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Full Length Article

Effects of Phosphorus Stress on Organic Acid Exudation from the Root System of *Pistia stratiotes* in Plateau Wetland

Xu Duan[1], Yang-Yi Zhao[2*] and Jiancong Zhang[2]
[1]*College of Forestry Department, Southwest Forestry University, Kunming 650224, China*
[2]*College of Ecology & Soil and Water Conservation department, Southwest Forestry University, Kunming 650224, China*
[*]For correspondence: yyz301@foxmail.com
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Abstract

Gas chromatography-mass spectrometry (GC-MS) was used to determine the *Pistia* in a plateau wetland under stress conditions of 0, 0.2, 1, 5, 10 and 20 mg/L phosphorus as well as the relative content and variation of eight organic acids secreted by its roots at 21 and 28 days. These results showed that the relative content of the phthalic acid (PA) and phenolic carbonate secreted by the roots of *Pistia* was significantly higher than that of other organic acids during the same concentration of P stress. After 7 days, the exudation of sulfite and benzene dicarboxylic acid (BCA) increased with the increase of the P concentration. The exudation of sulphuric acid (SA), carboxylic acid (CA), and benzoic acid (BA) increased with the increase of the P concentration. The oxalic acid (OA) decreased with the increase of the P concentration. Stone carbonate and benzene dicarboxylate demonstrated a significant ($P<0.05$) positive correlation between acid, BA and the P stress concentration as well as a significant ($P<0.05$) negative correlation between OA and the P stress treatments. With the prolongation of stress time, the exudation of phenolic acid, PA, OA, BCA, BA, and total organic acids appeared the least under the condition of no P stress; and PA, SA, The exudation of SA, CA, OA, BA, and total organic acids was positively correlated with the concentration of the P stress, $r=0.633$, $r=0.909$, $r=0.757$, $r=0.929$, $r=0.950$, $r=0.773$, and $r=0.642$. The regulation of PA exudation by *Pistia* under P stress is an important mechanism for actively adapting to the environment. The results of this study provide basic reference data for eutrophication and micro-control measures in plateau wetlands.

Keywords: Phosphorus; Organic acids; Root exudates; *Pistia stratiotes*

Introduction

Wetland eutrophication has gradually become one of the most important ecological environmental problems in the world (Tang *et al*. 2010a). Phosphorus (P) is one of the main limiting factors of wetland eutrophication (Qin *et al*. 2013; Roy 2017; Zhao *et al*. 2018) and is a necessary nutrient element for plant growth. At present, the eutrophication of water caused by P nutrients is becoming more and more serious, leading to abnormal ecosystem responses (Tang *et al*. 2010a; Hou *et al*. 2018; Zou *et al*. 2018). Due to the short flow of tributaries and the long period of water exchange, it is difficult to manage plateau wetlands (Yu *et al*. 2010; Zhou *et al*. 2016). Wetland plants can effectively purify sewage and root systems are a key link to material circulation and energy flow between wetland plants and the surrounding environment (You *et al*. 2013; Zhang *et al*. 2018a; Zhang *et al*. 2018b). Therefore, it is of great significance to study the root exudates of wetland plants.

Studies have shown that in root exudate, organic acids can affect the nutrients and energy of microorganisms. Furthermore, they can change the structure as well as biological activities of root-zone microorganisms; as such, the role they play is important (Kuang *et al*. 2003; Zhao *et al*. 2016). At present, research on organic acids secreted from root systems under P stress have mostly focused on the physiological and ecological changes of cash crops (Gardner 1983; Carvalhais *et al*. 2011; Khorassani *et al*. 2011; Xiao *et al*. 2014; Li *et al*. 2016), trees, and their effects on organic acid exudation (Xu and Ding 2006; Yu *et al*. 2017). Li *et al*. (2005) and Wang *et al*. (2014) studied environments such as the nutrient in rice roots that influence the exudation of organic acids under low P stress. Low P stress can accelerate the exudation of more organic acid from rice root systems. Deng *et al*. (2006) studied the influence of P concentration on *Pinus* seedling organic acid exudation. They showed that a P deficiency can lead to acid environment changes in the body. In addition, studies have shown that sweet potato and beet can promote the release of P in soil by changing root morphology and organic acid

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exudation composition under P-deficient environments (Ma *et al*. 2017). Chen (2009) studied the activation effect of P on root exudations in wheat and broad bean under P stress applied in a pot experiment, showing that the root system secreted low molecular weight organic acids to improve the biological efficiency of P. However, there is less research on wetland plant root exudation in specific organic acid content quality changes under P stress. Part of the study on wetland plant roots concentrated on the total root exudation and analysis of the influence of the rhizosphere effect (Tang *et al*. 2010b). Liu *et al*. (2009) compared water grass and willow roots exudation to determine various plant roots' exudation ability. Furthermore, they obtained three kinds of plant root exudation that were mainly composed of organic acids and aromatic proteins. Huang *et al*. (2014) discussed the change of root exudation amount in *Pinellia* and *Canna* with time. Although previous research on plant root exudation and P stress had certain understanding of its influence on plant roots, the wetland floating plant root exudation of organic acid changes has been rarely a research topic, specifically under P stress. This is especially true for wetland plant root exudation system research in *Pistia stratiotes*; therefore, this experiment has very high scientific value and practical significance.

With the increasing P accumulation in wetlands, the number of floating plants will increase and become the main source of primary productivity of wetland ecosystems (Scheffer *et al*. 2001). *P stratiotes*, a typical perennial floating herb, survives easily and has strong sewage purification capacity, which can effectively improve eutrophicated water bodies (Li *et al*. 2012; Victor *et al*. 2016). This research takes *Pistia* as experimental material, analyzes the wetland floating plants in different concentrations of P stress at different times that influences on root exudation of organic acids, and tries to find the floating plants' eutrophication adaptation mechanisms under P stress to provide basic reference data for root micromanagement measures for plateau wetlands.

Materials and Methods

Experimental details and treatments

Water culture and solution ratio: In this experiment, Hoagland nutrient solution was used in the Southwest Forestry University laboratory at the beginning of March 2018 to cultivate the seedlings at 20–30°C. P stress was carried out at the beginning of April, then, measured for 7, 14, 21 and 28 days. The concrete operation was as follows: 80 plastic buckets were taken as hydroponic equipment and the Hoagland nutrient solution was added. Approximately 160 healthy seedlings with the same age were selected and two seedlings were cultured in each plastic barrel. In the plastic barrel of hydroponically grown plants, a 300 mm rubber disc microporous aerator was installed to prevent the plant root system from rotting through the proper amount of aeration. To avoid the proliferation of algae in the barrel, aluminum foil was used for shading (Horchani *et al.* 2008). The culture medium was changed once in a week. After one month of culture, plants with good growth and similar plant height were selected and transplanted into the experimental device. KH_2PO_4 was used as the P source and the P concentration gradient was 0, 0.2, 1, 5, 10 and 20 mg/L. There were 72 plastic boxes (4 groups of experiments, 3 replicates in each group) and 2 plants were cultured in each box. Experimental design was that completely randomized design (CRD).

The composition of the Hoagland nutrient solution was: K_2SO_4 0.75×10^{-3} mol/L, $MgSO_4$ 0.75×10^{-3} mol/L, KCL 1×10^{-3} mol/L, $Ca(NO_3)_2$ 2.0×10^{-3} mol/L, H_3BO_3 1×10^{-5} mol/L, $CuSO_4$ 1×10^{-7} mol/L, $MnSO_4$ 1×10^{-6} mol/L, $ZnSO_4$ 1×10^{-6} mol/L, $(NH_4)6Mo_7O_{24}$ 5×10^{-6} mol/L, Fe-EDTA 1×10^{-4} mol/L. This was the mother liquor of the nutrient solution, diluted four times, and used as the solution of the hydroponic culture (Chen 2009).

