Research Article

Xu Duan, Yang-yi Zhao*, Jian-cong Zhang

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

https://doi.org/10.1515/chem-2020-0059
received November 26, 2018; accepted April 1, 2020

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
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 under phosphorus deficiency stress. We can 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 phosphorus-deficient 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-ji 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: K₂SO₄ 0.75 × 10⁻³ mol/L, MgSO₄ 0.75 × 10⁻³ mol/L, KCl 1 × 10⁻³ mol/L, Ca(NO₃)₂ 2.0 × 10⁻³ mol/L, H₂BO₃ 1 × 10⁻³ mol/L, CuSO₄ 1 × 10⁻⁷ mol/L, MnSO₄ 1 × 10⁻⁶ mol/L, ZnSO₄ 1 × 10⁻⁶ mol/L, (NH₄)₆Mo₇O₂₄ 5 × 10⁻⁶ mol/L, 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: H₂BO₃ 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 a 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 × 250 µm × 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.

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. striatiotes*, *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. striatiotes* 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 striatiotes*

The chemical composition of the *P. striatiotes* root exudates exposed to a concentration gradient of phosphorus and the relative contents of their individual components were determined by the artificial analysis mass spectrum database, 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 |
|                    | Tetradeyl 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 |
| 0.2                | 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 | Octadecan                            | 1.07 ± 0.03 | Oxalic acid                         | 0.42 ± 0.02 |
| 0.5                | 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 cyclohexasane                | 2.89 ± 0.18 | Naphthylamine                        | 4.27 ± 0.07 | Benzene dicarboxylic acid           | 4.11 ± 0.17 |
| 1                  | Dodecyl cyclopentane                 | 2.73 ± 0.07 | Diiiso-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 | Diiiso-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 | Diiiso-octyl phthalate               | 2.09 ± 0.05 | Formic acid                         | 1.59 ± 0.00 |
|                    | Octadecane                           | 2.43 ± 0.05 | Phenethylamine                       | 3.27 ± 0.00 | Benzene dicarboxylic acid           | 3.79 ± 0.38 |
|                    | 20-Carbon alkane                     | 4.20 ± 0.11 |                          |           |                                      |         |
|                    | Dodecyl cyclopentadimethylsiloxane   | 0.90 ± 0.13 | Cyclohexasiloxane                    | 6.09 ± 0.30 | Palmitic acid                       | 0.84 ± 0.01 |
|                    | Dodecyl cyclohexasiloxane            | 6.95 ± 0.01 | Cyclohexasiloxane                    | 0.80 ± 0.01 | Phthalic acid                       | 3.43 ± 0.40 |
|                    | Octadecyl                            | 0.95 ± 0.02 | Diiiso-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 cyclopentadimethylsiloxane     | 0.71 ± 0.01 | Silane                              | 16.24 ± 0.14 | Palmitic acid                        | 1.52 ± 0.02 |
|                    | Cycloheptane                         | 14.61 ± 0.12 | Cyclohexasiloxane                   | 5.26 ± 0.52 | Stearic acid                         | 0.73 ± 0.00 |
|                    | Octadecyl cyclooctadimethylsiloxane  | 0.61 ± 0.00 | Di-iso-octyl phthalate              | 6.24 ± 0.21 | Phthalic acid                        | 5.96 ± 0.72 |
|                    | Dodecyl cyclohexane                  | 5.59 ± 0.13 | Carboxylic acid                     | 1.34 ± 0.07 | Formic acid                          | 1.60 ± 0.01 |
| 0.2                | Decyl cyclopentadimethylsiloxane     | 0.95 ± 0.05 | Diphenyl sulfone                    | 7.40 ± 0.32 | Phthalic acid                        | 21.64 ± 0.91 |
|                    | Cycloheptane                         | 4.47 ± 0.17 | 1-Methylpyrrolidone                 | 3.19 ± 0.16 | Benzoic acid                         | 3.08 ± 0.23 |
|                    | Cyclotetrasiloxane                   | 8.43 ± 0.25 | Propionamide                        | 1.2 ± 0.09 | Acetic acid                          | 2.42 ± 0.15 |
|                    | Cycloheptane                         | 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 |
| 1                  | Cyclohexasiloxane                    | 4.71 ± 0.32 | Diphenyl sulfone                    | 6.59 ± 0.19 | Sulfurous acid                       | 1.48 ± 0.08 |
|                    | Cyclohexasiloxane                    | 3.87 ± 0.54 | Cyclohexanol                         | 1.88 ± 0.08 | Phenylacetic acid                    | 3.32 ± 0.19 |
| 5                  | Cyclohexasiloxane                    | 3.31 ± 0.12 | Phthalic acid                       | 36.66 ± 0.80 | Acetic acid                          | 4.60 ± 0.33 |
|                    | Silane                              | 4.64 ± 0.31 | Pyrrolidone                         | 4.23 ± 0.31 | Formic acid                          | 1.87 ± 0.02 |
| 10                 | 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 |                                      |          |
|                    | 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                 | 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                     | 7.67 ± 0.09 | 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 |                                      |          |
|                    | 2-Chloroacetaldehyde                 | 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                     | 0.27 ± 0.03 | Acrylic acid                        | 1.25 ± 0.01 | 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 dicarboxylic acid           | 3.25 ± 0.21 |                                      |          |

