

TEMPORAL AND SPATIAL DISTRIBUTION CHARACTERISTICS OF RHIZOSPHERE ORGANIC ACIDS UNDER WATER LEVEL FLUCTUATIONS IN THREE TYPES OF LAKES IN CHINA

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Abstract. Organic acids are influenced by many factors, such as season variations, water levels, vegetation and others. In the study, the rhizosphere soils of lakeshore plants in Poyang Lake, Chaohu Lake and Wuchang Lake in China were sampled under different water level fluctuations, and the amounts of organic acids were measured. Water level fluctuations affect the temporal and spatial characteristics of rhizosphere organic acids. The annual average of the total amount of organic acids in the rhizosphere soil of lakeshore plants in Wuchang Lake was higher than that in the other lakes. The seasonal variations in the amounts of organic acids were the same in the three lakes. The total amounts of organic acids in the rhizosphere soils at high water level sites were higher than those in the low water level sites of the three lakes. The effects of the interactions of season variations and water level fluctuations on the amount of organic acids in the rhizosphere were significant. The results revealed that the lake types changed in response to anthropogenic influence; Wuchang Lake, as a quasi-natural lake, suffered from much more human pollutants than Chaohu Lake as a reservoir-like lake and Poyang Lake as an intermittent lake.

Keywords: *rhizosphere soils, lake type, lakeshore, season variations*

Abbreviations: ANOVA, Analysis of variance; CA, Citric acid; CHL, Chaohu lake; MA, Malic acid; MC, Moisture content; OA, Oxalic acid; PYL, Poyanghu lake; SA, Succinic acid; SOM, Soil organic matter; TA, Tartaric acid; TN, Total nitrogen; TP, Total phosphorus; ToA, Total amount of organic acids; WCL, Wuchanghu lake

Introduction

A suitable water level is an important factor for maintaining the structure, function and biological integrity of lake ecosystems (Coops et al., 2003; Wantzen et al., 2008; Leira and Cantonati, 2008). Water level fluctuations are defined as the differences or changes in the water level within a period of time and are influenced by many factors, such as the water storage capacity, rainfall, and human factors involved in dam, highway and railway construction (Beckmann et al., 2005; Wang et al., 2007). Water level fluctuations have an important influence on the diversity of aquatic organisms; many studies have shown the effect of water level fluctuations on the digestion and retention of pollutants, the stabilization of the lakeshore, the maintenance of the water self-purification capacity, and the support of regional biological communities (Naiman and Décamps, 1997; Venkatachalam et al., 2005). For instance, water level fluctuations were found to influence the mobilization and transformation of nutrients such as nitrogen, phosphorus

and chlorophyll a, as well as the diversity of phytoplankton and zooplankton (Sherr et al., 2003; Zhang et al., 2013; Liu et al., 2018). However, few studies have focused on the relationship between water level fluctuations and the root-soil environment, especially the effect of water level fluctuations on organic acids in rhizosphere soil.

Low molecular weight organic acids, such as citric acid, oxalic acid, and malic acid, are three of the most active forms of carbon in the plant-rhizosphere-soil interface and have great importance in physiological and biochemical processes, such as microbiological activity, nutrient acquisition, and the detoxification of heavy metals (Bais et al., 2006; Keiluweit et al., 2015; Chen et al., 2017). Numerous studies have shown that the allocation, transformation and exudation characteristics of organic acids are important mechanisms in response to environmental stresses. For instance, the roots of dicotyledonous species such as lupine (*Lupinus albus* L.), rape (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.) exuded large amounts of organic acids under phosphorus deficiency; this organic acid mobilizes the insoluble phosphorus compound in rhizosphere soils, which increases the release of inorganic phosphorus and promotes phosphorus uptake (Haichar et al., 2014; Zhao and Wu, 2014; Baetz and Martinoia, 2014). Lakeshore plant species such as umbrella sedge and *Pistia stratiotes* adapt to phosphorus deficient environments via the regulation of the composition and amount of root-exuded organic acids (Zhang et al., 2019a,b). The roots of three lakeshore plants, *Canna indica*, *Zizania aquatic* and *S. warburgii* Seemen exude many organic compounds, mainly low molecular weight organic acids and aromatic proteins in root exudations; furthermore, the amount of such exudates was found to have a positive correlation with the biomass of the three plants (Lu et al., 2009). The mangrove *Kandelia candel* exudes high amounts of formic acid, butyric acid, malic acid, citric acid and lactic acid, and these low molecular weight organic acids increased the release of available heavy metals in wetland sediments (Lu et al., 2007). The composition and amount of organic acids in rhizosphere soils via root exudation can directly influence many biological processes in the rhizosphere-soil-plant interface under environmental stresses (López-Bucio et al., 2000; Zhao and Wu, 2018). Therefore, investigation of the composition and amount of organic acids in rhizosphere soils of lakeshore plants will provide important information on nutrient acquisition, heavy metal detoxification and plant growth.

