of Bp in +Zn0 treatment; the organic acids in the leaves in +Zn10 treatment and in the roots in -Zn10 treatment did not correlate with those in the root exudates at P < 0.05 (Table 3). The fitted values as expressed by the linear curves are presented in Table 4. The organic acids allocated in plant organs had significance in Ma in four treatments at P < 0.05. Except for the relationship of organic acids in roots and leaves under +Zn10 treatment and in roots and stems (leaves) under -Zn10 treatment, the

organic acids allocated in plant organs of *Bp* had significant correlation in four treatments (Table 4).

4 Discussion

4.1 Organic acid accumulation and exudation in response to Zn deficiency or excessive HCO₃⁻ concentration

Organic acids accumulated in the organs of two Moraceae plants reveal the species-specific difference under Zn deficiency or excessive HCO3⁻ stress. Zn deficiency inhibited the two plants' growth, regardless of whether or not the plant contained HCO₃⁻. Under no HCO₃⁻ conditions, Zn deficiency induced root-exuded organic acids actively in two Moraceae plants. Bp was more sensitive than Ma because the capacity of root-exuded organic acids in Bp was stronger than that in Ma, and then much more nutrition such as P, Zn and necessary microelements can be utilized by Bp under Zn deficiency (Rengel 2015; Hoffland et al. 2006). Under HCO_3^- treatments, the bioaccumulation effect of organic acids did not reduce their root activity and actually accelerated the organic acids exudation in two plants. The two Moraceae plants had a slightly resistance ability on Zn deficiency. But Bp was more adaptable than Ma under -Zn10, which was characterized by its organs accumulating a large amount of organic acids in the leaves of Bp. The toxic effect of excessive organic acid accumulation did not inhibit the growth of Bp and did not significant damage its root activity; however the amount of root-exuded organic acids (dominantly OA and CA) in Bp was higher than that in Ma. Bp preferred to detoxify bioaccumulated metals by pumping them into epidermal vacuoles and storing them there as weakly bounded by organic acids (Sun et al. 2006).

Treatments	Plant species	Organs	OA (%)	CA (%)	MA (%)	SA (%)	TA (%)	Total amount of organic acids (%)
+Zn0	Вр	Roots	31.90	33.12	28.35	29.31	28.89	30.65
		Stems	32.61	33.83	35.37	33.06	32.12	33.62
		Leaves	35.49	33.05	36.28	37.63	38.99	35.73
	Ма	Roots	33.69	30.14	31.67	27.13	27.68	30.44
		Stems	31.46	30.82	38.54	38.85	43.91	35.85
		Leaves	34.85	39.04	29.79	34.02	28.41	33.71
+Zn10	Вр	Roots	24.28	20.69	36.65	27.36	21.69	26.03
		Stems	27.95	27.05	27.44	32.43	39.39	29.70
		Leaves	47.77	52.26	35.91	40.21	38.92	44.27
	Ма	Roots	34.17	29.61	30.52	32.24	34.04	31.79
		Stems	32.27	32.40	40.39	33.31	35.19	34.83
		Leaves	33.55	37.99	29.09	34.45	30.77	33.38
-Zn0	Вр	Roots	34.28	30.73	29.68	43.67	24.76	32.44
		Stems	28.53	33.57	25.43	23.96	25.61	28.73
		Leaves	37.19	35.70	44.89	32.37	49.63	38.83
	Ма	Roots	34.69	28.58	37.61	35.29	36.66	34.21
		Stems	26.26	28.17	25.82	33.92	23.33	27.19
		Leaves	39.05	43.25	36.57	30.79	40.01	38.60
-Zn10	Вр	Roots	17.89	15.03	29.04	36.94	38.50	23.42
		Stems	18.92	28.47	19.31	32.18	28.52	24.62
		Leaves	63.19	56.50	51.66	30.88	32.98	51.97
	Ма	Roots	26.96	25.89	30.22	37.35	34.04	29.75
		Stems	26.00	31.68	30.03	31.05	32.23	29.97
		Leaves	47.03	42.42	39.75	31.61	33.73	40.28

Table 2 Allocation percentage of organic acids per plant in the organs of the two Moraceae plants exposed to four different treatments (n = 3)

Table 3 Correlation coefficient (Pearson) and *P*-value of the five types of organic acids in plant organs (roots, stems, and leaves) and in root exudates exposed to four different treatments (n = 5)

			Вр			Ма		
			Roots	Stems	Leaves	Roots	Stems	Leaves
	+Zn0	R	0.276	0.414	0.328	0.897	0.760	0.829
		Р	0.319	0.125	0.232	0.000	0.001	0.000
Root exudates	+Zn10	R	0.817	0.662	0.509	0.897	0.677	0.794
		Р	0.000	0.007	0.053	0.000	0.006	0.000
	-Zn0	R	0.744	0.712	0.706	0.737	0.856	0.672
		Р	0.001	0.003	0.003	0.002	0.000	0.006
	-Zn10	R	0.473	0.602	0.829	0.866	0.768	0.724
		Р	0.075	0.017	0.000	0.000	0.000	0.002

