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Research Article

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Characteristics of the root exudate release system of typical plants in plateau lakeside wetland under phosphorus stress conditions

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Abstract: In this study, the root exudates of wetland plants, Pistia stratiotes, black algae, and Cyperus alternifolius, exposed to six phosphorus concentration gradients (0, 0.2, 1, 5, 10, and 20 mg/L) were characterized. The experimental seedlings were cultivated in Hoagland solutions, which were then extracted, decompressed, and concentrated with CH₂Cl₂; subsequently, a gas chromatography-mass spectrometry (GC-MS) analysis was performed to study the root exudates effects under different phosphorus concentrations. Results showed the existence of several organic compounds, such as alkanes, esters, alcohols, amines, benzene, and acids (phthalic acid, cycloheptasiloxane, benzoic acid, and cyclopentasiloxane) in the root exudates of the wetland plants. The relative contents of phthalate, benzene dicarboxylic acid, and cyclohexasiloxane in the root exudates first increased, and then decreased, with the change in phosphorus concentration. The relative contents of three compounds in *Pistia* were the highest at 1 mg/L of phosphorus, and the lowest relative contents of phthalic acid and benzene dicarboxylic acid were observed at 20 mg/L of phosphorus. However, the relative content of cyclohexasiloxane was the lowest in the absence of P stress. In black algae, the relative contents of the three compounds were 36.66, 16.24, and 14.61%, respectively. The relative content of cyclohexasiloxane in the black algae first decreased and then increased, with its lowest relative content occurring at 5 mg/L of phosphorus and the highest at 10 mg/L of phosphorus. In Cyperus alternifolius, the highest relative concentrations of the four compounds: phthalic acid, dimethyl phthalate, octadecane, and diphenyl sulfone in Cyperus were observed at 5 mg/L phosphorus and the lowest at 10 mg/L phosphorus.

Keywords: phosphorus deficiency, *Pistia stratiotes*, *black* algae, Cyperus alternifolius, root exudates

1 Introduction

Root secretion comprises the metabolites secreted by plant roots in the process of growth through the bleeding effect releasing to growth matrix. Plant roots are the main carbon source for microorganism in the surrounding environment, which is essential to maintain the rhizosphere micro-ecological characteristics and ecological system stability [1,2]. Root secretion could increase the dissolution rate of the root nutrient elements, thereby promoting nutrient absorption in plants and microbial activity through passing the rhizosphere effect root, which can also take the initiative to resist and adjust the adverse environmental conditions; it is very important to maintain the rhizosphere micro-ecological characteristics and ecological system stability [3,4]. Root secretion can also activate plant internal tissues lacking nutrition elements, thus promoting the growth of plants [5]. It is an important medium of material and information communication between plants and living environment, and it is closely related to the phytoremediation of organic pollutants [6]. In addition, the change of organic acids in root secretion is an important mechanism of plant roots responding to nutrient, heavy metal, water, and other environmental stresses. The organic acids secreted by the roots are released into the rhizosphere, where they change the root microbial structure and the physical and chemical properties of soil [7].

Plant root secretion is mainly dependent on the plant species; different metabolites are secreted by

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different plants. However, external environmental stresses can also affect the root secretion to a certain extent. During adverse environmental stresses, plants release some compounds to influence the growth of other surrounding plants or change their root microbial structure and the physical and chemical properties, reach their maximum absorption and use of nutrients [8]. When encountering metal stress, the root system secretes organic acids and other compounds to change the pH value of the rhizospheric environment, thereby lowering the activity of the metal and reducing its uptake of metals by plants [7].

Wetland is a special ecological system having a strong ecological purification function. Wetland plants are an important part of an effective wastewater purification system, with their roots being an important bridge for material circulation and energy flow between them and the surrounding environment [9]. At present, several wetlands exist in the state of eutrophication, and phosphorus is the conditionality factor for lake eutrophication [10]. The absorption and removal of phosphorus in wetland plants mainly occurs through the root, with root secretions playing a major role in the whole process [11]. Therefore, this paper studies the species and composition characteristics of root secretion in wetland plants that have great significance phosphorus deficiency stress. We further understand the physiological and biochemical processes of roots and their regulatory mechanisms in wetland.

In recent years, more and more scholars at home and abroad have paid great attention to the study on root exudates, the changes and effects of different phosphorus stress on root exudates. For example, Li-yuan et al. [12] studied the secretion of organic acid by rice roots under the low phosphorus stress conditions and the secretion differences among the different varieties of rice. In addition, a previous study showed that the sweet potatoes and beets grown in a phosphorusdeficient environment showed a change in their root exudates, leading to a change in their root morphology and composition, which in turn promoted the release of phosphorus in the soil [13,14]. Niu et al. [15] studied the secretion of organic acids in Eucalyptus under different phosphorus levels and found that the concentration of the secreted organic acids under low phosphorus levels was higher than that under high phosphorus levels. Wang et al. [16] concluded that different crops have different morphological and physiological characteristics under low phosphorus conditions. In addition, studies have shown that phosphorus deficiency could affect

root development and nutrient absorption in sweet potato [17]. Rui-ji Yang and Jun-yi Niu [18] studied the effects of phosphorus stress and its duration on the amount of secretion in the roots of rapeseed.

However, for now, the effects of phosphorus stress on plant root secretion has been mainly concentrated on the crops, and the effects of low phosphorus stress or phosphorus deficiency stress have been mainly concentrated on terrestrial plant root secretion. The effects of nutrient stress on plant root secretion mainly focus on the low-phosphorus stress on the secretion of organic acids and morphological changes in plant roots. Although researchers have a certain understanding of the changes in plant roots under phosphorus stress, but the roots secretion system in lake plateau wetland plant has been rarely studied, the characteristics and effects of wetland plant root secretion under phosphorus stress have been rarely reported.

Pistia stratiotes is a perennial floating water herb, which grows easily and has a strong capacity to purify sewage and effectively improve eutrophic water quality [19,20]. Hydrilla verticillata, commonly called as seagrass, can transform the phosphorus present in a wetland substrate into its available state and is capable of purifying water quality in a eutrophic sewage [21,22]. As the root parts in the sediment submerged aquatic plants completely, the flood water and soil should be adopted from an environment closer to the original ecological environment, but the flooded soil cultivation method should be used to collect the root secretion solution, containing the inclusions and the bleeding xylem sap of the root system itself; due to a big error in the root secretion analysis, hydroponics can avoid the plant roots injury effectively, not including plant roots itself [23,24], Cyperus alternifolius is a kind of widely distributed wetland hydrophyte, which survives easily and has a great effect on wetland management. In this experiment, we took three wetland plants as test materials, set in an indoor hydroponic cultivation system, and analyzed their root secretions, the relative contents of their characteristic chemical components, and the effects of different concentrations of phosphorus on their root secretions. Thus, our study provides a theoretical basis for wetland floating plants, influencing their root secretions factors, and on each secretion relationship for further research. Furthermore, our study reveals the root-secreted defense compounds in response to external stress in wetland plants to find the rhizosphere regulation measures against wetland pollution.