Collection and isolation of root exudates: The entire root system of the plant cultured under P stress was washed with deionized water for 7, 14, 21 and 28 days respectively, and then collected with root exudates (Collection liquid ratio: H_3BO_3 5 μmol/L, $CaCl_2$ 600 μmol/L, KCl 100 μmol/L, $MgCl_2$ 200 μmol/L, pH 5.6). After three repeated washings, the entire root system was covered with a black plastic bag and transferred to a beaker containing 50 mL of root exudates. The root exudates were collected under natural light for 4 h (9:00–13:00), transplanted to a solution containing 1 L of 0.5 mmol/L $CaCl_2$ for 4 h (13:00–17:00). Then the extraction solution was obtained by a CH_2Cl_2 extraction and root lotion, three times (40 mL/times) (Tian *et al*. 2003; Zhang *et al*. 2007). Finally, a 200 mL extraction solution was extracted at 38°C, dried with anhydrous Na_2SO_4, and concentrated to dry reserve by rotating evaporation with a vacuum rotary evaporator (Wei *et al*. 2016).

Determination of root exudates: The liquid was extracted with a syringe after a full shake of the CH_2Cl_2, which was added to the rotating evaporation bottle operated by 1.2 knots for 0.5 mL, through a 0.45 μm needle filtration membrane. At the same time, the membrane was filtered by 0.45 μm needle and then put into a small brown bottle for GC-MS analysis. The organic acids in the root exudates were determined by GC-MS (Agilent 7890B). The chromatographic conditions (Yu *et al*. 2013; Liu *et al*. 2017) were as follows: The capillary column was an HP-5 ms column (30 m × 250 μm × 0.25 μm); the injection port temperature was 260°C, the carrier gas was He (purity is not less than 99.99%), the flow rate 1 mL/min, the injection 1 μL, the flow valve was opened after 1 min, the column temperature was programmed, the starting temperature was 50°C, and the flow rate was 2 min, 20°C per min, programmed to 150°C, @ 5°C per min, programmed to 220°C, then, 6°C per min, programmed to 250°C, for 15 min.

The mass spectrometer conditions (Liu *et al*. 2017) were: The electron bombardment source (Ei), ionization energy was 70 eV, ion source temperature was 200°C, interface temperature 280°C, quadrupole temperature 150°C, solution delay time 3.75 min, scanning mode (SCAN), the scanning range M/Z 33–453, and the tuning file was standard tuning.

Statistical analysis

The identification of organic acids in the root exudates was done by artificial analysis of total ion flow map and checked with the standard map of the NIST08 mass spectrometry database; the determination of the root exudates was carried out by computer search. The relative content of substances was calculated according to the peak area (%) of the components that was detected in the chromatogram. In this study, Excel WPS2016 and SPSS21 software were used for data processing and statistical analysis. The Kernelized Stein Discrepancy (KSD) test was used for multiple comparisons. The significant level α was 0.05, highly significant level α was 0.01, and the scanning map was drawn by Origin 8.5.

Results

GC-MS scanning pattern of root exudates from *Pistia*

In this study, only the scanning atlas in four different periods when the P concentration was 1 mg/L is listed. The scanning results showed that the scanning spectra were different in different periods; the number of characteristic peaks and the area of high and low peaks were not consistent. Each characteristic peak represented a compound, so it can be seen from the spectra that root exudation under P stress is different in different time periods from *Pistia* (Fig. 1).

Differences in organic acids in root exudates under different P treatments and stress time

Changes in organic acid exudation under different P treatments during the same stress period: Table 1 shows that, except for the difference of the relative content of phthalic acid in the different concentrations on the 7th day, there were significant differences ($p<0.05$) in the amount of organic acid secreted in each period of time. When the P stress time was 7 days, the exudation of sulfurous acid (SA) and phenyldicarboxylic acid (PCA) increased first and then, decreased with the increasing P concentration. The minimum exudation of SA was 1.06% when the concentration was 0 mg/L. When the maximum relative content was 1.39% in 5 mg/L, the content of sulfite in 5 mg/L was significantly ($P<0.05$) higher than that in 0 and 0.2 mg/L and the relative content of sulfite in 20 mg/L was significantly ($P<0.05$) lower than that in 5 mg/L. There was no significant ($P>0.05$) difference in the relative content between other phosphorous sulphite concentrations. The excretion of PCA began to decrease when concentration was 10 mg/L, and the minimum exudation was 0.28% at 0 mg/L concentration. The relative content of carbonated stone and BA increased with the increasing P concentration and the relative content of carbonic acid in 20 mg/L and 10 mg/L was significantly ($P<0.05$) higher than that of the other concentrations.

There was no significant ($P>0.05$) difference in the exudation at 0, 1 and 0.2 mg/L concentration, However, when the concentration was 20 mg/L, BA exudation increased significantly ($p<0.05$). When the P concentration was 0.2 mg/L, 1 mg/L, 5 mg/L and 10 mg/L, there was no significant ($P>0.05$) difference in the exudation of BA, CA, while OA tended to decrease with the increasing concentration. At most, when the concentration of CA was 10 mg/L, the amount of CA secreted by 0.2 mg/L was significantly ($P<0.05$) different from that the P concentrations of 1, 5 and 10 mg/L. There was no significant ($P>0.05$) difference in CA exudation between 0, 0.2 and 20 mg/L, and between 1, 5 and 10 mg/L. OA exudation was mainly in the range of 1–20 mg/L P concentration, and when the concentration of OA was 1 mg/L, the OA exudation decreased significantly ($p<0.05$). When the total organic acid content of root exudates was the highest at 1 mg/L under P stress, the minimum exudation was 0.2 mg/L concentration. There was no significant ($P>0.05$) difference between 0, 5, 10 and 20 mg/L concentrations in organic acid exudation (Fig. 2).

When the time of P stress was 14 days, except for the minimum exudation of SA that was 0.49% in 0.2 mg/L, the exudation of other organic acids and total organic acids were least in the condition of no P stress. The contents of carbonate, CA, and PA appeared at the same time. The minimum exudation of OA, PCA, BA, and total organic acid was as follows: 1.24, 0.44, 5.84, 0.18, 0.41, 0.24, and 9.36%. When the highest concentration of phosphorous acid was 1 mg/L, the exudation was significantly ($P<0.05$) higher than that at 0, 0.2, 5 and 20 mg/L (Fig. 3). The exudation of carbonates and CAs increased significantly ($P<0.05$) at the concentration of 0–1 mg/L ($p<0.05$), the exudation of 1–5 mg/L decreased significantly, and the exudation increased in the range of 5–20 mg/L. When the concentration was 20 mg/L, the content of CA was 11.53 and 4.71% at 1 mg/L (Table 1). The exudation of both PA and PCA increased 0–1 mg/L. The 1–5 mg/L exudation decreased significantly ($p<0.05$), and the 5–10 mg/L exudation increased significantly ($p<0.05$). 10–20 mg/L decreased significantly ($p<0.05$) and the maximum exudation occurred at the time of the 10 mg/L P concentration. OA increased gradually with an increase in P concentration; the relative content of 20 mg/L concentration was 1.055. Furthermore, it was significantly ($p<0.05$) higher than that of other concentrations. There was no significant ($P>0.05$) difference in the exudation of