In the present study, the composition and amount of organic acids under different water level fluctuations in different seasons were investigated in the rhizosphere soils of lakeshore plants in three different lakes in the lower-middle reaches of the Yangtze River basin of China. Furthermore, the effects of the physicochemical parameters of the rhizosphere soils on the amounts of organic acids were analyzed, and the responses of organic acids in different lakes under different water level fluctuations were investigated.

Materials and Methods

Study areas

Three lakes, Poyang Lake (PYL), Chaohu Lake (CHL) and Wuchang Lake (WCL), in the middle and lower reaches of the Yangtze River that are affected by human disturbance were investigated (Fig. 1). PYL is located in Jiangxi Province, and CHL and WCL are located in Anhui Province. The three lakes are at relatively low elevations. CHL and WCL are regulated by the Yuxi and Wanhe sluices, which were constructed as a consequence of human disturbance, and the water surface areas of PYL have been greatly reduced. The climate of three lakes is a typical subtropical monsoon climate characterized by hot and

rainy summers and cold and dry winters. The high temperature period is concentrated in July and August, the rainy season is from April to June, and the cold season occurs in January and February. The annual precipitation and annual air temperature at PYL, CHL and WCL are 1600.0 mm, 995.7 mm and 1299.6 mm, and 17.2 °C, 16.1 °C and 16.5 °C, respectively (Zhang, 2013; Zhang et al., 2018).

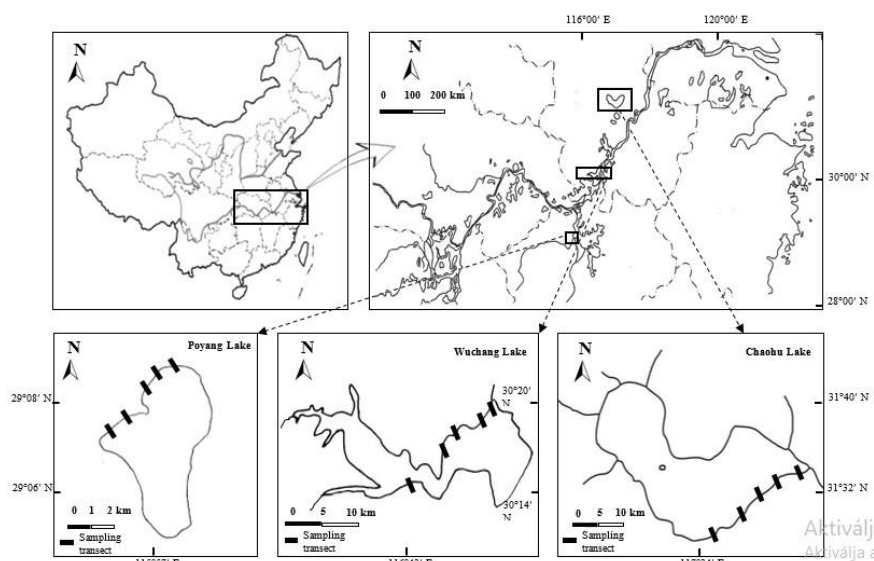


Figure 1. Study area and soil sampling sites of three lakes

PYL with a mean bottom elevation is 14.67 m amsl, and the water levels are low and stable with a mean water depth of less than 1.0 m. The mean bottom elevation of CHL is 5.0-6.0 m above mean sea level (amsl), and the water level is often maintained at 7.5-8.0 m amsl. The annual average water level of CHL was raised about 1.5 m after the building of the sluice (Zhang et al., 2014). The mean bottom elevation of WCL is 10.0 m amsl, and the water level is often controlled at 11.5 m amsl. The main uses of WCL are for aquaculture and flood control (Zhang et al., 2016). The other limnological parameters of the three lakes are shown in *Table 1*.

Table 1. Limnological parameters of the three lakes. Note: Data are from Wang and Dou (1998), Zhang (2013), and Zhang et al. (2018)

	Poyang Lake	Chaohu Lake	Wuchang Lake
Area (km ²)	30	769.6	100.5
Mean water depth (m)	0.70	2.69	3.43
Maximum water depth (m)	4.43	3.77	4.31
Annual precipitation (mm)	1600	995.7	1299.6
Annual air temperature (°C)	17.2	16.1	16.5
Recharge coefficient of lake	-	12.0	10.8
Catchment area (km ²)	-	9258	1083.7
Volume (m ³)	-	20.7×10 ⁸	3.45×10 ⁸

The floristic composition along the shores of the three lakes are significantly different (Zhang et al., 2015, 2016). The vegetations along the shores of CHL and PYL are relatively simple, and the dominant species of the lakeside zone near CHL is mainly

Cynodon dactylon, while *Carex sp.* is the dominant species in PYL. The floristic composition along the shore of WCL are relatively diverse, with *Cynodon dactylon* and *Carex sp.* covering some of the area, and the wild emergent aquatic plant species *Zizania latifolia* having a large distribution area.