			E	3p	N	1a
			Roots	Stems	Roots	Stems
	Stems	R	0.954		0.790	
+Zn0		Р	0.000		0.000	
	Leaves	R	0.936	0.986	0.889	0.614
		Р	0.000	0.000	0.000	0.015
	Stems	R	0.553		0.885	
7.10		Р	0.033		0.001	
$+\Sigma m10$	Leaves	R	0.477	0.942	0.875	0.713
		Р	0.072	0.000	0.000	0.003
	Stems	R	0.927		0.660	
7.10		Р	0.000		0.007	
$+\Sigma m10$	Leaves	R	0.840	0.938	0.746	0.852
		Р	0.000	0.000	0.001	0.000
	Stems	R	0.284		0.918	
7.10		Р	0.305		0.000	
-2010	Leaves	R	0.500	0.813	0.905	0.883
		Р	0.058	0.000	0.000	0.000

The difference in organic acid responses to different Zn and HCO_3^- levels between Bp and Ma may be responsible for their different abilities in relation to Zn and bicarbonate accumulation and tolerance. Bp transferred higher amounts of OA and CA from the roots to the rhizosphere than Ma. OA and CA exhibited a stronger complexation ability than MA, SA, and TA with Ca^{2+} and Fe^{3+} to overcome nutrient deficiency in calcareous soils (Parker et al. 1995; Wang et al. 2015). OA and CA were the most active forms of carboncontaining compounds as photosynthetic intermediates translocated from aboveground parts to belowground parts in Bp (Kuzyakov and Domanski 2000). On the other hand, Bp exhibited a greater HCO_3^- use capacity than Ma, resulting in a decrease in the toxicity of HCO_3^- (Zhao and Wu 2017). Furthermore, inorganic carbon accumulation increased in Bp leaves and promoted photosynthesis of Bp (Wu and Xing 2012; Wu et al. 2009). HCO_3^- treatment promoted the two plants' growth and organic acids accumulation in two plants organs under Zn-sufficiency conditions. While their root organic acid secretion did not significantly improve, the illustrated HCO₃⁻ use capacity was enhanced in two Moraceae plants, and Zn was a necessary element in influencing plant growth and development under natural conditions.

4.2 Production and translocation of organic acids

Organic acids in plants are mainly produced in the mitochondria via the TCA cycle in dark respiration and in

the glyoxysome as the intermediates of the glyoxylate cycle in photorespiration (López-Bucio et al. 2000; Haichar et al. 2014). Organic acids in plants are originally from photosynthetically fixed carbon (Jones 1998). Leaves are the main organs involved in photosynthesis, and stems are the secondary organs involved in the same process. The amount of organic acids decreased from aboveground (leaf and stem) parts to belowground (root and rhizosphere) parts, and this can be interpreted to mean that root-exuded organic acids are likely derived from the leaves because these plant organs are the main parts involved in photosynthesis and the site of the primary metabolism of organic compounds (Hohmann-Marriott and Blankenship 2011; Jones et al. 2009). Organic compounds were transferred from aboveground parts (leaf) to belowground parts (root) via stem. The variation of organic acids in the organs was similar to that in the root exudates of the two Moraceae plants. The leaves were the main organ in which organic acids were accumulated and allocated. The organic acids that accumulated in the leaves were involved in TCA cycle and glyoxylate cycle; these acids were primarily produced during photosynthesis (Baetz and Martinoia 2013). Organic acids were transferred from the leaves to the root. The composition and concentration of organic acids in the roots and root exudates also changed. The organic acid contribution of dark respiration to photorespiration increased from the leaves and stems to the root.

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Therefore, the sources of organic acids differ in the leaves and the stems. In particular, organic acids in the leaves may have been derived from dark respiration and photorespiration. By comparison, organic acids in the stems, roots, and root exudates may have been derived from dark respiration and organic acid translocation from the leaves.

5 Conclusions

The allocated and exuded organic acids in plant organs and root exudates varied with species and environmental stresses. Root-exuded organic acids may have been originally derived from the leaves. The sources of organic acids differed in the leaves, stems, root, and root exudates. Leaves were the main organ involved in organic acid production. Organic acid accumulation, translocation, and exudation in the plant-soil system were influenced by Zn deficiency, excessive HCO3⁻, and both, respectively. An increase in organic acid in the leaves resulted in an increase in root-exuded organic acids. OA and CA were the key intermediates influencing organic acid translocation and exudation; they regulated nutrient deficiency in the rhizosphere of Bp under excessive HCO_3^- conditions. The results and information generated in this study are valuable for future effective configuration of species diversity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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