Table 1: Comparison of relative content of organic acids in root exudates under different phosphorus concentration and time treatment (%)

Organic acid	Concentration (mg/L)	Duress time (day)			
		7	14	21	28
Sulfurous acid	0	1.06±0.07 b-A	1.01±0.04 b-A	0.51±0.03 b-C	0.89±0.09 d-B
	0.2	1.19±0.16 b-A	0.49±0.04 c-BC	0.36±0.06 c-C	0.56±0.09 d-B
	1	1.32±0.08 ab-A	1.19±0.04 a-B	0.42±0.05 bc-D	0.99±0.05 c-C
	5	1.39±0.15 a-B	1.01±0.12 b-C	0.55±0.08 ab-D	2.00±0.10 a-A
	10	1.35±0.04 ab-B	1.11±0.11 a-C	0.60±0.06 ab-D	1.51±0.09 b-A
	20	1.11±0.11 b-A	0.52±0.04 c-B	0.65±0.07 a-B	1.06±0.11 c-A
Phenol	0	0.21±0.02 d-C	1.24±0.11 f-B	2.05±0.06 c-A	0.08±0.01 e-D
	0.2	0.30±0.04 cd-C	1.58±0.08 e-B	10.26±0.06 ab-A	0.21±0.04 d-C
	1	0.34±0.04 cd-C	5.57±0.07 c-B	11.21±0.74 a-A	0.24±0.04 d-C
	5	0.43±0.12 c-B	4.00±0.23 d-A	10.99±0.46 ab-A	0.35±0.06 c-B
	10	0.61±0.06 b-B	10.20±0.18 b-A	10.09±1.13 b-A	0.48±0.04 b-B
	20	0.84±0.13 a-C	11.53±0.15 a-A	10.19±0.15 ab-B	0.58±0.06 a-D
Carboxylic Acid	0	1.12±0.11 ab-A	0.44±0.04 d-C	0.32±0.03 cd-D	0.68±0.04 d-B
	0.2	1.03±0.07 b-B	0.58±0.05 d-C	0.30±0.03 d-D	1.61±0.14 b-A
	1	1.16±0.03 a-C	4.71±0.03 a-A	0.40±0.04 c-D	1.49±0.11 b-B
	5	1.20±0.05 a-A	0.91±0.14 c-B	0.69±0.08 b-C	1.18±0.09 c-A
	10	1.21±0.03 a-B	1.01±0.13 c-C	1.26±0.04 a-B	2.16±0.07 a-A
	20	1.14±0.13 ab-B	1.20±0.11 b-B	1.34±0.08 a-B	2.20±0.11 a-A
Phthalic acid	0	3.22±0.04 a-C	5.84±0.11 e-B	6.11±0.09 c-A	0.43±0.08 d-D
	0.2	3.21±0.22 a-B	27.60±1.38 b-A	28.74±1.02 a-A	0.49±0.03 d-C
	1	3.37±0.18 a-C	28.14±1.05 b-B	29.68±0.93 a-A	0.88±0.09 c-D
	5	3.43±0.06 a-C	9.81±0.17 d-B	28.25±1.04 b-A	2.12±0.11 b-D
	10	3.41±0.11 a-C	32.10±0.51 a-A	28.11±0.80 b-B	3.66±0.22 a-C
	20	3.25±0.14 a-C	23.01±0.86 c-B	27.58±0.46 b-A	2.06±0.04 b-D
Oxalic acid	0	0.69±0.08 a-A	0.18±0.03 c-B	0.10±0.02 d-B	0.21±0.09 d-B
	0.2	0.45±0.04 c-B	0.21±0.03 c-C	0.15±0.04 d-C	1.04±0.08 a-A
	1	0.76±0.07 a-A	0.25±0.03 c-C	0.27±0.05 c-B	0.78±0.12 b-A
	5	0.56±0.06 b-B	0.32±0.04 bc-C	0.42±0.04 b-C	0.79±0.09 b-A
	10	0.42±0.06 c-B	0.40±0.11 b-B	0.43±0.04 b-B	0.61±0.07 c-A
	20	0.39±0.05 c-B	1.05±0.06 a-A	0.62±0.05 a-B	0.94±0.08 ab-A
Benzene dicarboxylic acid	0	0.28±0.08 c-B	0.41±0.04 e-A	0.32±0.03 d-AB	0.11±0.02 c-C
	0.2	0.31±0.03 c-D	2.59±0.08 b-A	2.39±0.06 c-B	0.57±0.07 b-C
	1	0.53±0.05 b-C	2.51±0.10 b-B	3.09±0.10 a-A	0.64±0.08 ab-C
	5	0.58±0.03 b-C	1.96±0.04 c-B	3.02±0.15 a-A	0.70±0.05 a-C
	10	0.74±0.03 a-B	3.21±0.11 a-A	3.07±0.16 a-A	0.62±0.11 ab-B
	20	0.61±0.03 b-B	0.57±0.09 d-B	2.65±0.12 b-A	0.55±0.07 b-B
Benzoic Acid	0	0.15±0.02 d-B	0.24±0.08 e-A	0.15±0.05 d-B	0.13±0.02 d-B
	0.2	0.33±0.03 c-D	5.55±0.10 a-A	0.92±0.04 bc-B	0.52±0.05 c-C
	1	0.38±0.03 bc-C	1.04±0.07 c-A	0.81±0.08 c-B	1.09±0.02 a-A
	5	0.40±0.03 b-D	2.28±0.07 b-A	1.34±0.10 a-B	1.01±0.21 a-C
	10	0.32±0.03 c-D	0.57±0.04 d-A	0.94±0.06 b-B	0.75±0.13 b-C
	20	0.60±0.04 a-B	0.49±0.10 d-B	1.34±0.07 a-A	0.53±0.08 c-B
Total organic acid	0	8.41±0.29 b-B	9.36±0.19 e-A	9.57±0.17 c-A	2.53±0.20 f-C
	0.2	7.08±0.48 c-C	43.10±1.48 c-B	54.71±1.09 a-A	6.15±0.35 d-C
	1	9.36±0.24 a-C	45.68±1.14 b-B	49.78±1.36 b-A	8.69±0.32 c-C
	5	8.20±0.31 b-D	25.02±0.71 d-B	48.91±1.34 b-A	9.82±0.37 b-C
	10	8.71±0.16 b-D	52.62±0.71 a-A	49.40±1.29 b-B	11.37±0.42 a-C
	20	8.95±0.10 ab-D	41.78±0.95 c-B	48.97±0.45 b-A	10.20±0.23 b-C

Note: The figures in the table were all repeated mean ±standard errors in three groups. The lowercase letters after the numbers in the table indicate the differences (longitudinally) in exudations between different concentrations of phosphorus in the same organic acids at the same time of stress, the uppercase letters represent the differences (horizontal) between different stress time treatments of the same organic acid at the same phosphorus concentration. In the same column (or row), the same letter means that the difference is not significant ($p>0.05$), and the difference between the letters is significant ($p<0.05$)

BA between 0 and 2 mg/L, but there was a significant ($p<0.05$) difference between other P concentrations. There was no significant ($P>0.05$) difference in the total organic acid exudation except for the 0.2 and 20 mg/L concentrations. The relative contents of total OAs in the other P treatments were significantly ($p<0.05$) different, and the total OA excretions most frequently occurred at a 10 mg/L concentration; the exudation was as high as 52.62% (Table 1).