Rhizosphere soil sampling

The rhizosphere soils of the lakeshore plants around lakes that exhibited three different types of fluctuations were investigated in the summer of 2016 (August, Summer), the autumn of 2016 (November, Autumn), the winter of 2017 (February, Winter) and the spring of 2017 (May, Spring). The main vegetation that grew in the soil samples were *Cynodon dactylon* in CHL, *Carex sp.* in PYL, and *Zizania latifolia* in WCL. The sections with limited artificial interference were selected in the three lakes, the five typical sample points were evenly distributed throughout this section, ranging from the lowest perennial average water level to the highest perennial average water level of the lake, which were called 1, 2, 3, 4 and 5 successively. The distance between two adjacent sample points remained equal. According to the water level fluctuations of the three lake types in the normal hydrological year 2015, water levels in these lakes are higher in winter and spring, and low in late spring to early summer, which is opposite to the natural condition (Zhang et al., 2018). Therefore, the elevations of the sample points in PYL were 14.75 m (PYL1), 15.56 m (PYL2), 16.38 m (PYL3), 17.19 m (PYL4) and 18.00 m (PYL5). The elevations of the five sample points in CHL were 8.15 m (CHL1), 8.61 m (CHL2), 9.07 m (CHL3), 9.52 m (CHL4) and 9.98 m (CHL5). The elevations of the sample points in WCL were 10.82 m (WCL1), 11.52 m (WCL2), 12.22 m (WCL3), 12.91 m (WCL4) and 13.61 m (WCL5).

The rhizosphere soil samples were randomly collected in triplicate in the three different lakes owing to the obvious lakeshore plant layer. The soil samples were collected always at the same site, the sampling sites of WCL, CHL and PYL were shown in *Figure 1*.

The soils within 0.5 cm from the root system were collected after excavating the roots and the aboveground parts of plants following the method described by Gollany et al. (1997), and the rhizosphere soil samples were divided into two parts.

One part was stored at -24 °C as fresh soil. The other part was dried and sieved through 2 mm mesh, and the soil physicochemical parameters, such as pH, moisture content (MC), soil organic matter (SOM), total nitrogen (TN) and total phosphorus (TP), were determined. The pH and MC were measured by the potentiometric method and the oven-drying method, respectively; SOM was measured by the K₂Cr₂O₇ titration method; the TN and TP contents were measured using the Kjeldahl method and molybdenum blue colorimetry, respectively (Lu, 2000).

Extraction and purification of organic acids in rhizosphere soils

A total of 5 g of fresh rhizosphere soil samples with the root chips, residues and others large particulate matter removed were placed in a 50 mL Erlenmeyer flask, and 25 mL 0.1% H₃PO₄ extracted solution was added. The soils and the extracted solution were mixed with a glass rod, the mixed soil solution was oscillated for 2 h; then, the mixture was transferred to a 50 mL centrifuge tube. The tube underwent centrifugation at 10000 r min⁻¹ for 10 min; then, the supernatant was collected and passed through a 0.22 µm water phase filtration membrane.

The solutions containing organic acids were 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 solutions were passed through an anion exchange column (12 mm × 15 mm) filled with 3 g of Dowex 1 × 8 resin (100 mesh to 200 mesh; OH⁻ form; Acros Co.). The solutions were evaporated under rotary conditions of 40 °C after steaming to nearly dry, and the residue was obtained in 1 mL water for a quick rhizosphere soil sample test.

Determination of the amounts of organic acids in rhizosphere soils

The amounts of organic acids, including oxalic acid (OA), citric acid (CA), malic acid (MA), tartaric acid (TA) and succinic acid (SA), in the rhizosphere soils of the three lakes were analyzed by Waters Acquity H-Class ultra-performance liquid chromatography coupled with a four element pump system, automatic injection system, ultraviolet detector system, solvent system and chromatography column system (Zhao et al., 2019). The standard samples of OA, CA, MA, TA and SA were obtained from Sigma-Aldrich-Fluka (ref. 02288, 27488, 75688, 251380, and S7501, respectively). The concrete analytical conditions were the following: chromatography column, BEH C18 column (2.1 mm i.d. × 500 mm, 1.7 µm); eluent solution, 10 mM KH₂PO₄ solution (adjust pH to 2.20±0.10 by 1 mol L⁻¹ H₃PO₄ solution); flow rate, 0.30 mL min⁻¹; temperature of the chromatography column, 30 °C and injection volume, 1 µL. The eluent solution and analyte were filtered using a 0.22 µm membrane and then ultrasonically degassed before use.