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, diocetyl phthalate, and acrylic acid, which showed the relative contents 56.07, 9.03, 5.13, and 5.30%, respectively. At the concentration of 0.2 mg/L phosphorus, the *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 contained 29 chemical components, 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*

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; $F = 14.1576 > F_{\text{crit}} = 6.3589$; 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...
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    | Naphthalene      | 2.36 ± 0.03    | Acrylic acid     | 5.30 ± 0.23    |
|                     | Octadecane       | 2.28 ± 0.13    | Butyric acid     | 3.82 ± 0.14    | Oxalic acid      | 1.74 ± 0.04    |
| 0.2                 | 19 alkanes       | 2.81 ± 0.08    | 1-Methylypyrrolidone | 3.02 ± 0.13  | Oxalic acid      | 1.93 ± 0.08    |
| 5                   | 21 alkanes       | 2.88 ± 0.21    | Diocetyl phthalate | 5.13 ± 0.19  | Benzene dicarboxylic acid | 3.39 ± 0.26  |
| 1                   | 20-carbon alkane | 1.96 ± 0.02    | Diphenyl sulfone | 9.03 ± 0.38    | Benzoin acid     | 4.69 ± 0.17    |
|                     | Cylohexasiloxane | 2.19 ± 0.09    | Phthalic acid    | 56.07 ± 2.24   | Silicate         | 2.89 ± 0.12    |
| 5                   | 26 alkanes       | 2.93 ± 0.12    | Diphenyl sulfone | 4.38 ± 0.19    | Benzoin acid     | 3.84 ± 0.14    |
|                     | 20-Carbon alkane | 6.18 ± 0.23    | Naphthalene      | 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    | Acetic acid      | 3.10 ± 0.02    | Silicate         | 2.89 ± 0.12    |
|                     | 5                | 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    | Methoxycetic acid | 2.57 ± 0.17   |
|                     | 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  | Nitrate         | 4.60 ± 0.12    |
|                     | 20-Carbon alkane | 3.08 ± 0.13    | Phthalic acid    | 60.53 ± 2.75   | Silicate         | 2.00 ± 0.07    |
|                     | Ethane           | 0.97 ± 0.02    | Naphthalene      | 4.02 ± 0.11    | Oxalic acid      | 2.10 ± 0.07    |
|                     | 0.2              | 2.15 ± 0.14    | Naphthalene      | 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    |
|                     | Cylohexasiloxane | 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    | 1.04 ± 0.11    | Caproic acid     | 1.60 ± 0.14    |
|                     | Pyrrolidone      | 2.85 ± 0.22    | Pyrimidine       | 0.94 ± 0.09    | Acetic acid      | 1.60 ± 0.03    |
|                     | Hexanone         | 2.83 ± 0.18    | Propionic acid   | 1.04 ± 0.12    | 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    | Naphthalene      | 4.70 ± 0.24    | Acetic acid      | 1.96 ± 0.02    |
|                     | 20                | 3.58 ± 0.21    | Naphthalene      | 2.63 ± 0.10    | Propionic acid   | 1.58 ± 0.8     |
|                     | Epoxy ethane     | 1.66 ± 0.03    | Naphthalene      | 2.25 ± 0.21    | Butanoic acid   | 1.29 ± 0.03    |
|                     | Eicosane         | 2.29 ± 0.30    | Phenethylamine   | 1.66 ± 0.08    | Sulfurous acid   | 1.61 ± 0.10    |
|                     | Octadecane       | 1.08 ± 0.00    | Glyoxal          | 3.52 ± 0.18    | Methoxycetic acid | 2.21 ± 0.17   |
|                     | 19 alkanes       | 2.34 ± 0.05    | Trifluoroacetyl group | 3.52 ± 0.13  | Acetic acid      | 1.44 ± 0.09    |
|                     | 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    |
and benzene dicarboxylic acid were the lowest when the concentration of phosphorus was 20 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 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 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 mg/L and between 5 and 10 mg/L. When the phosphorus concentration was between 1 and 20 mg/L, and 0 and 10 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 phenyl dicarboxylic 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 phenyl dicarboxylic acid was also