2 Materials and methods

2.1 Hydroponic cultivation and solution ratio

Twenty-one plastic buckets containing the Hoagland nutrient solution were used as a hydroponic device. Forty-two healthy seedlings, each of *Pistia stratiotes*, *black* algae, and Cyperus alternifolius plants, were used. Each plastic bucket was equipped with an aerator to prevent root rot by allowing proper aeration. In order to avoid the proliferation of algae in the barrel, aluminum foil was used for the shading treatment [25]. Subsequently, we selected the plant size, growth period, and plant height and transplanted the plants into the test device, in accordance with the phosphorus gradient set six cases concentration variant, each bucket containing three test plants and three replicates for each plant species.

The plastic bucket was placed in a greenhouse. The composition of the Hoagland nutrient solution was as follows: $K_2SO_4 \ 0.75 \times 10^{-3} \ \text{mol/L}, \ MgSO_4 \ 0.75 \times 10^{-3} \ \text{mol/L},$ KCL 1 \times 10⁻³ mol/L, Ca(NO₃)₂ 2.0 \times 10⁻³ mol/L, H₃BO₃ 1 \times $10^{-5} \, \text{mol/L}$, CuSO₄ 1 × $10^{-7} \, \text{mol/L}$, MnSO₄ 1 × $10^{-6} \, \text{mol/L}$, $ZnSO_4 1 \times 10^{-6} \text{ mol/L}, (NH_4)6Mo_7O_{24} 5 \times 10^{-6} \text{ mol/L}, \text{ and Fe-}$ EDTA 1×10^{-4} mol/L. The nutrient solution was diluted four times to make a hydroponic solution [26].

2.2 Collection and separation of root exudate

The whole root systems of the plants were washed with deionized water and then treated with the root exudate collection fluid (composed of collection fluid: H₃BO₃ 5 μmol/L, CaCl₂ 600 μmol/L, KCl 100 μmol/L, and MgCl₂ 200 µmol/L, pH 5.6). The roots were rinsed thrice within a black plastic bag to cover the entire root, and the roots were moved to a 50-mL beaker; the collected liquid root secretion in the beaker is collected for 60 days, with the condition of lamplight illuminate for 4 h, and the plant was transplanted to 11 0.5 mmol/L CaCl2 solution and cultivated for 4 h (9:00-13:00 h), with CH₂Cl₂ extract the root lotion, and each treatment need to repeat three times. Three drops of microbial inhibitors were added to inhibit the decomposition of organic acids by microorganisms; finally, 200 mL of root exudate solution was collected using vacuum pan evaporation at 38°C, reducing the concentration turn to 10 mL before setting it aside.

2.3 Determination of root exudate

Rotary evaporation was used to concentrate the root secretion extract using a 0.45-µm needle filter membrane, followed by reduced pressure concentration to dry the extract and addition of CH₂Cl₂ through a 0.45-µm needle membrane. Finally, 0.5 mL of the extract was taken in a small brown bottle for conducting a GC-MS analysis. The compositions of the root exudates in this study were determined by gas chromatography/mass spectrometry (Agilent 7890B type instrument). The following chromatographic conditions [27,28] were used: an HP-5ms capillary column (30 m \times 250 μ m \times 0.25 µm) was used. The injection port temperature was 260°C, and He was used as the carrier gas (purity was not less than 99.999%) with a flow rate of 1 mL min⁻¹, sample quantity 1 µL, splitless injection, and 1 min to open the bypass valve. The column temperature was programmed as follows: the initial temperature was 50°C for 2 min, temperature programmed to 150°C per minute with 20°C first, then the temperature programmed to 220°C with 5°C per minute, and finally, the temperature programmed to 250°C, with per minute 6°C, and keep for 15 min.

Mass spectrometry conditions: source of electron impact (EI), ionization energy was 70 eV; the ion source temperature was 200°C; the interface temperature was 280°C; the quaternary pole temperature was 150°C; the solution delay time was 3.75 min; the scanning mode was full SCAN mode (SCAN) and the SCAN range was M/Z33-453; and a standard tuning file was used. A manual analysis and ion flow diagram of the NIST08 mass spectrum database standard map were used to check and to determine the relative content (%) of unknown material, using the computer retrieval according to the chromatogram peak area of each detected component.

2.4 Statistical analysis

In this study, excel wps2016 and spss21 software were used for data processing and statistical analysis. The lsd method was used for multiple comparisons. The significant level α was 0.05, the extremely significant level α was 0.01.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Results of GC-MS identification of root exudate in three wetland plants

3.1.1 GC-MS scanning of root exudate in three wetland plants

In this study, three wetland plants P. stratiotes, black algae, and Cyperus alternifolius were exposed to the hypotonic solutions containing 0 (phosphorus-free) 0.2, 1, 5, 10, and 20 mg/L phosphorus. The GC-MS scan map of the root secretion samples showed the intensive distribution and characteristic peaks of the more obvious chemical components. The baseline drift was not large; therefore, the test results can be trusted.

The scan map of the plants root exudates exposed to different phosphorus concentrations showed obvious differences. The P. stratiotes root secretion showed a more obvious characteristic peak in the absence of the phosphorus stress than in the presence of the phosphorus stress. When exposed to 5 mg/L phosphorus, the black algae root secretion showed a more obvious characteristic peak when exposed to other phosphorus concentrations.

3.1.2 GC-MS characterization of root exudate in Pistia stratiotes

The chemical composition of the P. stratiotes root exudates exposed to a concentration gradient of phosphorus and the relative contents of their individual components were determined by the artificial analysis mass spectrogram, checked against the NIST08 mass spectrum database of standard map analysis. According to the map and MS database analysis, the substance was detected according to the matching degree greater than 85% and the relative content greater than 0.5% for further analysis, which was shown in Table 1.