Statistical analysis

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

Results

Soil physicochemical parameters

The pH values were significantly different among the three lakes in the same season; the difference in MC was not significant in winter but exhibited significant differences in the other three seasons; SOM, TN and TP were not significantly different, except for SOM and TN, which were higher in PYL than in WCL and CHL in spring (*Table 2*). MC was not significantly different in the four seasons in WCL, while the other soil parameters were different (*Table 2*). The pH in autumn and winter was lower than that in spring and summer, while MC, SOM, TN and TP were not significantly different in CHL and PYL (*Table 2*).

The total amount of organic acids in the rhizosphere soil of lakeshore plants in different seasons

The total amount of organic acids (ToA), including OA, CA, MA TA and SA, in the rhizosphere soil of the lakeshore plants in winter was lower than that in the other three seasons in the three lakes, which was higher in summer than in the other three seasons in

CHL and PYL and was highest in WCL (Fig. 2). The ToA in WCL was significantly higher than that in CHL and PYL during the same season (Fig. 2). The annual averages of the total amounts of organic acids in WCL, CHL and PYL were 2.783 mg kg⁻¹, 1.668 mg kg⁻¹, and 1.954 mg kg⁻¹, respectively.

Table 2. The soil parameters at the sampling sites in Wuchanghu Lake, Chaohu Lake and Poyang Lake

Lake name	Soil parameters	Season			
		Spring	Summer	Autumn	Winter
Wuchang Lake	pH	7.22±0.80Aa	7.37±0.30Aa	6.05±0.18Ab	5.800±0.30Ab
	MC (%)	22.13±4.88Aa	24.41±4.60Aa	26.06±5.04Aa	29.47±18.29Aa
	SOM (mg kg ⁻¹)	1.03±0.22Aa	1.01±0.48Aa	1.63±0.58Aab	2.57±1.93Ab
	TN (mg kg ⁻¹)	0.72±0.11Aa	0.62±0.26Aa	1.00±0.30Aab	1.65±0.88Ab
	TP (mg kg ⁻¹)	0.44±0.09Aa	0.33±0.09Ab	0.32±0.05Ab	0.42±0.08Aab
Chaohu Lake	pH	8.68±0.20Ba	8.29±0.15Bb	7.58±0.05Bc	7.68±0.11Bc
	MC (%)	18.67±4.16Aa	21.13±4.07Aa	18.31±5.46Ba	16.60±2.84Aa
	SOM (mg kg ⁻¹)	0.84±0.34Aa	1.96±1.54Aa	1.08±0.81Aa	1.44±1.28Aa
	TN (mg kg ⁻¹)	0.54±0.16Aa	1.14±0.72Aa	0.80±0.48Aa	0.97±0.84Aa
	TP (mg kg ⁻¹)	0.58±0.24Aa	1.12±1.19Aa	0.69±0.51Aa	1.16±1.18Aa
Poyang Lake	pH	5.65±0.12Ca	6.06±0.62Ca	4.90±0.14Cb	4.68±0.19Cb
	MC (%)	28.56±4.52Ba	30.73±4.07Ba	28.57±3.55Aa	27.55±4.98Aa
	SOM (mg kg ⁻¹)	1.46±0.40Ba	1.65±0.44Aa	1.74±0.62Aa	1.88±0.12Aa
	TN (mg kg ⁻¹)	1.05±0.26Ba	1.17±0.22Aa	1.13±0.38Aa	1.3±0.17Aa
	TP (mg kg ⁻¹)	0.39±0.09Aa	0.37±0.06Aa	0.38±0.06Aa	0.38±0.06Aa

Values are means ± SE (n=3). The different capital letters indicate a significant difference in the three lakes in the same season at p<0.05. The different lowercase letters indicate a significant difference in the four seasons at the same lake at p<0.05. MC: moisture content; SOM: soil organic matter; TN: total nitrogen; TP: total phosphorus

