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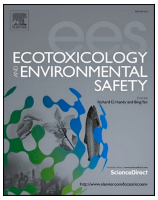

Influence of cadmium and microplastics on physiological responses, ultrastructure and rhizosphere microbial community of duckweed

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ABSTRACT

The combined contamination of heavy metals and microplastics is widespread in freshwater environments. However, there are few researches on their combined effects on aquatic plants. In this study, the effects of single and combined stress of 0.01 mg L^{-1} cadmium (Cd), 50 mg L^{-1} polyethylene and 50 mg L^{-1} polypropylene for 15 days on the physiological response, ultrastructure and rhizosphere microbial community of duckweed were investigated. The results showed that Cd and microplastics single or combined stress inhibited the growth of duckweed, shortened the root length and decreased the chlorophyll content. Compared with single Cd treatments, the combination of microplastics and Cd increased duckweed growth rate and increased superoxide dismutase activity and malondialdehyde content and reduced chloroplast structural damage, indicating that the combined stress could reduce the toxicity of heavy metals to duckweed. Through the study of rhizosphere microbial diversity, 1381 Operational Taxonomic Unit (OTUs) were identified and rich microbial communities were detected in the duckweed rhizosphere. Among them, the main microbial communities were *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*. Compared with Cd single stress, the ACE and chao index of rhizosphere microbial community increased under combined stress, indicating that the diversity and abundance of microbial communities were improved after combined stress treatment. Our study revealed the effects of heavy metals and microplastics on aquatic plants, providing a theoretical basis for duckweed applications in complex water pollution.

1. Introduction

In recent years, microplastics and heavy metals in oceans, lakes, rivers, soil, and the atmosphere have been continuously detected by researchers. This combined pollution has gradually attracted widespread attention (Luo et al., 2021; Wu, 2020). Microplastic and heavy metal pollution, mainly caused by industrial emissions, can slow down the growth of aquatic plants and animals, or even kill them and can also affect human health through diet and drinking (Cao et al., 2021). Heavy metals are highly toxic metals that are difficult to degrade, mainly including cadmium (Cd), mercury, arsenic, copper, and lead (Jaiswal et al., 2018). The 2020 National Eco-Environmental Quality Profile released by the Ministry of Ecology and Environment of China showed that Cd is the main heavy metal pollutant in Chinese farmland (Sun, 2021). Cd is a non-essential element with potential carcinogenic, teratogenic, and mutagenic effects. Cd in the environment can easily accumulate in grains, accumulate through the food chain and be ingested by humans, endangering human health (Wang et al., 2019; Xu et al., 2018; Zhang et al., 2020b). Microplastics (MPs) are an emerging contaminant that includes mainly polyethylene (PE), polypropylene (PP), and polystyrene (Bradney et al., 2019; Cui et al., 2020; Thompson et al., 2004). The tiny MPs are easily transferred in the food chain, affecting the growth, development, reproduction, and survival of organisms (Ma et al., 2016). MPs have a large specific surface area and surface functional groups and can act as carriers of heavy metals for migration to different environments (Zhou et al., 2019).

Recently, the combined pollution of MPs and Cd has become a hot topic and some studies have shown that different types of MPs can

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facilitate the accumulation of Ni, Cu, Zn, Cd, and other heavy metals (Ashton et al., 2010; Tang et al., 2020, 2021). Since MPs are carriers of heavy metal in water, they can facilitate the entry of heavy metals into the food chain and continues to accumulate toxicity through biomagnification. The higher the food chain level, the more toxic the organisms accumulate, leading to higher ecotoxicity (Bradney et al., 2019; Tauqeer et al., 2022). Furthermore, their small size, only 100 nm to 5 mm, may be absorbed by aquatic organisms, which hinder the normal metabolic processes of organisms (Jaiswal et al., 2018). The growth, development, and physiological and biochemical processes of organisms may be inhibited, even to death (Yang et al., 2021b). Because these metals can also be accumulated through the food chain, this poses a threat to the entire aquatic ecosystem (Okereafor et al., 2020). In addition, MPs can affect plant physiological responses such as root biomass, root length and tissue density, potentially altering Cd uptake by plant roots (Zhou et al., 2021). The effect of MPs on Cd adsorption differs under different conditions, and the binding of MPs to Cd may change the toxicity to organisms (Bhagat et al., 2021). Therefore, it is crucial to study the combined effects of MPs and Cd in water and to treat the combined pollution.

The remediation of heavy metals and MPs combined pollution is a key development direction in the field of environmental treatment (Wei et al., 2021). Phytoremediation has the advantages of low costs, easy operation, and allows for the easy control of secondary pollutants. It can effectively reduce pollutants in the environment through the absorption, volatilization, filtration and retention of plant roots. Consequently, it has become an important method for remediating polluted water (Wang and Chao, 2020). Studies have shown that plant roots can release a variety of exudates that alter soil properties and rhizosphere microbial community composition and regulate the relationship between plants and microorganisms (Feng et al., 2021; Olanrewaju et al., 2019; Vives-Peris et al., 2020). This effect can promote host plant growth and enhance its ability to adapt to stress (Ceng et al., 2017). Therefore, rhizosphere microorganisms play an important role in plant growth.