When the P stress lasted for 21 days, except for CA and SA, a least amount of exudates appeared at the P concentration of 0.2 mg/L, lithic acid, PA, OA, and PCA. Least amount of BA and total organic acid appeared when the concentration was 0 mg/L and the relative contents were: 2.05, 6.11, 0.10, 0.32, 0.15 and 9.57%. In the concentration range of 0–1 mg/L, the exudation of sulfite was significantly ($P<0.05$) decreased, the amount of 10 mg/L was less than that of 5 mg/L, and the exudation of 5 mg/L concentration was significantly ($p<0.05$) higher than that at 1 mg/L (Fig. 4). The exudation of CA and PCA

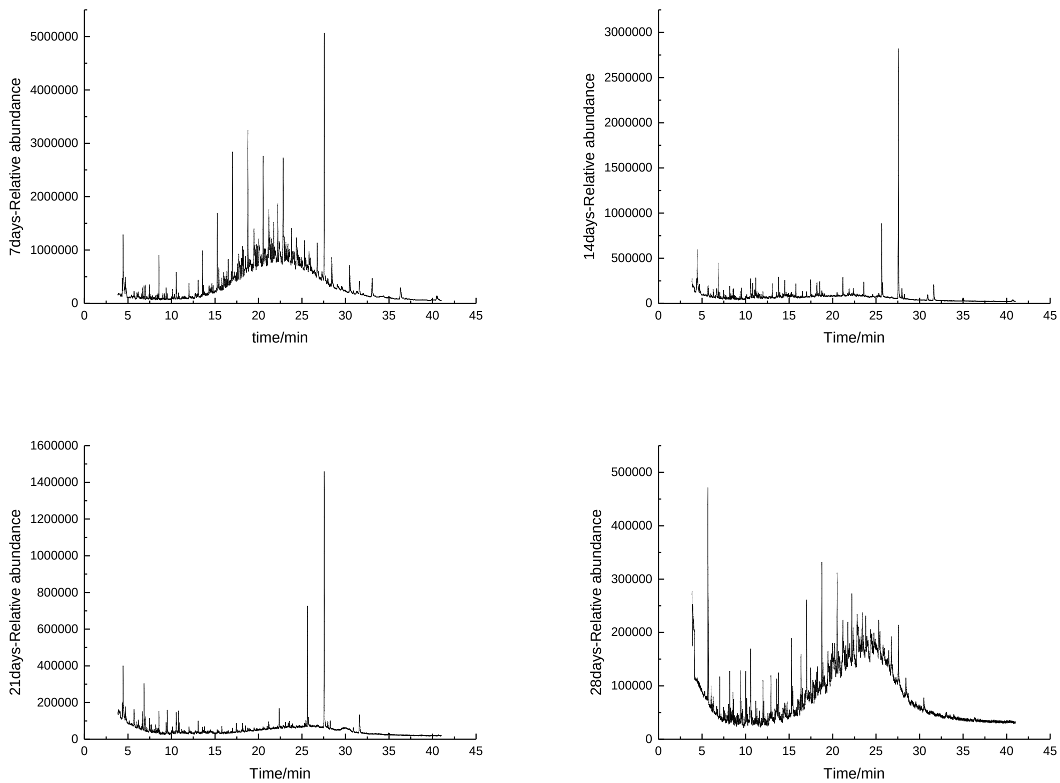

Fig. 1: Scanning map of root exudates of *P stratiotes* at different times of 1 mg/L phosphorus concentration

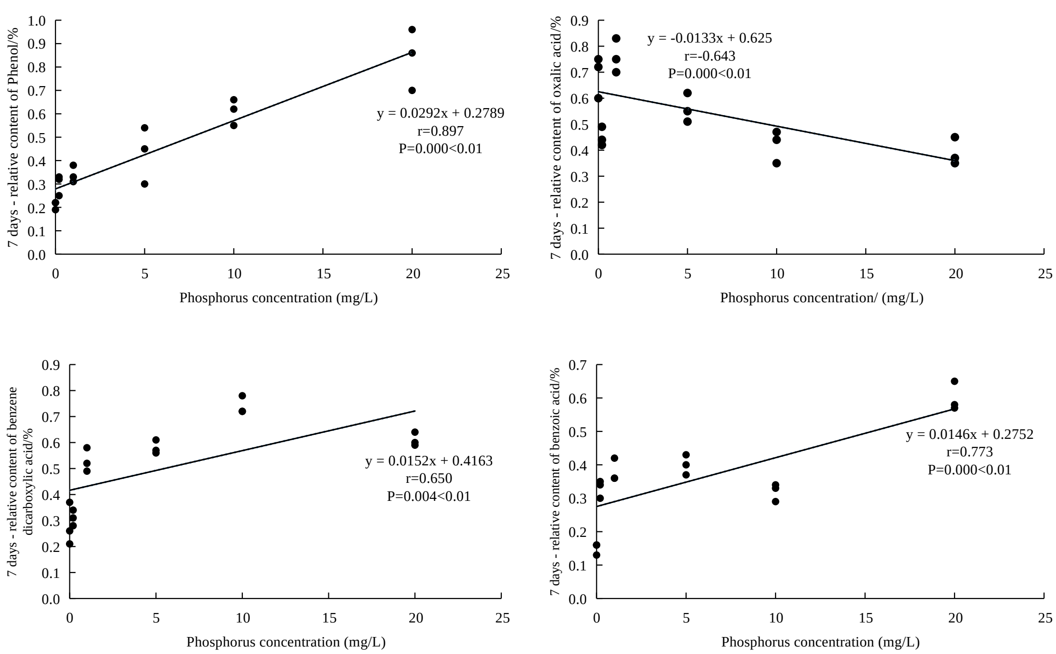

Fig. 2: Correlation analysis between phosphorus concentration and organic acid at 7 days of stress duration

increased significantly ($p < 0.05$) at the concentration of 0–1 mg/L. The exudation of 5 mg/L was significantly ($P<0.05$) lower than that of the concentration of 1 mg/L. The concentration of 10 mg/L was lower than that of 5 mg/L ($p<0.05$). The content of 20 mg/L was higher than that of 10 mg/L. The amount of BCA (10 mg/L) was higher than that of 5 mg/L and the amount of 20 mg/L was lower than that of 10 mg/L. When the maximum exudation amount of the

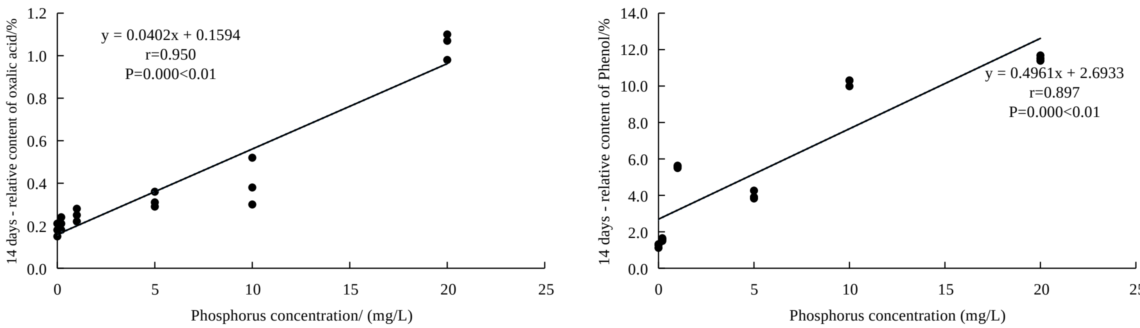

Fig. 3: Correlation analysis between phosphorus concentration and organic acid at 14 days of stress duration