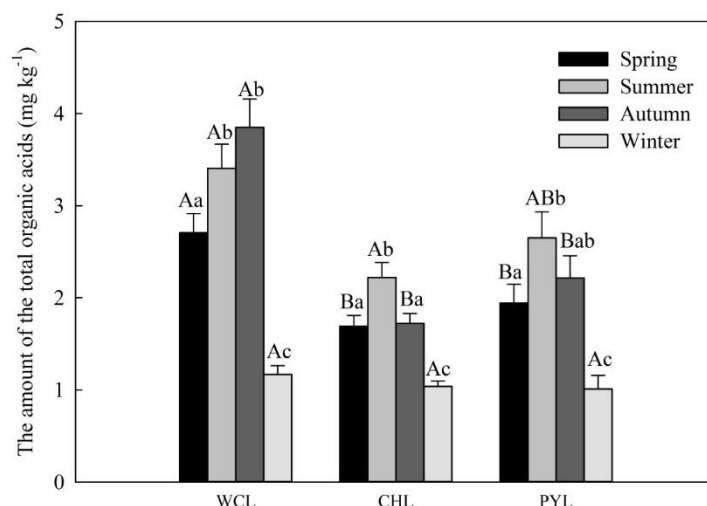


Figure 2. The variations in the total amount of organic acids in the rhizosphere soil of lakeshore plants in three lakes in different seasons. Note: Columns with bars indicate means ± SD (n= 12). The different capital letters indicate a significant difference in the three lakes in the same season at p<0.05. The different lowercase letters indicate a significant difference in the four seasons under the same lake at p<0.05. PYL: Poyanghu Lake; CHL: Chaohu Lake; WCL: Wuchanghu Lake

The effect of water level fluctuations on the characteristics of the ToA in the rhizosphere soil in different seasons

The seasonal variations in organic acids in the rhizosphere soil were significant at the different water levels in the three lakes, as shown in Fig. 3. At the different water level fluctuations, the ToA was between 0.607 mg kg⁻¹ and 6.817 mg kg⁻¹ in WCL, which was highest in summer, and lowest in winter, and the ToA at high water levels, such as at WCL3, WCL4, and WCL5, was higher than that at low water levels, such as WCL1 and WCL2 (Fig. 3). At the different water level fluctuations, the ToA was between 0.712 mg kg⁻¹ and 3.798 mg kg⁻¹ in CHL. The ToA had was not significantly different in the sites with low water level fluctuations, including CHL1 and CHL2, in the different seasons; however, the ToA values were higher at the sites with high water level fluctuations, including CHL4 and CHL5. The ToA was the maximum at CHL4, which was two-fold higher than that at CHL1 in the all seasons except winter (Fig. 3). The ToA in the sites with different water level fluctuations in PYL was between 0.410 mg kg⁻¹ and 3.909 mg kg⁻¹. The seasonal variations in the amount of organic acids were the following: summer > autumn > spring > winter. The ToA at PYL4 was higher than that at the other sites.

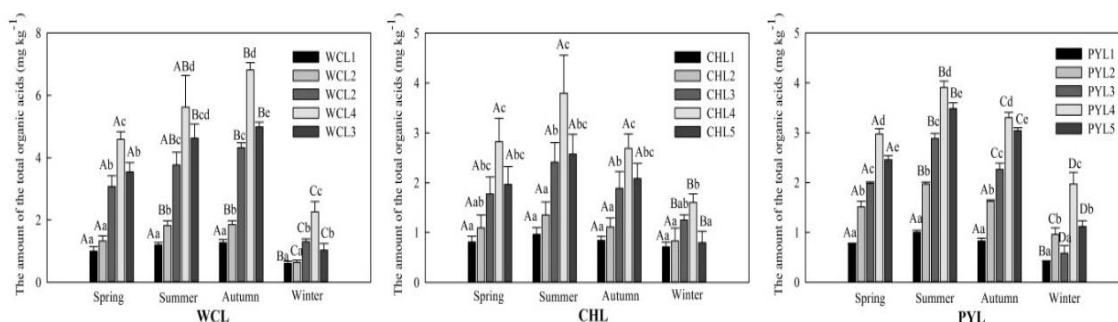


Figure 3. The effect of water level fluctuations on the characteristics of the total amounts of organic acids in rhizosphere soil in different seasons in the three lakes. Note: Columns with bars indicate means $\pm$ SD ($n=3$). The different capital letters indicate a significant difference in the four seasons under the same water level fluctuations in the same lakes at $p<0.05$. The different lowercase letters indicate a significant difference in the water level fluctuations in the same season in the same lakes at $p<0.05$. PYL: Poyanghu Lake; CHL: Chaohu Lake; WCL: Wuchanghu Lake

The proportions of the five organic acids in the rhizosphere in different seasons in the three lakes

The contributions of OA, CA, MA, TA and SA to the ToA in the three lakes differed in the different seasons. The proportions of the five organic acids varied with season in WCL, and the proportion of CA increased, which accounted for 40% in winter (Fig. 4). The dominant organic acids were CA and MA in CHL, and the proportion of CA and MA was over 60% in all four seasons (Fig. 4). CA, MA and SA were the dominant organic acids in the rhizosphere soils of PYL, accounting for more than 75% of the ToA, especially more than 90% in winter (Fig. 4).