### 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 \( (P < 0.05) \).
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_{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. Phenyl(dicarboxylic 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 phenyl(dicarboxylic acid when the phosphorus concentration was between 5 and 10 mg/L, 0 and 0.2 mg/L, and 10 and 20 mg/L. When the concentration of phosphorus was between 20 and 5 mg/L, 5 and 1 mg/L, and 1 and 0.2 mg/L, no significant decrease was observed in the relative content of phenyl(dicarboxylic acid (P < 0.05). The relative content of cyclohexasiloxane increased significantly at 0.2, 5, and 10 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 phenyl(dicarboxylic 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 phenyl(dicarboxylic 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 alternifolius*, 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, phenyl(dicarboxylic 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 phenylidicarboxylic 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 phenylidicarboxylic 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 phenylidicarboxylic 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 phenylidicarboxylic 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.](image)
acid and alkane compounds in the root exudate of Cyporus 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 Cyporus.

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 cyclohexasiloxane 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 cyclohexasiloxane 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 hostile environment [42]. However, the detailed 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.

Acknowledgments: This research was supported by National Science Foundation of China (No. 31860225; 31760149), Fund to Key Research and Development Program of Yunnan Provincial (No. 2018BB018), Fund Project to The forestry science and technology innovation platform project of The State Forestry Administration (2019132161; 2019-YN-13).

Conflict of interest: Authors declare no conflict of interest.