From the analysis results shown in Table 1, the root secretions and the differences in their relative qualities under different phosphorus concentrations are more apparent. The hydroponic solution containing no phosphorus resulted in the root secretion, containing 30 kinds of chemical compounds, including alkanes, esters, alcohols, amines, benzene, and acid compounds. The acid compounds included phthalates, siloxane, naphthylamine, and 26 alkanes, and their highest relative contents were 20, 8.47, 6.39, and 4.12%, respectively. Under the phosphorus stress of 0.2 mg/L, the root secretions showed the presence of 19 kinds of chemical compounds, including alkanes, esters, amines, and acids. Among these, phthalic acid showed the highest relative content, accounting for 49.83% of the total content. At the concentration of 1 mg/L phosphorus, the root secretion contained 16 kinds of chemical compounds, whereas at a concentration of 5 mg/L phosphorus, the root secretion contained 14 chemical compounds, including alkanes, ester, and acid compounds. The highest relative contents of phthalates at 1 and 5 mg/L phosphorus were 56.86 and 40.65%, respectively. At 10 mg/L, 22 kinds of chemical compounds were detected in the root secretions with high relative contents of alkanes, esters, amines, and acids. Among acids, the relative content of phthalic acid was the highest, accounting for 34.65%. At 20 mg/L of phosphorus, the root secretions showed the presence of 11 compounds, among which the relative content of cyclopentasiloxane was the highest being 16.11%, and the contents of other compounds were relatively low. A significant difference was observed in the types of compounds and in the relative contents of the individual compounds that detected in the root secretions of the wetland plants under six different phosphorus treatments. The experimental data showed a reduction in the relative contents by many types of compounds in the root secretion. The wetland plants *Pistia* under phosphorus stress adapt to the environment by self-regulating the composition of their root secretions, especially by secreting a large amount of phthalic acid.

3.1.3 GC-MS identification of root exudate in black algae

The results presented in Table 2 show a significant difference in the number of species and relative quality of the black algae root secretions under different phosphorus concentrations. When exposed to a hydroponic solution containing no phosphorus, the root secretion of black algae contained 15 chemical components, including alkanes, ester, and acid compounds, including silane, siloxane, phthalate, and diisooctyl phthalate, which showed the highest relative content, accounting for 16.24, 14.61, 6.24, and 5.96% of all compounds. The relative content of other compounds was relatively low. At the concentration of 0.2 mg/L phosphorus, the root secretion contained 16 different compounds, including alkanes, sulfur, ketone, acid, and amine compounds. Among these compounds, phthalic acid showed the highest relative content, accounted for 21.64%, followed by heptyl siloxane and diphenyl

Table 1: Major root exudates of Pistia stratiotes exposed to a concentration gradient of phosphorus

Concentration mg/L	Chemical name	Area%	Chemical name	Area%	Chemical name	Area%
0	Cyclotetrasiloxane	3.68 ± 0.36	9-Octylhexadecane	0.58 ± 0.04	Naphthylamine	6.39 ± 0.19
	Tetradecyl cyclohexasiloxane	1.18 ± 0.58	Hexacosane	5.65 ± 0.25	Naphthyl acetamide	1.27 ± 0.18
	Cyclohexasiloxane	2.17 ± 0.23	Heptacosane	4.26 ± 0.32	Butylated hydroxytoluene	2.45 ± 0.11
	17 alkanes	0.72 ± 0.12	24 alkanes	1.96 ± 0.08	Erucic acid amide	4.45 ± 0.39
	Octadecane	1.44 ± 0.09	Cyclopentasiloxane	8.47 ± 0.25	Benzene dicarboxylic acid	1.35 ± 0.28
	2,6,10,14-1- Tetramethylhexadecane	0.73 ± 0.06	Docosanoic	1.37 ± 0.04	Palmitic acid	2.57 ± 0.23
	43 Alkanes	0.63 ± 0.01	Octacosane	1.41 ± 0.05	Phthalic acid	20 ± 0.63
	20-carbon alkane	3.27 ± 0.29	Dibutyl phthalate	4.12 ± 0.48	Sulfurous acid	0.82 ± 0.32
	Eicosane	0.76 ± 0.29	Dodecanol	1.66 ± 0.07	Benzoic acid	1.65 ± 0.06
	21 alkanes	2.74 ± 0.12	Octadecanol	1.07 ± 0.03	Oxalic acid	0.42 ± 0.02
0.2	Cyclohexasiloxane	3.67 ± 0.26	Silane	2.43 ± 0.19	Phthalic acid	49.83 ± 0.56
	Octadecane	1.52 ± 0.01	20-Carbon alkane	6.63 ± 0.03	Terephthalic acid	7.94 ± 0.18
	26 alkanes	2.75 ± 0.12	Heptacosane	3.74 ± 0.05	Benzoic acid	4.98 ± 0.01
	21 alkanes	2.26 ± 0.04	Dibutyl phthalate	1.97 ± 0.08	Methoxyacetic acid	1.31 ± 0.02
	2,6,10,15-1- Tetramethylhexadecane	2.03 ± 0.01	Acetamide	2.62 ± 0.01	Silicate	2.62 ± 0.05
	Dodecyl cyclohexane	2.89 ± 0.18	Naphthylamine	4.27 ± 0.07	Benzene dicarboxylic acid	4.11 ± 0.17
	19 alkanes	2.56 ± 0.12				
1	Dodecyl cyclopentane	2.73 ± 0.07	Diiso-octyl phthalate	3.51 ± 0.13	Phthalic acid	56.86 ± 0.25
	Dodecyl cyclohexasiloxane	1.39 ± 0.11	Oxalic acid	2.26 ± 0.09	Benzene dicarboxylic acid	5.36 ± 0.11
	Cycloheptane	1.90 ± 0.01	Benzoic acid	7.05 ± 0.03	Glutaric acid	1.89 ± 0.09
	26 Alkanes	2.27 ± 0.21	Acetic acid	1.78 ± 0.06	Salicylic acid	6.23 ± 0.01
	44 Alkanes	2.04 ± 0.03	Phenol	2.27 ± 0.01	Arsenite	4.12 ± 0.02
	Cyclohexasiloxane	6.39 ± 0.35				
5	Tetradecyl polysilicon oxide	6.35 ± 0.01	Silane	1.76 ± 0.00	Phthalic acid	40.65 ± 1.02
	19 alkanes	2.08 ± 0.03	Cyclohexasiloxane	5.02 ± 0.15	Benzene and sulfonic acid	1.38 ± 0.00
	20-Carbon alkane	1.56 ± 0.01	Eicosane	1.83 ± 0.03	Sulfurous acid	2.47 ± 0.01
	Tetradecyl cyclohexasiloxane	4.11 ± 0.09	Diiso-octyl phthalate	4.25 ± 0.15	Benzene dicarboxylic acid	3.15 ± 0.27
	Cycloheptane	9.45 ± 0.03	Methoxyacetic acid	2.49 ± 0.04		
10	Cyclohexasiloxane	2.45 ± 0.53	Eicosane	1.85 ± 0.11	Naphthylamine	6.1 ± 0.06
	19 alkanes	2.90 ± 0.12	Silane	9.92 ± 0.23	Phthalic acid	34.65 ± 0.72
	36 Alkanes	3.17 ± 0.03	Heptacosane	3.67 ± 0.01	Sulfurous acid	3.21 ± 0.15
	Dodecyl cyclohexasiloxane	1.72 ± 0.04	29 alkanes	4.46 ± 0.05	Silicate	1.57 ± 0.01
	Cycloheptane	3.03 ± 0.01	21 alkanes	3.31 ± 0.04	Palmitic acid	0.59 ± 0.00
	17 alkanes	1.50 ± 0.03	Diiso-octyl phthalate	2.09 ± 0.05	Formic acid	1.59 ±
	Octadecane	2.43 ± 0.05	Phenethylamine	3.27 ± 0.00	Benzene dicarboxylic acid	3.79 ± 0.38
	20-Carbon alkane	4.20 ± 0.11				
20	Dodecyl cyclopentadimethylsiloxane	0.90 ± 0.13	Cyclohexasiloxane	6.09 ± 0.30	Palmitic acid	0.84 ± 0.01
	Dodecyl cyclohexasiloxane	6.95 ± 0.01	Cyclotetrasiloxane	0.80 ± 0.01	Phthalic acid	3.43 ± 0.40
	Octadecyl cyclotetramethylsiloxane		Diiso-octyl phthalate	0.81 ± 0.00	Benzene dicarboxylic acid	0.71 ± 0.35
	Cycloheptane	16.11 ± 0.14	Benzoic acid	15.82 ± 0.22	•	