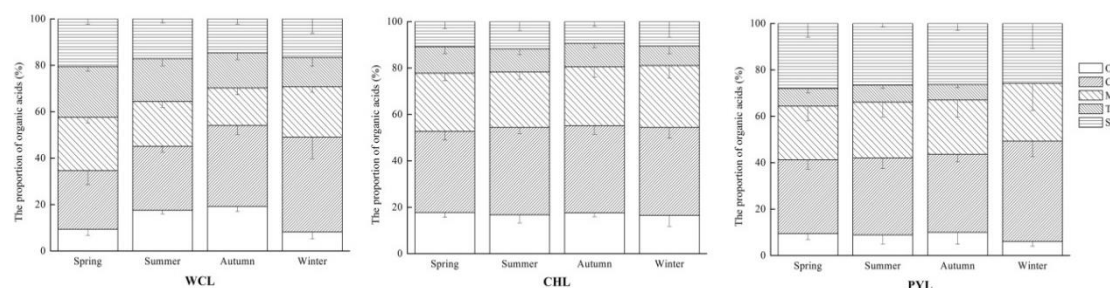


Figure 4. The proportions of the five organic acids in rhizosphere soil in different seasons in the three lakes. Note: Columns with bars indicate means $\pm$ SD ($n = 3$). PYL: Poyanghu Lake; CHL: Chaohu Lake; WCL: Wuchanghu Lake; OA: oxalic acid; CA: citric acid; MA: malic acid; TA: tartaric acid; SA: succinic acid

The interaction of seasonal variation and water level fluctuations with the characteristics of organic acids in rhizosphere soil

The effect of the interaction of the seasonal variation and water level fluctuations on the characteristics of the amounts of five organic acids and the ToA in rhizosphere soil of lakeshore plants in three lakes was significantly different based on the two-way ANOVA. The mean square value was between 0.002 and 4.839, and the F value was between 5.602 and 123.355 in the three lakes ($p < 0.001$) (Table 3). The mean square value or F value of ToA in WCL was higher than that in PYL and CHL, which indicated the effect of interaction of seasonal variations and water level fluctuations on WCL was stronger than that on other two lakes (Table 3).

Table 3. The interaction of seasonal variations and water level fluctuations on the characteristics of organic acids in rhizosphere soil of lakeshore plants based on two-way ANOVA

Lake name	Organic acids	Degree	Mean square (MS)	F	Significance (p value)
Wuchanghu Lake	OA	12	0.263	123.355	<0.001
	CA	12	0.649	29.793	<0.001
	MA	12	0.115	23.254	<0.001
	TA	12	0.276	16.617	<0.001
	SA	12	0.180	15.361	<0.001
	ToA	12	4.839	47.289	<0.001
Chaohu Lake	OA	12	0.069	5.800	<0.001
	CA	12	0.175	5.129	<0.001
	MA	12	0.073	14.791	<0.001
	TA	12	0.013	5.863	<0.001
	SA	12	0.019	5.602	<0.001
	ToA	12	1.090	10.744	<0.001
Poyanghu Lake	OA	8	0.002	13.366	<0.001
	CA	8	0.035	17.620	<0.001
	MA	8	0.065	30.201	<0.001
	TA	8	0.002	7.860	<0.001
	SA	8	0.022	10.523	<0.001
	ToA	8	0.100	13.622	<0.001

OA: oxalic acid; CA: citric acid; MA: malic acid; TA: tartaric acid; SA: succinic acid; ToA: total amount of organic acids

The correlations between the amounts of organic acids and environmental factors in the three lakes

Table 4 shows the correlations between the amounts of organic acids and physicochemical parameters of rhizosphere soils such as pH, MC, SOM, TN and TP in the three lakes. The amount of one organic acid exhibited a linear correlation with the other organic acids and the ToA. The Pearson correlation coefficient (R) ranged from 0.502 to 0.963 in the rhizosphere soils of lakeshore plants in the three lakes ($p < 0.01$). The soil parameters had different influences on the amount of organic acids in the rhizosphere soils of lakeshore plants in the three different lakes. There were no significant differences between the amounts of five organic acids or the ToA and MC, SOM, TN and TP, except for the significant difference between TN and OA ($p < 0.05$). There were highly negative linear correlations between the individual organic acids or the ToA and pH ($p < 0.01$), except for the nonsignificant difference between TA and pH ($p > 0.05$) in PYL. In CHL, the soil physicochemical parameters included pH, MC and SOM, and the individual organic acids or the ToA exhibited significant differences. The differences between the individual organic acids or the ToA and TN were highly significant ($p < 0.01$), except for the nonsignificant difference between TA or SA and TN ($p > 0.05$). Meanwhile, there was no significant difference between the individual organic acids or the ToA and TP ($p > 0.05$), except for the highly significant differences between OA and TP ($p < 0.01$). In WCL, the soil physicochemical parameters, including MC, SOM, TN and TP, exhibited a negative linear correlation with the individual organic acids or the ToA ($p < 0.01$), while pH had an influence on the amounts of MA, TA and SA.