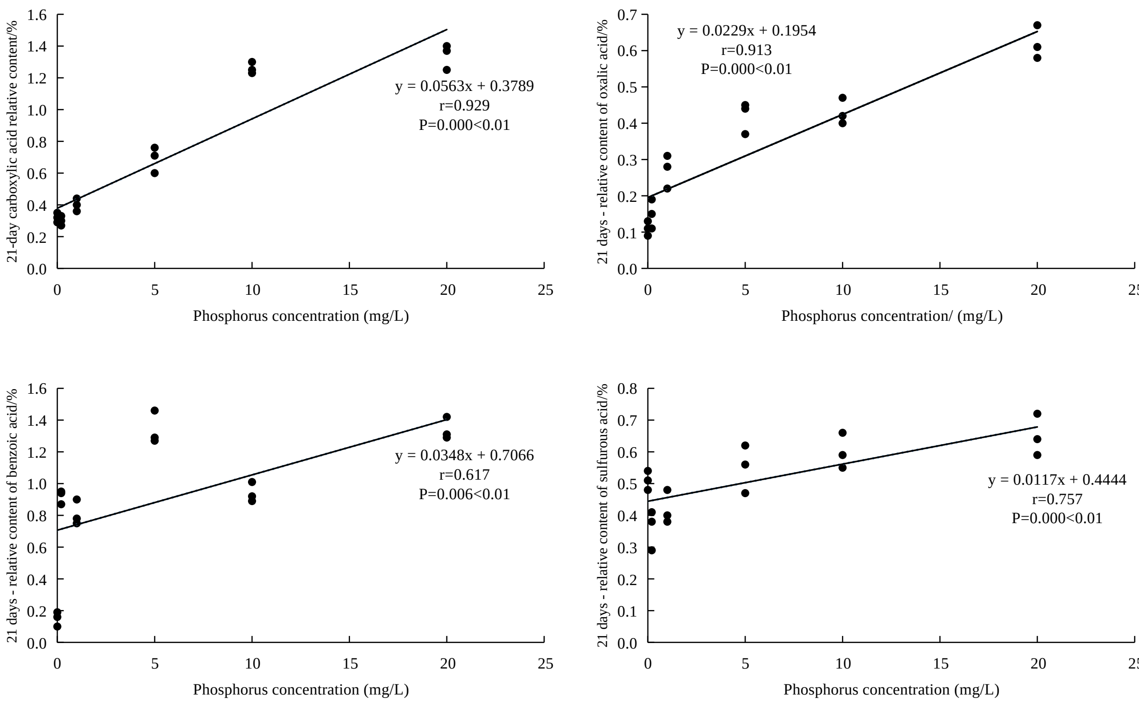

Fig. 4: Correlation analysis between phosphorus concentration and organic acid at 21 days of stress duration

stony carbonate and PCA was both 1 mg/L concentration, it was 11.21 and 3.09%, respectively (Table 1).

OA excretion increased gradually with the increase of the concentration. In the range concentration of 0.2–20 mg/L, the exudation of OA increased significantly ($P<0.05$) except for 5 mg/L and the maximum exudation was 0.62. There was no significant ($P>0.05$) difference in the exudation of PA between 0.2 and 1 mg/L, the three concentrations of 5, 10 and 20 mg/L and the highest relative content was 29.68% when the concentration of 1 mg/L was the highest (Table 1). The amount of CA secreted from 0.2–20 mg/L increased gradually with the increase of P concentration, and the maximum exudation of CA was 1.34 with 20 mg/L. The highest level of BA exudation was 1.34, and the difference of BA exudation was significant ($p<0.05$) at the concentrations of 0, 1, 5, and 10 mg/L. The total OA content of 0.2 mg/L was significantly ($P<0.05$) higher than that of 0, 5, 10, and 20 mg/L ($p<0.05$), which was lower than that of 0.2 mg/L at 54.71% (Table 1).

When the P stress lasted for 28 days, except for the SA exudation of at least 0.56% that appeared in the concentration in 0.2 mg/L, the minimum content of other acids was 0.08%; carbonic acid, 0.68%; CA, 0.43%; PA, OA 0.21%; PCA 0.11%; BA 0.13%; and total organic acid 2.53% in the treatment of concentration in 0 mg/L. At the concentration of 5 mg/L, the exudation of sulfite was the largest (2%). The exudation of sulfite increased significantly ($P<0.05$) in the 0.2–5 mg/L region, and decreased significantly ($p<0.05$) in the 5–20 mg/L region. With the increase of the P concentration, the exudation of the stone carbonate increased significantly (p<0.05), which accounted for 0.58% at the concentration of 20 mg/L (Table 1).

With the increase of P concentration, the exudation of CA, except for 0.2, 1, 10 and 20 mg/L, increased significantly (p< 0.05). The relative content of CA was 2.2% when the concentration was 20 mg/L. When the P concentration was 10 mg/L, PA had the highest exudation, accounting for 3.66%. The exudation of 10–20 mg/L decreased significantly and 1–10 mg/L increased significantly (p<0.05). The relative content of OA decreased significantly (p<0.05) from 0.2 and 1 mg/L as well as 5 and 10 mg/L and increased significantly (p<0.05) from 0 and 0.2 mg/L as well as 10 and 20 mg/L (p<0.05). The exudation of PCA increased significantly (p<0.05) in the range of 0–5 mg/L concentration (p<0.05), while that of the 5–20 mg/L decreased gradually. The highest amount was 0.7% under the treatment of the 5 mg/L concentration. The 0–1 mg/L range of BA increased significantly (p<0.05). The content of 1–20 mg/L BA gradually decreased with the increase of the P concentration. The relative content of BA secreted at the 1 mg/L concentration was 1.09%. The exudation of the total organic acids increased significantly (p<0.05) in the range of 0–10 mg/L (p<0.05) and the exudation from 10–20 mg/L decreased significantly (p<0.05). Under the treatment of concentration 10 mg/L, the total organic acids secreted the most at 11.37% (Table 1).

Changes of organic acid exudation in different time periods under the same P stress treatments: When the concentration was 0 mg/L, the organic acids that were secreted the most at 7 days of stress were sulfite (1.06%), CA (1.12%), and OA (0.69%). The highest exudations of PCA and BA were 0.41% and 0.24%, respectively, at 14 days of stress and the highest exudation of carbonic acid was 2.05 and 6.11 for PA, and 9.57% for the total organic acids at 21 days of stress. SA, OA, and CA had the lowest exudations of 0.51, 0.1, and 0.32%, respectively, at 21 days after stress. The total organic acid exudations of carbonated acid, PA, BA, and total organic acid were the lowest in the four stress periods at 28 days and the relative content was 0.08, 0.43, 0.11, 0.13, and 2.53% respectively. The amount of sulfite secreted on day 14 was significantly (p<0.05) lower than that of day 7 (p<0.05) and the amount at day 21 was significantly (p<0.05) lower than that at day 14. The relative contents of carbonic acid and PA were significantly (p<0.05) higher at 21 days than in 7 days and 28 days. CA exudation decreased significantly (p<0.05) with the increase of stress duration. OA excretion at 7 days was significantly (p<0.05) higher than that in the other three periods (p<0.05). The exudation of PCA on day 28 was significantly (p<0.05) lower than those of days 14 and 21 and the exudation of BA on day 14 was significantly (p<0.05) higher than that at the other three periods. The total organic acid excretion at 7 days was significantly (p<0.05) higher than that at 14 and 21 days, and the total organic acid content decreased significantly (p<0.05) from 21 to 28 days (Table 1).