Note: the figures in the table are all 3 groups of repeated mean value plus or minus standard error.

Table 2: Major root exudates of black algae exposed to a concentration gradient of phosphorus

Concentration mg/L	Chemical name	Area%	Chemical name	Area%	Chemical name	Area%
0	Decyl	0.71 ± 0.01	Silane	16.24 ± 0.14	Palmitic acid	1.52 ± 0.02
	cyclopentadimethylsiloxane					
	Cycloheptane		Cyclohexasiloxane	5.26 ± 0.52	Stearic acid	0.73 ± 0.00
	Octadecyl	0.61 ± 0.00	Diiso-octyl phthalate	6.24 ± 0.21	Phthalic acid	5.96 ± 0.72
	cyclotetramethylsiloxane					
	Dodecyl cyclohexane		Carboxylic acid	1.34 ± 0.07	Formic acid	1.60 ± 0.01
	17 alkanes	0.54 ± 0.06	Benzene dicarboxylic acid	0.63 ± 0.19	Stearic acid	0.73 ± 0.02
0.2	Cyclopentadimethylsiloxane	0.95 ± 0.05	Diphenyl sulfone	7.40 ± 0.32	Phthalic acid	21.64 ± 0.91
	Dodecyl cyclohexane	4.47 ± 0.17	1-Methylpyrrolidone	3.19 ± 0.16	Benzoic acid	3.08 ± 0.23
	Cycloheptane	8.43 ± 0.25	Propionamide	1.2 ± 0.09	Acetic acid	2.42 ± 0.15
	Cyclotetrasiloxane	1.53 ± 0.07	Phenylacetic acid	6.58 ± 0.11	Carboxylic acid	1.69 ± 0.21
	Silane	4.86 ± 0.17	Formic acid	1.95 ± 0.06	Benzene dicarboxylic acid	1.24 ± 0.10
	Cyclohexasiloxane	4.71 ± 0.32			aciu	
1	Cyclohexasiloxane		Diphenyl sulfone	6.59 ± 0.19	Sulfurous acid	1.48 ± 0.08
1	Cycloheptane		Cyclopentanol	1.88 ± 0.08		3.32 ± 0.19
	19 alkanes	3 54 + 0 65	Naphthylamine	5.21 ± 0.00	Carboxylic acid	1.31 ± 0.25
	Eicosane	3.76 ± 0.28		1.90 ± 0.17	Silicate	2.62 ± 0.13
	21 alkanes	3.31 ± 0.12	Phthalic acid	36.66 ± 0.80	Acetic acid	4.60 ± 0.33
	Silane		Benzoic acid	5.34 ± 0.13	Formic acid	1.87 ± 0.02
	1-Methylpyrrolidone		Methoxyacetic acid	1.47 ± 0.02	Torrine dela	1.07 ± 0.02
5	Cycloheptane		Pyrrolidone	4.23 ± 0.31	Phenylacetic acid	6.50 ± 0.31
	17 alkanes	2.05 ± 0.20	Phenethylamine	4.46 ± 0.22	Carboxylic acid	1.63 ± 0.12
	Methyl 19		Naphthylamine	4.10 ± 0.27	Silicate	1.61 ± 0.15
	20-carbon alkane	5.64 ± 0.59	, ,	1.34 ± 0.35	Acrylic acid	1.34 ± 0.21
	Cyclotetrasiloxane		Nonyl aldehyde caprylic aldehyde	1.58 ± 0.05	Arsenite	1.60 ± 0.01
	Silane	2.92 ± 0.17	Benzene dicarboxylic acid	2.52 ± 0.21	Benzoic acid	1.52 ± 0.07
	Cyclohexasiloxane	2.74 ± 0.38	Phthalic acid	31.64 ± 0.94	Sulfurous acid	2.71 ± 0.19
	Diphenyl sulfone	8.08 ± 0.33	Oxalic acid	1.44 ± 0.12		
10	Cyclohexasiloxane	5.65 ± 0.31	Formic acid	1.89 ± 0.20	Phthalic acid	36.54 ± 1.45
	18 alkanes	0.61 ± 0.03	Silicate	1.20 ± 0.09	Benzene dicarboxylic acid	2.81 ± 0.36
	20-Carbon alkane	0.56 ± 0.00	Benzoic acid	3.20 ± 0.11	Oxalic acid	1.04 ± 0.02
	Cycloheptane	9.59 ± 0.27	Acrylic acid	2.35 ± 0.08	Silane	5.56 ± 0.32
	Diphenyl sulfone		Salicylic acid	6.01 ± 0.21	Acetic acid	3.32 ± 0.14
	1-Methylpyrrolidone	1.44 ± 0.01	Sulfurous acid	0.58 ± 0.00	Propionic acid	0.70 ± 0.00
	phenethylamine	6.01 ± 0.32	Benzoic acid	0.84 ± 0.09		
20	Cyclohexasiloxane	3.78 ± 0.32	2-Chloroacetaldehyde	3.30 ± 0.14	Phthalic acid	43.4 ± 0.35
	Cycloheptane	5.96 ± 0.28	Phenethylamine	1.57 ± 0.01	Benzoic acid	1.55 ± 0.19
	20-carbon alkane	2.07 ± 0.03	Acrylic acid	8.25 ± 0.31	Silicate	0.70 ± 0.00
	Diphenyl sulfone	5.96 ± 0.31	Silicate	3.42 ± 0.03	Sulfurous acid	2.48 ± 0.02
	1-Methylpyrrolidone	5.03 ± 0.04	Benzene	3.25 ± 0.21		
			dicarboxylic acid			

Note: the figures in the table are the mean values plus or minus standard errors of the three replicates of each group.

sulfone, accounting for 8.43 and 7.40%, respectively. Under the stress of 1 mg/L phosphorus, the root secretions contained 20 kinds of compounds, among which alkanes, sulfur, amine, alcohol, and acid compounds showed relatively high contents, with phthalic acid showing the highest relative content, accounting for 36.66%. At 5 mg/L phosphorus, the root secretions showed the presence of 23 kinds of compounds, including alkanes, aldehydes, amines, alcohols, sulfur, and acids. At 10 mg/L phosphorus, the root secretions contained 20 kinds of compounds, with high contents of alkanes, sulfur, amine, ketone, and acids, among which the relative content of phthalic acid was the highest, accounting for 36.54%. At 20 mg/L phosphorus, the root secretions contained 14 kinds of compounds, among which the relative content of phthalic acid was the highest, accounting for 43.4%.