Table 4. The Pearson correlation coefficients between the amounts of organic acids and soil physicochemical parameters of rhizosphere soils in Poyang Lake, Chaohu Lake and Wuchang Lake

		CA	MA	TA	SA	ToA	pH	MC	SOM	TN	TP
Poyang Lake	OA	0.739**	0.722**	0.904**	0.769**	0.827**	-0.502**	0.075	-0.219	-0.290*	-0.106
	CA	1	0.864**	0.837**	0.892**	0.959**	-0.381**	-0.015	0.086	-0.096	-0.014
	MA		1	0.818**	0.841**	0.942**	-0.372**	-0.012	-0.059	-0.242	0.020
	TA			1	0.749**	0.877**	-0.270	0.006	0.111	-0.017	-0.031
	SA				1	0.951**	-0.513**	0.068	0.009	-0.164	-0.046
	ToA					1	-0.465**	0.026	-0.017	-0.195	-0.019
Chaohu Lake	OA	0.822**	0.757**	0.636**	0.526**	0.895**	-0.278**	0.230**	0.458**	0.375**	0.205**
	CA	1	0.853**	0.619**	0.578**	0.963**	-0.287**	0.341**	0.427**	0.314**	0.124
	MA		1	0.620**	0.502**	0.923**	-0.269**	0.386**	0.460**	0.331**	0.096
	TA			1	0.508**	0.720**	-0.403**	0.452**	0.208**	0.127	-0.109
	SA				1	0.649**	-0.337**	0.430**	0.154*	0.028	-0.106
	ToA					1	-0.330**	0.390**	0.449**	0.325**	0.105
Wuchang Lake	OA	0.827**	0.693**	0.788**	0.760**	0.881**	0.013	-0.209**	-0.228**	-0.332**	-0.557**
	CA	1	0.800**	0.827**	0.822**	0.947**	-0.135	-0.357**	-0.180*	-0.235**	-0.477**
	MA		1	0.913**	0.878**	0.913**	-0.196**	-0.457**	-0.342**	-0.415**	-0.337**
	TA			1	0.890**	0.946**	-0.185*	-0.367**	-0.317**	-0.416**	-0.388**
	SA				1	0.929**	-0.157*	-0.373**	-0.295**	-0.376**	-0.372**
	ToA					1	0.053	-0.379**	-0.278**	-0.363**	-0.469**

OA: oxalic acid; CA: citric acid; MA: malic acid; TA: tartaric acid; SA: succinic acid; ToA: total amount of organic acids; MC, soil moisture content; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus. ** indicates a significant difference at $p < 0.01$; * indicates a significant difference at $p < 0.05$

Discussion

The influence of lake types on the amount of organic acids

According to the different water level fluctuation patterns, the lakes in this study area could be divided into three types (Zhang, 2013). WCL is a quasi-natural fluctuating lake, which includes most lakes and some barrier lakes, and the water level fluctuations are similar to those of the Yangtze River. CHL is a reservoir-like fluctuating lake, which is mainly obstructed by other lakes and maintains a high water level all year round. The water level decreases due to irrigation or flood storage from April to June. PYL is an intermittent fluctuating lake, which is similar to floodplains and maintains stable low water levels most of the time, except in the high water level period at the Yangtze River trunk stream.

The composition and content of organic acids in the rhizosphere soil of the different lakes were different, and the annual average values of the ToA varied greatly. The annual average ToA in WCL was higher than that in PYL and CHL, which indicated that the carbon storage capacity in the rhizosphere soil of the quasi-natural lake wetland was higher than that in the intermittent lake and reservoir-like lake. The amount of organic acids in rhizosphere soils responded to the carbon storage and transformation capacities (Jones et al., 2009; Kuzyakov and Razavi, 2019). Due to the limited variations in the water level fluctuations and limited anthropogenic impacts, the carbon storage capacity in the rhizosphere soils of the WCL was higher than that in the CHL and PYL. CHL, as a reservoir-like lake, has a large amount of runoff, and the water that is stored in the lake is not enough to meet the demands because of the large amount of industrial and agricultural water consumption in the region, leading to the loss of carbon in rhizosphere soils. Hence, the annual average organic acid values were low in this lake. Furthermore, the dominant plant species and their distribution in the three lakes influenced the amount of organic acid in the rhizosphere. *Zizania latifolia*, as the dominant species in WCL, had the maximum biomass; *Carex sp.*, as the dominant species in PYL had intermediate biomass, and the biomass of *Cynodon dactylon*, as the dominant species in CHL, was minimal (Zhang et al., 2018). The photosynthetically fixed carbon increased as the biomasses of these annual or biennial herbaceous plants increased. The carbon transformation increased from the aboveground parts (leaf, stem) to the belowground parts (root), resulting in an increase in root-exuded organic acids in the rhizosphere soils, which explained the higher ToA in the rhizosphere of quasi-natural lakes such as WCL than in the other two types of lakes.