When the concentration was 0.2 mg/L, the exudation of carbonated acid, PA, and total organic acid was the highest at 21 days, the relative contents were 10.26, 28.74, and 54.71%, respectively, and the smallest one was at 28 days. The exudation values were 0.21, 0.49, and 6.15%, the highest exudation of PCA and BA was at 14 days and the least at 7 days, and OA exudation was highest at 28 days and least at 21 days. The relative content of sulfite was a maximum of 1.19% at 7 days and 0.36% at the minimum at 21 days. The exudation of SA and CA decreased significantly (p<0.05) at 21 days and 7 days, respectively, and increased significantly (p<0.05) at 28 days compared with 21 days. The exudation of PCA and BA increased significantly (p<0.05) from 7 to 14 days and decreased significantly (p<0.05) at 28 days compared to 21 days (p<0.05). The carbonate and total organic acids increased significantly (p<0.05) from 7 days to 21 days and the exudation at 28 days was significantly higher than that of the 21 day (p<0.05). The exudation of OA decreased significantly (p<0.05) from 7 days to 21 days, while that of the PA increased significantly (p<0.05) between 7 days and 21 days (Table 1).

When the concentration was 1 mg/L, the exudation of carbonic acid, PA, and total organic acids increased significantly (P<0.05) from 7 days to 21 days and the exudation of 28 days was significantly (p<0.05) lower than that of 21 days (P<0.05). The exudation of all four acids was the highest at 21 days and the lowest at 28 days. The exudation of BA and CA was significantly (p<0.05) higher at 14 days than that at 7 days and higher at 28 days higher than at days 21 and 14 (P<0.05). However, CA exudation was greatest at 14 days and the least at 21 days. The greatest BA exudation was at 28 days and the least at 7 days. OA decreased significantly (p<0.05) from 7 days to 21 days (P<0.05) and the exudation was the most at 28 days and the least at 14 days (Table 1).

When the concentration was 5 mg/L, the relative contents of PA, carbonic acid, and total organic acids increased significantly (P<0.05) from 7 days to 21 days; at least 7 days for PA and total organic acid and 28 days for PA and carbonic acid. The relative contents of SA and CA decreased significantly (P<0.05) from 7 days to 21 days. The exudation of both acids was least 28 days compared with that of the 21st day. The maximum exudations of SA and CA were at 28 and 7 days, respectively. The maximum relative content of OA was found at 28 days and the least at 14 days; there was no significant (p>0.05) difference in this value between days 14 and 21. The exudation of BA was the most at 14 days and the least at 7 days. The exudations of days 14, 21, and 28 days were significantly (P<0.05) higher than that of the P stress on day 7 (Table 1).

When the concentration was 10 mg/L, the exudations of PA, carbonic acid, PCA, and total organic acids at 14 days were significantly (P<0.05) higher than those at day 7. There was no significant (p>0.05) difference between lithic acid and PCA on the 14th day and 21st day. The maximum exudations of stone carbonate and PCA were both at 14 days

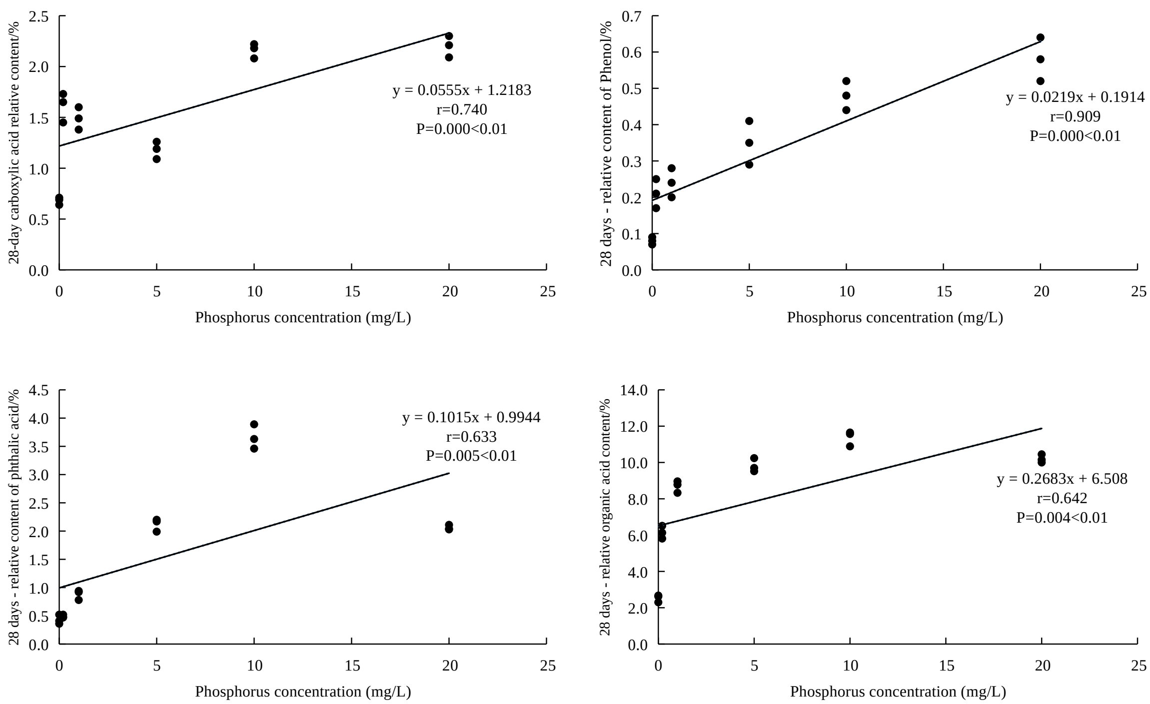

Fig. 5: Correlation analysis between phosphorus concentration and organic acid at 28 days of stress duration

and the minimum exudations were at 28 days. The maximum exudations of PA and total organic acids were at 14 days. At 7 days, the relative contents of the two acids at 21 days were significantly ($P<0.05$) lower than those at 14 days. On the 28th day, the amounts of SA, CA, and OA were the highest, while the lowest exudations of CA and OA appeared at 14 days. The exudation of SA was the least at 21 days as it decreased significantly ($P<0.05$) from day 7 to day 21. The amount of OA secreted at day 28 was significantly ($P<0.05$) higher than that on day 21 but there was no significant ($P>0.05$) difference in the exudation of OA at days 7, 14, and 21. The exudation of BA increased significantly from 7 days to 21 days ($P<0.05$) and was significantly ($P<0.05$) lower at 28 days than that on day 21 (Table 1).

At the concentration of 20 mg/L, the highest exudation of organic acids for PA, PCA, BA, and total organic acids were as follows: For the total organic acid and PA, this occurred from days 7–21. The exudations of the two acids at 28 days was significantly ($P<0.05$) less than at 21 days, the total organic acid content was the least 7 days and at 28 days for PA. The exudation of PA and PCA were the least 14 days. There was no significant ($P>0.05$) difference between days 7 and 14 and the exudation of 21 days was significantly ($P<0.05$) larger than that of days 7 and 14. The maximum exudation of stony carbonate and OA were both at 14 days. The exudation of both acids on 14 days was significantly ($P<0.05$) higher than that at 7 days and the exudation at 21 days was significantly ($P<0.05$) lower than that at 14 days. The minimum exudation of stone carbonate was at 28 days; the same for OA at 7 days of stress. The exudation of SA at 7 days was significantly ($P<0.05$) higher than that of the other three periods and there was no significant ($P<0.05$) difference between days 14 and 21; the minimum exudation appeared at 14 days. The amount of CA secreted at 28 days was significantly ($P<0.05$) higher than that on days 7, 14, and 21. There was no significant ($P>0.05$) difference in the relative content of CA between days 7, 14, and 21; the minimum amount of exudation occurred at day 7 (Table 1).