Other compounds were relatively low in content. A significant difference was observed in the types of compounds and in the relative contents of the individual compounds, which were detected in the root secretions of wetland plants exposed to six different phosphorus treatments. The experimental data showed that the content of phthalic acid was prominent and relatively high under each phosphorus stress treatment. The results showed that the content and quantity of organic acids in root secretions could be adjusted by the root system in *black algae* under phosphorus stress.

3.1.4 GC-MS characterization of root exudate in Cyperus alternifolius

According to the analysis results presented in Table 3. the number of species and relative qualities of the C. alternifolius root exudates grown were significantly different under different phosphorus concentrations. When C. alternifolius was grown in a hydroponic solution containing no phosphorus, its root secretion contained 17 chemical components, including alkanes, ketone, amine, ester, sulfur, and acid compounds, including phthalates, diphenyl sulfone, dioctyl phthalate, and acrylic acid, which showed the relative contents 56.07, 9.03, 5.13, and 5.30%, respectively. At concentration of 0.2 mg/L phosphorus, C. alternifolius produced 17 different root secretions including alkanes, ketone, ester, sulfur, and acid compounds, among which phthalic acid, dimethyl phthalate, diphenyl sulfone, and bromine butyric acid showed the highest relative content, accounting for 50.16, 13.43, 5.59, and 5.04%, respectively. Under the

stress of 1 mg/L phosphorus, the Cyperus alternifolius root exudate contained 22 chemical components, mainly including alkanes, ketones, sulfur, amine, ester, and acid compounds. The content of phthalic acid was the highest, being of 54.62%. At the concentration of 5 mg/L phosphorus, the root exudate showed the presence of 17 chemical components, including alkanes, ketone, sulfur, ester, and acid compounds, with phthalic acid showing the highest relative content of 60.53%. Under 10 mg/L phosphorus stress, the C. alternifolius root exudate showed the presence of 31 chemicals, including alkanes, sulfur, amine, and acid compounds. The phthalic acid was the highest, accounting for 39.22%. At 20 mg/L phosphorus, the root exudate contained 29 chemical components, including alkanes, ketone, sulfur, amine, aldehyde, acids, and other compounds, among which phthalic acid showed the highest relative content, accounting for 44.28%. For a specific compound under six different stress conditions, the relative content varied greatly. However, phthalic acid showed the highest relative content in root secretions under each stress condition. This indicates that *C. alternifolius* can adapt to the environment by self-regulating the content and quantity of organic acids in root exudate under phosphorus stress.

3.2 Relative contents of root exudates in three different wetland plants

3.2.1 Relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretion of *Pistia stratiotes*

tlsb 03w?Through the analysis and comparison of the relative compounds contents listed in Table 1, three compounds with high relative contents were obtained under different phosphorus concentrations (Figure 1). The single factor analysis of variance showed significant differences in the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretions of three wetland plants (P-value = 0.0004 < 0.05; $F = 14.1576 > F_{crit} = 6.3589$; alpha = 0.005). In the figure, the relative contents of all three compounds first increased and then decreased with the change in phosphorus concentrations. Under phosphorus stress, the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane were the highest when the concentration of phosphorus was 1 mg/L, and the relative contents of phthalic acid

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Table 3: Major root exudates of Cyperus alternifolius exposed to a concentration gradient of phosphorus