References

[1] Xiao-Ping W, Xiao X, Tian-Wen T, Yun-Xiang L, Juan X. Seasonal changes of the input of root exudates and its driving characteristics of Rhizosphere microbe in a ceridiphyllum japonicum sieb. plantation. Bull Botanical Res. 2018;38(1):47–55.
[2] Chunxia L, Fengzhi W. Advances of root exudates collection and root exudates mediated interspecific interactions. Acta Agric Boreali-Occidentalis Sin. 2016;25(6):795–803.
[3] Xian X. Effect of chemical forms of cadmium, zinc, and lead in polluted soils on their uptake by cabbage plants. Plant Soil. 1989;113(2):257–64.
[4] Gransee A, Wittenmayer L. Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J Plant Nutr Soil Sci. 2015;163(6):381–5.
[5] Chancui W, Yangjin H, Caijiao H, Shanshan W. Mobilization of insoluble Cr by wetland plant Leersia hexandra Swartz root exudates. J Quanzhou Norm Univ. 2016;34(6):1–4.
[6] Sheng-Wang P, Xin Y, Can L, Yan-Lan L, Ting Y, Hai-Yuan T. Effects of benzo[a]pyrene on the organic compounds of low molecule weight excreted by root systems in five Festuca species with different remediation potentials. Chin J Plant Ecol. 2016;40(6):604–14.
[7] Zhao K, Zhou B, Wanzheng MA, et al. The influence of different environmental stresses on root-exudated organic acids: a review. Soils. 2016;48(2):235–40.
[8] Cai-xia W, Hua F. Effects and roles of root exudates. Pratacult Sci. 2009;26(9):24–9.
[9] Xiang-jing S, Sheng-nan L, Jia G, Wei W, Yi-lei Y. Response of wetland plant roots to environmental factors: A. J Hydrocol. 2017;38(2):1–9.
[10] Roy ED. Phosphorus recovery and recycling with ecological engineering: a review. Ecol Eng. 2017;98:213–27.
[11] Li T, Qi Y, Jiang-ping Q, Xu-dong L. Influence of root exudates from phragmites typha orientalis presl. J Harbin Univ Commerce (Nat Sci Ed): 2010;26(4):425–9.

[12] De-hua L, Chun-lei X, Yi-quan J, Zai-hua G, Li-yuan H. Physiological characteristic of roots of different rice variety under the stress of low phosphorus. J Huazhong Agric Univ. 2005;21(11):186–8.

[13] Zhou J, Wang X, Deng Y, et al. Effects of phosphorus stress on the root morphology and root exudates in different sugar beet genotypes. Chin Agric Sci Bull. 2011;1151–4.

[14] Zhi-hai Z, Yan C, Sheng-fang H, Meng-chen Z, Dong-mei W. Effect of P deficiency stress on soybean root system and its secretion of H+ and organic acid. Chin J Oil Crop Sci. 2011;33(2):135–40.

[15] Niu FH, Zhi-Hui Li, Chen SX. Effects of phosphorus levels on organic acids in Eucalyptus dunnii root exudates. Eucalypt Sci Technol. 2017;(1):1–8.

[16] Wang YL, Almvik M, Clarke N, et al. Contrasting responses of root morphology and root exuded organic acids to low phosphorus availability in three important food crops with divergent root traits. AoB Plants. 2015;7:7. 2016-8-17

[17] Ruonan M, Qing L, Huan L, Yanxi S, Juan L. Impact of phosphorus deficiency stress on root development and nutrient absorption of sweet potato. Acta Agric Boraeil Sin. 2017;32(5):171–6.

[18] Rui-jj Y, Jun-yi N. Effects of phosphorus deficiency on root exudation of rape (Brassica campestris L.). J Southwest Agric Univ (Nat Sci). 2006;28(6):895–9.

[19] Meng L, Xu-zhou M, Wux W. Effects of pistia stratiotes L. on removal rate for nitrogen and phosphorus in polluted water body. Resour Environ Yangtza Basin. 2012;21(9):1137–42.

[20] Victor KK, Seka Y, Norbert KK. Phytoexcretion of waste-water toxicity using water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes). Int J Phytoexcretion. 2016;18(10):949–55.

[21] Cahoon LB, Boller MA, Labry MOD, et al. Phosphorus partitioning a storm water pond. J North Carolina Acad Sci. 2015;131(2):25–8.

[22] Haichao Z, Shengrui W, Xiangcan J, Qingyun B, Jinghui L. Availability and transferring of phosphorus forms by Hydrilla verticillata in the sediment and soil. J Lake Sci. 2008;20(3):315–22.