The variational characteristics of the amount of organic acids under water level fluctuations in four seasons

The effects of seasonal variations on the amount of organic acids in the rhizosphere soil were evident, and the effects were irrelevant of the lake type. The amounts of organic acids were higher in summer and autumn than in spring and winter in the three lakes, resulting from the rapid growth and strong photosynthesis of wetland plants in summer and autumn, when plant roots exuded much more organic acids to rhizosphere soils. Due to the weak photosynthesis and low water level, the organic acid storage in the rhizosphere soil was low in winter (Wu and Zhao, 2013).

The amounts of organic acids at the sites with different water level fluctuations were different, and these were not related to the lake type. The amounts of ToA were higher at the sites with high water level fluctuations than that with low water level fluctuations.

These differences not only were relevant to the above-mentioned factors, such as photosynthesis and biomass, but also may be related to other factors, such as the soil sample location and root morphology. Previous studies have shown that the root morphological parameters such as root length, root density, root volume, and root surface area at the sites with low water fluctuations were lower than those at sites with moderate and high water fluctuation levels (Zhang et al., 2018). These findings were consistent with the variation characteristics of organic acids in rhizosphere soils in this study. The root morphological parameters were positively correlated with the amount of organic acids in the rhizosphere soils of lakeshore plants. When the root morphological parameters were high, much more root organic acids were exuded into the rhizosphere soils.

The differences in dominant organic acids

The dominant types of organic acid were different in the three lakes. These were CA, MA and SA in PYL and CA and MA in CHL. In WCL, the distributions of the five organic acid contents were average, and only the CA content was higher in the high water level area. CA is the dominant organic acid among the three lakes in our study, which is the main organic acid in the Krebs cycle, not only accumulates in plant organs and tissue, but also is released into the rhizosphere via root exudates (Sweetlove et al., 2010; Araújo et al., 2012; Yu et al., 2017).

The correlation between the amount of organic acids and the soil physicochemical parameters

There were significant positive correlations among the amounts of organic acids in the three lakes, which indicated that a single organic acid was not exuded into soils, while multiple organic acids exuded simultaneously into the rhizosphere soil through the plasma membrane via diffusion and anion channels (Ryan et al., 2001; Adeleke et al., 2017; Silva and Lambers, 2020). There was a negative correlation between the amount of organic acids and pH, which indicated that an increase in the amount of organic acids led to an increase in H⁺ release, decreasing the pH; however, this condition did not change the adsorption sites. MC and SOM had the same influences on the amount of organic acids, which included no influence on the amount of organic acids in PYL, a positive influence in CHL, and a negative influence in CHL because of the different heights of the water level fluctuations. TN and TP, as indicators of pollution sources, had limited impacts on the release of organic acids in PYL and CHL because the two lakes were greatly affected by human activities and exhibited low release of organic acids. TN and TP exhibited significant negative correlations with the amount of organic acids in the rhizosphere soil of WCL, which indicated that an increase in the exogenous input of nitrogen and phosphorus decreased the amount of organic acids in the rhizosphere soil. Thus, WCL, as a quasi-natural lake, was greatly influenced by human activities, and this lake may be converted to another type of lake and lose the function of the natural wetlands.

Conclusion

The organic acids of rhizosphere soil in different types of lakes were influenced by many factors, such as season variations, water levels, vegetations and the soil

environment. The annual average amount of the total organic acids in the rhizosphere soil in the quasi-natural WCL was higher than that in the intermittent PYL and reservoir-like CHL, which was related to the vegetation density, photosynthesis and root morphological parameters of WCL. The seasonal differences in the amounts of organic acids were independent of lake type, and the amount of organic acids in the three lakes in winter was lower than that in the other three seasons. The dominant organic acids were different, and CA was the dominant organic acid in the three lakes. The CA accumulation in plant tissue can promote the release into rhizosphere soil. There were positive correlations among the amounts of organic acids in the three lakes. pH exhibited a negative correlation with the amount of organic acids. MC and SOM had no effect on the amount of organic acids in PYL, a positive effect in CHL and a negative effect in WCL. TN and TP had a greater impact on the amount of organic acids in WCL than in PYL and CHL, which would lead to changes to WCL as a result of anthropogenic influence.

In future studies, it will be necessary to consider the following two points: first, quantitatively analyze the organic acids characteristics in plant-rhizosphere soil- aquatic environment, the carbon distribution and transfer of organic acids should be focused via stable carbon isotope tracer technology. Second, in order to better understand the function of organic acid in plants especially root-exuded organic acids, the heavy metal concentration, nutrition such as phosphorus and nitrogen, and microbial activities should be investigated in rhizosphere soil-aquatic environment, and discuss the influence of anthropogenic intake pollution source.

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