Concentration mg/L	Chemical name	Area%	Chemical name	Area%	Chemical name	Area%
0	Cycloheptane	4.38 ± 0.11	Naphthylamine	2.36 ± 0.03	Acrylic acid	5.30 ± 0.23
	Octadecane	2.28 ± 0.13	Acetamide	3.82 ± 0.14	Acetic acid	1.74 ± 0.04
	19 alkanes	2.81 ± 0.08	1-Methylpyrrolidone	3.02 ± 0.13	Oxalic acid	1.93 ± 0.08
	21 alkanes	2.88 ± 0.21	Dioctyl phthalate	5.13 ± 0.19	Benzene dicarboxylic acid	3.39 ± 0.26
	20-carbon alkane	1.96 ± 0.02	Diphenyl sulfone	9.03 ± 0.38	Benzoic acid	4.69 ± 0.17
	Cyclohexasiloxane	2.19 ± 0.09	Phthalic acid	56.07 ± 2.24		
0.2	Cyclohexasiloxane	1.53 ± 0.15	Pyrrolidone	3.40 ± 0.11	Phthalic acid	50.16 ± 0.77
	Cycloheptane	3.58 ± 0.20	Dimethyl phthalate	13.43 ± 0.24	Bromine butyric acid	5.04 ± 0.31
	Cetane	1.98 ± 0.00	Dibutyl phthalate	2.13 ± 0.17	Benzene dicarboxylic acid	3.60 ± 0.37
	Octadecane	2.08 ± 0.03	Diphenyl sulfone	5.59 ± 0.31	Sulfurous acid	1.76 ± 0.06
	20-Carbon alkane	2.99 ± 0.14	Acetic acid	3.20 ± 0.08	Silicate	2.89 ± 0.12
	2,6,10,15-1-	2.99 ± 0.21	Acetic acid	3.10 ± 0.02		
	Tetramethylhexadecane					
1	Cycloheptane	3.70 ± 0.11	Octadecane	2.05 ± 0.19	Benzoic acid	3.55 ± 0.41
	Eicosane	2.57 ± 0.09	Tetradecane	1.78 ± 0.05	Sulfurous acid	1.59 ± 0.06
	Cyclohexasiloxane	1.69 ± 0.09	Methyl 19	2.75 ± 0.16	Silane	3.55 ± 0.19
	Cetane	1.97 ± 0.02	Pyrrolidone	2.67 ± 0.08	Bromide acetic acid	4.66 ± 0.13
	26 alkanes	2.93 ± 0.12	Diphenyl sulfone	4.38 ± 0.19	Benzene dicarboxylic acid	3.84 ± 0.14
	20-Carbon alkane	6.18 ± 0.23	Naphthylamine	4.78 ± 0.24	Phthalic acid	54.62 ± 1.23
	21st alkanes	5.08 ± 0.23	Dimethyl phthalate	1.92 ± 0.11	Terephthalic acid	3.22 ± 0.16
	Heptacosane	2.50 ± 0.03				
5	Cycloheptane	5.12 ± 0.21	Pyrrolidone	4.15 ± 0.27	Benzoic acid	3.83 ± 0.25
	Silane	4.34 ± 0.22	Diphenyl sulfone	5.96 ± 0.29	Methoxyacetic acid	2.57 ± 0.17
	Cyclohexasiloxane	2.27 ± 0.13	Dibutyl phthalate	2.22 ± 0.04	Acetic acid	2.39 ± 0.31
	17 alkanes	2.04 ± 0.01	Antibutenedioic acid	3.16 ± 0.14	Oxalic acid	2.34 ± 0.39
	19th alkanes	3.24 ± 0.03	Benzene dicarboxylic acid	4.44 ± 0.10	carbonate	4.60 ± 0.12
	20-Carbon alkane	3.08 ± 0.13	Phthalic acid	60.53 ± 2.75		
10	Ethane	0.97 ± 0.02	Diphenyl sulfone	4.02 ± 0.11	Oxalic acid	2.10 ± 0.07
	Cycloheptane	2.15 ± 0.14	Naphthylamine	1.29 ± 0.02	Propionic acid	1.13 ± 0.05
	Propane	2.43 ± 0.18	Benzoic acid	1.08 ± 0.07	Oxalic acid	0.66 ± 0.00
	Eicosane	0.98 ± 0.08	Acetic acid	0.91 ± 0.01	Pelargonic acid	2.05 ± 0.16
	Cyclohexasiloxane	0.93 ± 0.06	Propionic acid	1.12 ± 0.19	Acetoacetate	0.73 ± 0.02
	Tetradecane	0.65 ± 0.01	Sulfurous acid	0.69 ± 0.05	Silicate	0.74 ± 0.01
	20-carbon alkane	1.60 ± 0.11	caprylic acid		Caproic acid	1.60 ± 0.14
	Pyrrolidone	2.85 ± 0.22	Pyrimidine formic acid	0.94 ± 0.09	Acetic acid	1.60 ± 0.03
	Hexanone	2.83 ± 0.18	Propionic acid	1.04 ± 0.02	Acrylic acid	0.90 ± 0.00
	Propylene	2.46 ± 0.23	Benzene dicarboxylic acid	3.09 ± 0.15	Phthalic acid	39.22 ± 1.82
	Cyclohexene	1.40 ± 0.08	,			
20	Cycloheptane	3.58 ± 0.21	Diphenyl sulfone	4.70 ± 0.24	Acetic acid	1.96 ± 0.02
	Epoxy ethane	1.66 ± 0.03	Naphthylamine	2.63 ± 0.10	Propionic acid	1.58 ± 0.8
	Eicosane	2.29 ± 0.30	Phenethylamine	2.25 ± 0.21	Butanoic acid	1.29 ± 0.03
	Octadecane	1.08 ± 0.00	Glyoxal	1.66 ± 0.08	Sulfurous acid	1.61 ± 0.10
	19 alkanes	2.34 ± 0.05	Trifluoroacetyl group	3.52 ± 0.18		2.21 ± 0.17
	20-Carbon alkane	2.58 ± 0.07	Benzoic acid	3.52 ± 0.13	Acetic acid	1.44 ± 0.09
	21 alkanes	2.29 ± 0.18	Trichloroacetic acid	1.78 ± 1.10	Acrylic acid	2.30 ± 0.13

Table 3: continued

Concentration mg/L	Chemical name	Area%	Chemical name	Area%	Chemical name	Area%
	Cyclohexasiloxane	1.32 ± 0.10	Propanesulfonic acid	2.08 ± 0.06	Oxalic acid	2.32 ± 0.21
	Propane	1.61 ± 0.03	Benzene dicarboxylic acid	3.56 ± 0.39	Phthalic acid	44.28 ± 2.79
	Pyrrolidone	3.33 ± 0.14				

Note: the figures in the table are the mean values plus or minus standard error of the three replicates of each group.

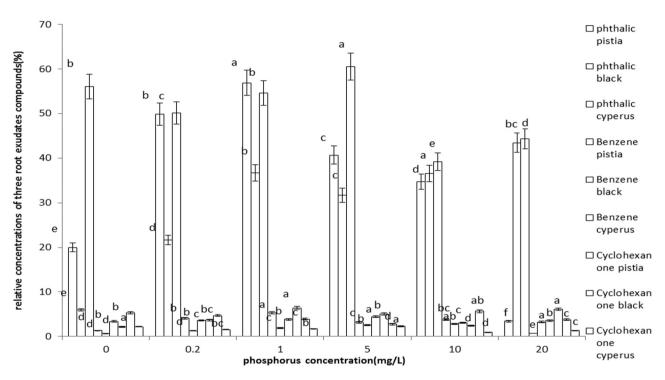


Figure 1: Different relative concentrations of the three root exudates compounds under phosphorus gradients stress. Note: the same letters in the figure indicate no significant difference (P > 0.05), while different letters indicate significant difference (P < 0.05).

and benzene dicarboxylic acid were the lowest when the concentration of phosphorus was $20\,\mathrm{mg/L}$. In the absence of phosphorus stress, the relative content of cyclohexanol siloxane was the minimum in root secretions. When the phosphorus concentration changed from 0 to $1\,\mathrm{mg/L}$, the relative content of phthalic acid in root secretions was significantly increased (P < 0.05) but was significantly decreased (P < 0.05) when the phosphorus concentration changed from 1 to $20\,\mathrm{mg/L}$. No significant difference was observed in the relative content of phthalic acid, when the concentration of phosphorus was between 0.2 and $10\,\mathrm{mg/L}$ and between 5 and $10\,\mathrm{mg/L}$. When the phosphorus concentration was between 1 and $20\,\mathrm{mg/L}$, and 0 and $10\,\mathrm{mg/L}$, the relative content of cyclohexanol siloxane showed no

significant difference. When the concentration of phosphorus was 1, 5, 0.2, and 10 mg/L, the relative content of cyclohexanol siloxane appeared significantly increased (P < 0.05).

The correlation analysis showed that the correlation coefficient between phosphorus concentration and the relative content of phthalic acid was -0.716, and the significance probability of nonlinear correlation was 0.001 < 0.01. The correlation coefficient between phosphorus concentration and the relative content of phenyldicarboxylic acid was -0.537, and the significance probability of nonlinear correlation was 0.022 < 0.05, indicating a significant negative correlation between the two. The significant positive correlation between phthalic acid and phenyldicarboxylic acid was also

obtained; in this case, the correlation coefficient was 0.946 and the probability of nonlinear correlation was 0.000 < 0.01. The correlation between phosphorus concentration and the relative contents of cyclohexasiloxane and other compounds was not significant (P > 0.05).