[23] Boeufetremblay V, Plantureux S, Guckert A. Influence of mechanical impedance on root exudation of maize seedlings at two development stages. Plant Soil. 1995;172(2):279–87.

[24] Can Z, Rui G, Ming-ming Z, Shi-hong Z, Rong D, Chun-sheng Y. Preliminary investigation of Lamio phломis rotata (Benth)Kudo root exudates from different stage of land deterioration. Sci Technol Eng. 2016;16(3):175–8.

[25] Horchan F, Gallusci P, Baldet P, et al. Prolonged root hypoxia induces ammonium accumulation and decreases the nutritional quality of tomato fruits. J Plant Physiol. 2008;165(13):1352–9.

[26] Bai Yan C, Yi Z, Li T. Mobilizing phosphorus in red soils by root exudates of wheat and broadbean under phosphorus stress condition. J Yunnan Agric Univ. 2009;6:869–75.

[27] Xuejing W, Mei L, Lei Z, Aizhong D, Yan L, Xue Z, et al. Effects of salt stress on root exudates in Phragmites australis. J Beijing Norm Univ (Nat Sci). 2016;52(1):44–8.

[28] Tingting L, Yuting Q, Yuying W, Jing Z, Yanrong Z. Solvent extraction and GC-MS analysis of organic sulfur-containing compounds from tillering onion. Food Sci. 2017;38(12):151–6.

[29] Weijie XU, Guo J, Zhao M, et al. Research progress of soil plant root exudates in heavy metal contaminated soil. J Zhejiang A&F Univ. 2017;34(6):1137–48.

[30] Rice EL. Allelopathy. 2nd ed. Physiological ecology. 1984.

[31] Latif S, Chiapisugo G, Weston LA. Allelopathy and the role of allelochemicals in plant defence. Adv Botanical Res. 2017;82:19–54.

[32] Song Xi, Li SN, Guo J, et al. Effects of different salt levels on root growth and physiological characteristics of Tamarix chinensis cutting seedlings. Eco-J. 2018;38:606–14.

[33] Ge RL, Liu YQ, H AR, et al. Quantitative characteristics of root system of three typical soil and water conservation plants. Sci S & W Cons Chi. 2018;16:88–95.

[34] Chen F, Meng YJ, Shuai HW, et al. Effects of plant allelochemicals on seed germination and its ecological significance. Chin J Eco-Agric. 2017;25:36–46.

[35] Qin L, Jiang H, Tian J, et al. Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. Plant Soil. 2011;349:25–36.

[36] Zhao K, Wu Y. 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. 2014;60(5):640–50.

[37] Wu FY, Chung AK, Tam NF, et al. Root exudates of wetland plants influenced by nutrient status and types of plant cultivation. Int J Phytoexrem. 2012;14(6):543–53.

[38] Feng C, Yongjie M, Haiwei S, Xiaofeng L, Wenguan Z, Jianwei L, et al. Effect of plant allelochemicals on seed germination and its ecological significance. Chin J Eco-Agric. 2017;25(1):36–46.

[39] Yanli N, Yi Z, Kehui L. Effect of root exudates on activation of phosphates in soils. J Yunnan Agric Univ. 2002;17(3):281–6.

[40] Zhong-ming L, Xin-jian L, Wei-guang Z, Hui Z, Yi-qun W. Effect of P deficiency on the emergence of Astragalus L. root exudates and mobilization of sparingly soluble phosphorus. Sci Agricult Sino. 2012;45(8):1521–31.

[41] Akmukanova NR, Zayadan BK, Sadvakasova AK, et al. Consortium of higher aquatic plants and microalgae designed to purify sewage of heavy metal ions. Russian J Plant Physiol. 2018;65(1):143–9.

[42] Chen ZJ, Tian YH, Zhang Y, et al. Effects of root organic exudates on rhizosphere microbes and nutrient removal in the constructed wetlands. Ecol Eng. 2016;92:243–50.