Correlation analysis between P concentration and relative organic acids content of roots

Through correlation analysis, a significant ($P<0.05$) positive correlation was found between the concentrations of carbonic acid, PCA, and BA in root exudates measured at 7 days (Fig. 2). The results showed that with the increase of the P stress concentration ($r=0.897$, $P=0.000$; $r=0.650$, $P=0.004$; and $r=0.773$, $P=0.000$), the exudation of carbonic acid, PCA, and BA also increased. OA had a highly significant ($P<0.01$) negative correlation ($r = -0.643$) with P stress concentration and OA decreased significantly ($P<0.05$) with the increase of concentration. There was no significant ($P>0.05$) correlation between the contents of SA, CA, and total organic acid at 7 days of culture and the concentration of stress. When the stress duration was 14 days (Fig. 3), only OA and carbonated acid, secreted from 7 acids, as well as total organic acids were positively correlated with the stress concentration ($r=0.950$, $P=0.000$; $r=0.897$, $P=0.000$), there was no significant ($p>0.05$) correlation between the concentrations of phosphorous acid, sulfuric acid, CA, PA, BA, and total organic acid. The exudation of SA, CA, OA, and BA was positively ($P<0.01$) correlated with the stress concentration at 21 days (Fig. 4). There was no significant ($p>0.05$) correlation between the content of PA, PCA, carbonate, and total organic acid and P concentration. When the stress duration was 28 days (Fig. 5), the exudation of CA, carbonate, PA, and total organic acid was positively correlated with the stress concentration ($r=0.740$, $P=0.000$; $r=0.909$, $P=0.000$; $r=0.633$, $P=0.005$; and $r=0.642$, $P=0.004$). There was no significant ($P>0.05$) correlation between the concentration and SA, OA, PCA, or BA.

Discussion

During the entire plant growth process, the root system is an important organ for the communication between the plant and the soil environment; it mainly relies on the root system to absorb the nutrients needed for the growth of the plant from the outside environment. At the same time, roots also secrete a large amount of organic-root exudates into growth media. Plants respond to environmental stress by adjusting the types and contents of root exudates. The diversity of root exudates is the embodiment of adaptation by different types of plants to their living environment (Song *et al*. 2017; 2018). The organic acids in root exudates are one of the main adaptive mechanisms of plant roots under environmental nutrient stress. Plant roots change the pH value of the rhizosphere by secreting low molecular weight organic acids. Insoluble P in the surrounding environment of roots is activated to improve the utilization efficiency of nutrient components in plants (Qin *et al*. 2011; Chen *et al*. 2017). In wetland ecosystems, the water quality eutrophication caused by P is becoming more and more serious; therefore, the study of the effects of P stress on plant root exudates to understand the specific changes of organic acids in root exudates is important. It is important to better understand the adaptation mechanism of plant roots to nutrient stress. At the same time, the secretory characteristics of specific plants in a specific environment (wetland, woodland, grassland, etc.) can provide basic reference materials for the rhizosphere measurements of environmental pollution control.

In this study, with the increase of culture time, there were significant differences in the amount of organic acid exudates in each time period and the relative contents of organic acids secreted by large root exudates under different P concentrations were also discernible. When the P concentration was 20 mg/L, the organic acids content secreted in each time period decreased, as P is the main nutrient element necessary for plant growth. The P concentration of 20 mg/L for wetland plants has exceeded the amount needed by plants, which may destroy plant root tissue and lead to a decline in plant root exudation ability. At 7 and 28 days of culture, most of the acids showed a decrease in exudation, which may be due to the fact that at 7 days, the plants did not fully adapt to the P stress and the exudation was not very stable. After approximately one month, some plants began to senesce, resulting in a decrease in exudation; the specific reasons for this need to be further verified. PA and carbonic acid are the main organic acids exuded by root systems at all culture levels. Previous studies have shown that many non-mycorrhizal plants, such as *white lupin and rape*, secrete a large amount of organic acids into the root environment under P deficiency stress (Tian *et al*. 2000). However, the main organic acids secreted by different plant roots are not consistent. Such is the case of *Brassica napus*, which secretes a large amount of organic malic acid in the absence of P (Duan 2003). *Alfalfa* secretes citric acid, malic acid, and succinic acid under P deficiency stress. Under P stress, oxalic acid, citric acid, and malic acid were the main organic acids in the root exudates of *Gymnaceae* and *Zhuge* (Zhao and Wu 2014). The content of organic acid in *soybean* root exudates increased under P stress and the relative content of malic acid was the highest (Zhang *et al*. 2011). It can be concluded that terrestrial plants mainly adjust malic acid and citric acid to adapt to environmental changes under the stress of nutrient elements. The reason for this is that each plant has its unique characteristics, for example, the *P stratiotes* is a typical wetland phytoplankton and the growth environment of terrestrial plants is different from that of terrestrial plants. The concentrations of P stress were P deficiency as well as low and high concentrations of P; the relative content of PA was the highest under different culture times, followed by carbonation. This indicates that the high exudation of PA is the adaptation mechanism of phytoplankton in wetlands under external nutrient stress.

Conclusion

Under P stress for 7 days, the exudation of SA, carbonic acid, BCA, and BA was the least in the condition of no P stress. Carbonate, PCA, BA increased substantially with the increase of stress concentration; OA significantly decreased with increasing stress. At 14, 21 and 28 days, the exudation of carbonate, PA, OA, PCA, BA, and total organic acid were the lowest under the condition of no P stress. On the 14th day, the exudation of OA and carbonic acid increased significantly with the increase of stress concentration; at 21 days, the exudation of SA, CA, OA, and BA increased significantly with the increase of stress concentration. At 28 days, the exudation of CA, carbonate, PA, and total organic acid increased with the increase of stress concentration. The results showed that the exudation of organic acids was closely related to the concentration of stress and the time of treatment. P stress could increase the exudation of organic acids in plant roots. PA is the main organic acid exuded by root systems at all culture levels, indicating that the major regulation of PA exudation by large roots under P stress is an important mechanism of active adaptation to the environment.

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Author Contributions

DX and ZJC planned the experiments, made the write up and statistically analyzed the data, ZYY and DX interpreted the result, statistically analyzed the data and made illustrations.

References

Carvalhais LC, PG Dennis, D Fedoseyenko (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

Chen BY (2009). Activation of phosphorus in red soil by root exudates of wheat *Vicia faba* under phosphorus stress. *J Yunnan Agric Univ (Nat Sci Edn)* 24:869–875

Chen F, YJ Meng, HW Shuai, XF Luo, WG Zhou, JW Liu, WY Yang, K Shu (2017). Effects of plant allelochemicals on seed germination and its ecological significance. *Chin J Ecol Agric* 25:36–46

Deng Y, HL Guan, KJ Dai, WJ Zhou, YX Shen (2006). Effects of phosphorus supply on morphogenesis and organic acid secretion in rhizosphere of *Pinus yunnanensis* seedlings. *J Yunnan Agric Univ (Nat Sci Edn)* 28:358–363

Duan HY (2003). Studies on nutritional physiology and genetic behavior of high phosphorus efficiency in *Brassica napus* L. Huazhong Agriculture University, Wuhan, China

Gardner WK (1983). The acquisition of phosphorus by Lupinus albus L. III. The probable mechanisms by which phosphorus movement in the soil/roots interface in enhanced. *Plant Soil* 70:391–402