3.2.2 Relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretion of *black algae*

By analyzing and comparing the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretions of black algae, it was concluded that all three compounds had high relative contents under different phosphorus concentrations (Figure 1). The single factor analysis of variance showed significant differences in the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretions of the three studied wetland plants (*P*-value = 0.0000 < 0.05; F = 25.4457 > $F_{\text{crit}} = 3.6823$; alpha = 0.005). It can be seen from the figure that the relative content of phthalic acid first increased and then decreased with the change in phosphorus concentration. Under the stress of 1 mg/L phosphorus, the relative content of phthalic acid was the highest. Phenyldicarboxylic acid increased with the increasing of the concentration gradient of phosphorus. Cyclohexasiloxane first decreased and then increased, and its relative content was the lowest under the stress of 5 mg/L phosphorus. The relative content of cyclohexasiloxane in the root secretions of black algae was the highest under 10 mg/L phosphorus stress. When the phosphorus concentration changed from 0 to 10 mg/L, the relative content of phthalate was significantly increased (P < 0.05) but was significantly decreased when the phosphorus concentration changed from 1 to 20 mg/L (P < 0.05). When the phosphorus concentration was between 1 and 20 mg/L and 5 and 20 mg/L, the phthalate relative content showed no significant difference.

No significant difference was observed in the relative content of phenyldicarboxylic acid when the phosphorus concentration was between 5 and $10\,\mathrm{mg/L}$, 0 and $0.2\,\mathrm{mg/L}$, and 10 and 20 mg/L. When the concentration of phosphorus was between 20 and $5\,\mathrm{mg/L}$, 5 and $1\,\mathrm{mg/L}$, and 1 and $0.2\,\mathrm{mg/L}$, no significant decrease was observed in the relative content of phenyldicarboxylic acid (P < 0.05). The relative content of cyclohexasiloxane increased significantly at 0.2, 5, and $10\,\mathrm{mg/L}$

(P < 0.05), while no significant difference was observed in its content when the phosphorus concentration between 0 and 0.2 mg/L, 1 and 10 mg/L, and 1 and 20 mg/L.

The correlation analysis showed that the correlation coefficient between the phosphorus concentration and the relative content of phthalic acid was 0.543, and the significance probability of a nonlinear correlation was 0.020 < 0.05, indicating a significant positive correlation between the two. The correlation coefficient between the phosphorus concentration and the relative content of phenyldicarboxylic acid was 0.835, and the significance probability of a non-linear correlation was 0.000 < 0.01, indicating a very significant positive correlation between the two. The significant positive correlation between phthalic acid and phenyldicarboxylic acid was also obtained, in which case the correlation coefficient was 0.850, and the probability of a nonlinear correlation was 0.000 < 0.01.

3.2.3 Relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretion of *Cyperus alternifolius*

By analyzing and comparing the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretions of Cyperus alternifoliuis, it was concluded that all three compounds had high relative contents under different phosphorus concentrations (Figure 1). The single-factor analysis of variance showed significant differences in the relative contents of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane in the root secretions of the three studied wetland plants (P-value = 0.0000 < 0.05; $F = 220.1757 > F_{crit} = 3.68232$; alpha = 0.005). It can be seen from the figure that the relative contents of phthalic acid, phenyldicarboxylic acid, and cyclohexasiloxane increased to the highest point and then decreased with an increase in the concentration of phosphorus. The highest relative contents of these three compounds were obtained at 5 mg/L phosphorus and the lowest at 10 mg/L phosphorus. When the phosphorus concentration changed from 0.2 to 5 mg/L, the relative content of phthalic acid was significantly increased (P < 0.05) but was significantly decreased when the phosphorus concentration changed from 5 to 10 mg/L and from 5 to 20 mg/L (P < 0.05); however, no significant difference was observed in the relative content of phthalic acid when the phosphorus concentration was between 0 mg/L and 1 mg/L. No significant difference was

observed in the relative content of benzoic acid, a compound secreted when the phosphorus concentration was 0, 0.2, 1, and 20 mg/L. A significant decrease was observed in the relative content of benzoic acid only when the concentration of phosphorus changed from 5 to 10 mg/L (P < 0.05). When the concentration of phosphorus was between 0 and 5 mg/L and 0.2 and 1 mg/L, the relative content of cyclohexanol siloxane showed no significant difference. However, when the phosphorus concentration was 5, 20, and 10 mg/L, the relative content of cyclohexanol siloxane decreased significantly (P < 0.05).

The correlation analysis showed that the correlation coefficient between the phosphorus concentration and the relative content of phthalic acid was -0.605, and the significance probability of a nonlinear correlation was 0.008 < 0.01, indicating a very significant negative correlation between the two. The correlation coefficient between the concentration of phosphorus and the relative content of cyclohexasiloxane was -0.528, and the significance probability of a nonlinear correlation was 0.024 < 0.05, indicating a significant negative correlation between the two. The significant positive correlation between phthalic acid and phenyldicarboxylic acid was also obtained, in which case the correlation coefficient was 0.620, and the probability of a nonlinear correlation was 0.06 < 0.01. The correlation coefficient between the relative contents of phthalic acid and cyclohexasiloxane was 0.881, and the significance probability of a nonlinear correlation was 0.000 < 0.01, indicating a significant positive correlation between them. The correlation coefficient between the relative contents of benzene dicarboxylic acid and cyclohexasiloxane was 0.585,

and the significance probability of a nonlinear correlation was 0.011 < 0.05, indicating a significant positive correlation between the two (Figure 2). The correlation between the phosphorus concentration and the relative contents of phenyldicarboxylic acid and other compounds was not significant (P > 0.05).

Organic acid secretion is the adaptation mechanism of a plant root system under the conditions of nutrient stress. The plant roots secrete low-molecular weight organic acids in the root exudate; these organic acids change the pH value of the plant rhizosphere and activate insoluble phosphorus in the surrounding environment so as to improve the nutrient-use efficiency of plants [29]. The relative contents of phthalic and phenyldicarboxylic acids change with a change in the concentration of phosphorus of C. alternifolius. At 5 mg/L phosphorus, the root exudate of C. alternifolius showed the largest degree of adjustment in organic acids, among which the relative contents of phthalic and phenyldicarboxylic acids were up to 60.53 and 4.44%. The phosphorus stress can affect the metabolism of C. alternifolius plants, and the secretion of organic acids in the root exudate can activate the hard-to-dissolve phosphorus, thereby improving the utilization rate of nutrients in a hydroponic fluid so as to promote the growth and development of plants.

Some scholars argue that benzoic acid and its derivatives, water-soluble organic acids, phenol, and alkane compounds belong to allelochemicals, which had allelopathy to plants, and might play a major allelopathy in relatively high content of material generally [30,31]. Therefore, in phosphorus-stressed environments, the presence of

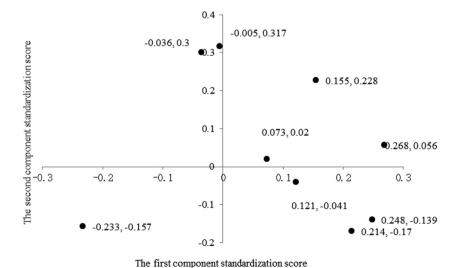


Figure 2: The bi-plot from a multivariate PCA analysis of the three root exudates compounds under phosphorus gradients stress.

acid and alkane compounds in the root exudate of *Cyperus sinensis* might produce chemosensitization effects. Moreover, when the concentration of phosphorus stress was 5 mg/L, the presence of phthalic acid, phenyldicarboxylic acid, and cyclohexasiloxane in the root secretion had a major influence on the microregulation of roots in *Cyperus*.

4 Discussion

During the whole process of plant growth, the root system is an important organ for the communication between the plant and the outside environment. It mainly relies on the root system to absorb the nutrients needed for the plant growth 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. Diversity of root exudates is the embodiment of adaptation by different types of plants to their living environment [9,32,33]. 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 rhizosphere by secreting low molecular weight organic acids. Insoluble phosphorus in the surrounding environment of roots is activated to improve the utilization efficiency of nutrient components in plants [34,35]. In wetland ecosystem, the water quality eutrophication caused by phosphorus is becoming more and more serious; hence, it is significant to study the effects of phosphorus stress on plant root exudates and the specific changes of organic acids in root exudates. 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 specific environment (wetland, woodland, grassland, etc.) can provide basic reference materials for the rhizosphere measures to environmental pollution control.

Previous studies suggest that the amount of organic acids in root secretion in *Broussonetia papyrifera*, *mulberry*, and *rape* plants under phosphorus stress was increased and consisted mainly of oxalic acid, citric acid, and malic acid [36]. However, in our study, the root secretion of organic acid content was higher under phosphorus stress and consisted mainly of phthalic acid and benzene dicarboxylic acid. Additionally, researchers studied cattail and vetiver root secretion using different concentrations of nitrogen and phosphorus and concluded that at low nutrient concentration, the secretion of dissolved organic carbon is higher than at high

nutrient concentration [37]; our results are not in agreement with the results of this study, and we found that the relative content of organic acid is higher in the presence of 1 mg/L phosphorus. The reasons for the differences can be attributed to the species and genotype of the plant as plants secretions are unique. Yang Runji et al. [19] used *rape* as the study material. Result shows that phosphorus was able to influence *P. stratiotes* root secretion by increasing the secretion of organic acids in self-regulation.

Therefore, the root secretion of phthalic acid, benzene dicarboxylic acid, and three types of cyclohexanol siloxane compounds by P. stratiotes might have allelopathic properties, and when P. stratiotes is under phosphorus stress, the secretion of phthalic acid, benzene dicarboxylic acid, and cyclohexanol siloxane may play a major role in the adjustment of rhizosphere [38]. Under environmental nutrient stress, root exudate may change to adapt to the environment [29]. Weijie XU conducted the research in the absence of phosphorus stress detected black algae root secretion that contained more alkanes, esters, acids, and silane and siloxane, phthalate, and diisooctyl phthalate relative content, which is higher; the differences in our results and the previous study's results could be the difference in species, black algae are wetland plants and are largely different from terrestrial plants. It may also be caused by inconsistencies in the methods of culture, extraction, and identification. Irrespective of the relative content substances detected in this study, the effect of these substances on plant growth and their substrates need to be further studied.

In the study of phosphorus stress on the effect of plant root secretion, Zhen-hai Zhang et al. [14] studied soybean root secretion under low phosphorus stress; the secretion of organic acids content increased, malic acid showed the highest relative content. In current study, the organic acids, phthalic acid and phenyldicarboxylic acid, were secreted by the root system and changed with different concentrations of phosphorus stress in black algae. When the phosphorus concentration was 1 mg/L, the organic acid of the root exudate in black algae was the most highly regulated, and the relative content of phthalic acid was 36.66%. The results showed that the external phosphorus supply could affect the metabolism of the algae, especially the secretion of organic acids. Therefore, by secreting a large amount of organic acids, the black algae can activate more nearby insoluble phosphorus and improve the utilization rate of nutrients in hydroponics to promote the growth and development.

Roots are the primary organs for plants to absorb soil nutrients, water, and environmental stimuli. The root system secretes a large amount of organic matter to

the surrounding area, so as to promote the release of phosphorus in the growth matrix, improve the uptake of phosphorus by plants, and alleviate the phosphorus stress of plants [39]. For example, Lan Zhong-ming et al. [40] found that under phosphorus deficiency stress, the organic acids secreted by the root system include oxalic acid, tartaric acid, citric acid, and malic acid. But in this study, the root secretion of Cyperus alternifolius and oilseed rape under phosphorus stress has many similar compounds, and its predecessors find the root secretion of oilseed rape have lots of allelopathy, so the material in this study whether have the allelopathy or have other function still remains should to be further studied, regardless of the relative content [4].

The current research on wetland plant root secretion is limited. We showed that C. alternifolius root can secrete a large number of organic compounds, such as phthalic acid, phthalic acid dibutyl, and diphenyl sulfone compounds which have a relatively higher content [41]. With the increase in phosphorus concentration in root exudates, the amount and type of organic compounds were higher than in the absence of phosphorus stress. This shows that C. alternifolius through enhanced secretion of root system regulated their physiological changes and adapted to the surrounding environment; additionally, by speeding up the root secretion under phosphorus stress adapted to the environment [42]. However, the mechanism of compound secreted by C. sinensis root under different environmental stress needs further research and exploration.

5 Conclusion

The phosphorus gradients have good correlation with the relative contents of phthalic acid and benzene dicarboxylic acid by Pistia and Black algae, and the relative contents of phthalic acid and cyclohexanone by Cyperus. The relative contents of phthalate, benzene dicarboxylic acid, and cyclohexasiloxane in the Pistia root exudates first increased and then decreased with the change in phosphorus concentration. The relative contents of four compounds in Pistia were the highest at 1 mg/L of phosphorus, and the lowest relative contents of phthalic acid and benzene dicarboxylic acid were observed at 20 mg/L of phosphorus. The cyclohexasiloxane was the lowest in the absence of P stress. While in black algae, the relative content of cyclohexasiloxane first decreased and then increased, with its lowest relative content occurring at 5 mg/L of phosphorus and the highest

at 10 mg/L of phosphorus. In Cyperus.alternifolius, the highest relative concentrations of the four compounds: phthalic acid, dimethyl phthalate, octadecane, and diphenyl sulfone in Cyperus were observed at 5 mg/L phosphorus and the lowest at 10 mg/L phosphorus. Therefore, based on these results, the allelopathy and other effects of the substances need to be further studied.

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