Horchani F, P Gallusci, P Baldet, C Cabasson, M Maucourt, D Rolin, S Aschi-Smiti, P Raymond (2008). Prolonged root hypoxia induces ammonium accumulation and decreases the nutritional quality of tomato fruits. *J Plant Physiol* 165:1352–1359

Hou XL, CHG Yuan, XP Li, YC Ren, YM Luo, DK Wang (2018). Effects of nitrogen and phosphorus concentrations in Dianchi Lake on the interannual changes of blue, green and diatom. *J Water Ecol* 39:16–22

Huang YF, QY Yang, TP Zhang, JT He (2014). Root exudation characteristics of two plants under hydroponics and their relationship with pollutant removal. *J Ecol* 33:373–379

Khorassani R, H Ursula, R Astrid, S Bernd, K Petr, C Norbert (2011). Citramalic acid and salicylic acid in sugar beet root exudates solubilize soil phosphorus. *BMC Plant Biol* 11; Article 121

Kuang YW, DZH Wen, CHW Zhong, GY Zhou (2003). Root exudates and their role in phytoremediation. *J Plant Ecol* 27:709–717

Li DH, CHL Xiang, YQ Jiang, LY He (2005). Difference of drganic acid secretion in different rice varieties under low phosphorus stress. *Chin Agric Sci Bull* 21:186–188

Li H, XRYang, B Weng, JQ Su, S Nie (2016). The phenological stage of rice growth determines anaerobic ammonium oxidation activity in rhizosphere soil. *Soil Biol Biochem* 100:59–65

Li M, X Zh Ma, W Wang (2012). Preliminary study on removal of nitrogen and phosphorus from water body by *Pistia stratiotes*. *Res Environ Yangtze River Basin* 21:1137–1142

Liu SL, HY Hu, YX Sun, J Yang (2009). Study on growth and root exudates of three wetland plants under hydroponics. *Environ Sci* 30:1901–1905

Liu TT, YT Qin, YY Wu, J Zhang, YR Zhang (2017). Solvent extraction of organic sulfur compounds from tiller onion and its GC-MS analysis. *Food Sci* 38:151–156

Ma RC, Q Liu, H Li, XY Shi, J Li (2017). Effects of phosphorus deficiency stress on root development and nutrient uptake of sweet potato in early stage. *J North Chi Agric* 32:171–176

Qin BQ, G Gao, GW Zhu, YL Zhang, YZ Song, XM Tang, H Xu, JM Deng (2013). Lake eutrophication and its ecosystem response. *Sci Bull* 58:855–864

Qin L, H Jiang, J Tian, J Zhao, H Liao (2011). Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. *Plant Soil* 349:25–36

Roy ED (2017). Phosphorus recovery and recycling with ecological engineering: A review. *Ecol Eng* 98:213–227

Scheffer M, S Carpenter, JA Foley, C Folke, B Walker (2001). Catastrophic shifts in ecosystems. *Nature* 413:591–596

Song XJ, SN Li, J Guo, W Wei, YL Yu (2017). Effect of environmental change on root system of wetland plants. *J Water Ecol* 38:1–9

Song XJ, SN Li, J Guo, YL Yu, ZW Liu, W Wei (2018). Effects of different salt levels on root growth and physiological characteristics of tamarix chinensis cutting seedlings. *Ecol J* 38:606–614

Tang X, G Gao, J Chao, X Wang, G Zhu, B Qin (2010a). Dynamics of organic-aggregate-associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in china. *Limnol Oceanogr* 55:469–480

Tang L, Q Yang, JP Qiu, XD Li (2010b). Comparative analysis of rhizosphere exudates of reed and typha and their effects on rhizosphere. *J Harbin Commer Univ (Nat Sci Edn)* 26:425–429

Tian ZM, FL Qin, B Wang (2003). Comparative study on collecting methods of root exudates from phosphorus-deficient white *lupin*. *J NW Univ Agric For Sci Technol (Nat Sci Edn)* 31:154–158

Tian ZM, CHJ Li, CH Wang, ZJ Zhao (2000). Comparison of organic acids secreted from root tips of phosphorus-deficient white lupin bean. *Mol Plant* 26:317–322

Victor KK, Y Seka, KK Norbert (2016). Phytoremediation of wastewaters toxicity using water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*). *Intl J Phytoremed* 18:949–955

Wang RL, JY Liu, TR Guo (2014). Effects of aluminum toxicity and low phosphorus stress on organic acid secretion from rice seedling growth roots. *Anhui Agric Sci* 6:1603–1606

Wei XJ, M Liu, L Zheng, AZ Ding, Y Li, X Zhao, MX Liu (2016). Effect of salt stress on root exudates of Reed. *J Beijing Normal Univ (Nat Sci Edn)* 52:44–48

Xiao XM, JSH Liu, CH Zhou, GX Wang, GY Chen, HJ Yue (2014). Difference of root exudates of macadamia nut seedlings under different phosphorus levels. *J Trop Crops* 35:261–265

Xu XH, GJ Ding (2006). Physiological and biochemical responses of *Pinus massoniana* to low phosphorus stress. *For Sci* 42:24–28

You HL, XU Li-Gang, JH Jiang (2013). Research Progress of growth dynamics and environmental adaptability of roots of wetland plants. *Res Environ Yangtze Basin* 1:52–58

Yu HY, G Shen, X Gao (2013). Determination of tobacco root exudates by GC-MS. *Acta Tabacaria Sin* 19:64–72

Yu JD, DY Yin, JM Wu, CHF Zhou, XQ Ma (2017). Research Progress on adaptation mechanism of low phosphorus stress in forest trees. *World For Res* 30:18–23

Yu Y, M Zhang, SHQ Qian, DM Li, FX Kong (2010). Present situation and evolution of lake water quality in Yungui plateau. *Lake Sci* 22:820–828

Zhang RM, D Zhang, HW Chen, J Bai, Y Gao (2007). Study on extraction method of root exudates from *Haloxylon ammodendron* Seedling. *Res Environ Arid Areas* 21:153–157

Zhang ZH, Y Chen, SF Han, MC Zhang, DM Wang (2011). Effects of low phosphorus stress on root growth characteristics, secretion and organic acids in soybean. *Chin J Oil Crops* 33:135–140

Zhang ZR, XF Zhang, J Guo, JQ Wang (2018a). Experimental study on purification of eutrophication water by three new floating bed plants. *J Univ Sci Technol Chin* 3:221–228

Zhang ZX, JK Liu, ZHM Zhang, MX Zhang (2018b). Comparison of removal efficiency of nitrogen and phosphorus in water by artificial floating Island planted with different plants and their combinations. *Wetland Sci* 16:273–278

Zhao K, BH Zhou, WZ Ma, LM Yang (2016). The Influence of different environmental stresses on root-exuded organic acids: A review. *Soils* 48:235–240

Zhao K, Y Wu (2014). Rhizosphere calcareous soil p-extraction at the expense of organic carbon from root-exuded organic acids induced by phosphorus deficiency in several plant species. *Soil Sci Plant Nutr* 60:640–650

Zhao X, DP Xu, XJ Liu, NN Zhang, ZJ Yang (2018). Effects of phosphorus supply on growth and foliar nutrition of *Dalbergia odorifera* seedlings. *Bull Bot Res* 38:218–224

Zhou C, LF Yu, GJ Cai, M An, PH Xia (2016). Study on temporal and spatial variation of water quality and water quality zonation of wetland Lakes in the Caohai plateau. *J Water Ecol* 37:24–30

Zou YH, RY Zhang, JA Chen, LY Wang, DP Lu (2018). Application of clay minerals in the control of phosphorus pollution in eutrophic water and sediment. *Adv Earth Sci* 33:578–589