Duckweed is a model plant popularly used in eco-environmental toxicology research. There are five genera of duckweed: *Lemna*, *Spirodela*, *Landoltia*, *Wolffiella*, and *Wolffia* (Yang et al., 2021a). Duckweed grows asexually and quickly, doubling its biomass in 2 d. Duckweed has a wide geographical distribution and strong ability to adapt to environment (Ekperusi et al., 2019). It has a simple structure where both the thallus and root can absorb water and ions, thereby effectively avoiding long-distance transport and improving the transport efficiency of ions (Yang et al., 2021b). Duckweed cultivation has the characteristics of low costs, less accompanying pollution, high efficiency in removing pollutants, and good effect on some heavy metals that are difficult to remove at low concentrations (Körner et al., 2003). Therefore, duckweed has a good potential in toxicity detection and wastewater treatment (Ekperusi et al., 2019).

In this study, we investigated the effects of pollutants in water on the physiological response, ultrastructural characteristics, and rhizospheric microbial communities of duckweed. These pollutants included Cd, PE, and PP, as well as Cd/PE and Cd/PP combination. The findings of this study may not only reveal the adaptability of duckweed in polluted water, but it may also provide new insights for the more efficient use of duckweed for energy and environmental remediation.

2. Materials and methods

2.1. Materials and culture conditions

Water samples and duckweed were collected from Guizhou Province, China (E106.671435, N26.432726), in while avoiding impurities such as sediment. The samples were sent to the laboratory within 1 h and cultured in plastic boxes (14.5 × 9.5 × 5.4 cm). The duckweed was cultured at 25 ± 1 ℃, with 16 h day/8 h night cycles, and a light intensity of 40 μmol m^{-2} s^{-1} for a week.

2.2. Experimental design

Duckweed with a fresh weight of 5 g was transplanted into a plastic box with 400 mL of the water sample. The experiment was divided into the following six groups: blank (CK) group, Cd group, PE group, PP group, Cd/PE group, and Cd/PP group, with three repetitions per group (Table 1). In total we used 90 g duckweed (fresh weight) and 18 plastic boxes.The culture conditions were 25 ± 1 °C, 16 h day/8 h night cycle, and 40 μmol m^{-2} s^{-1} light intensity for 15 days. After 15 days of treatment, we salvage the duckweed, rinse with distilled water, and shake off the water and measure the physiological indicators.

2.3. Determination of antioxidative enzyme activity and malondialdehyde content

Freeze 0.1 g duckweed in liquid nitrogen to prevent enzyme inactivation and 1 mL of the extract was added for ice bath homogenization. This mixture was centrifuged at 8000 rpm for 10 min at 4 °C. Thereafter, the supernatant was extracted and placed on ice for testing. Using 1 mL of supernatant, superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), and catalase (CAT, EC 1.11.1.6) activities were detected using kits (Solarbio, Beijing, China).

The malondialdehyde (MDA) content was determined using the thiobarbituric acid method (Draper et al., 1993). Weigh 0.5 g duckweed into a centrifuge tube, add 4 mL of 10 % trichloroacetic acid, grind in an ice bath, and centrifuge at 12,000 rpm at 4 °C for 10 min. The supernatant was diluted and fixed to 5 mL, 2 mL of which was taken (2 mL of distilled water for the control tube), and 2 mL of 0.6 % thiobarbituric acid was added and shaken well. This mixture was then boiled in a water bath for 30 min, cooled rapidly, and centrifuged at 4 ℃ for 10 min at 3000 rpm. The absorbance of the supernatant was measured at 440 nm, 532 nm, and 600 nm (MULTISKAN, Thermo Fisher Scientific, USA).

2.4. Transmission electron microscopy analysis

The duckweed leaves and roots were separated, pre-fixed with 2.5 % glutaraldehyde, re-fixed with 1 % osmium tetroxide, and gradually dehydrated with acetone. Transmission electron microscopy (TEM) was used to characterize the damage to duckweed mesophyll cells caused by exposure to the heavy metal and microplastics (JEM-1400FLASH, JEOL, Japan).

2.5. Processing of the duckweed microbial community

Place 1 g of duckweed in a sterile tube and add 10 mL of 0.1 mol L^{-1} phosphate buffer solution (PBS) for aseptic soaking, wash by shaking, and centrifuge at 180 rpm for 20 min to obtain a suspension (Repeat this step twice). The washed sample was removed, placed in a 50 mL centrifuge tube containing 20 mL of 0.1 mol L^{-1} PBS solution, and sonicated at 160 W for 30 s at 30 s intervals for 10 min. The three washes were mixed, passed through a 0.22 μm filter membrane, and the microorganisms were preserved on the membrane. Thereafter, the filter membrane was snap-frozen in liquid nitrogen, and stored at − 80 °C.

Table 1
Treatment conditions of duckweed.

Groups	Cd (mg L^{-1})	PE (mg L^{-1})	PP (mg L^{-1})
CK	0	0	0
Cd	0.01	0	0
PE	0	50	0
PP	0	0	50
Cd/PE	0.01	50	0
Cd/PP	0.01	0	50

2.6. DNA extraction and amplification

DNA extraction and amplification (Table 2), and high-throughput sequencing were carried out by the Shanghai Majorbio Company, and the structure and abundance of microbial communities were analyzed using the Major Microbial Diversity Cloud Platform.

Table 2
Primers of the 338F-806R sequence.

Primer name	Primer sequence (5′→3′)
338F	ACTCCTACGGGAGGCAGCAG
806R	GGACTACHVGGGTWTCTAAT

2.7. Data analysis

Graph Pad Prism 6.02 was used for plotting, and one-way analysis of variance (ANOVA) was used to compare the differences between the experimental groups (Cd, PE, PP, Cd/PE, Cd/PP) and the control group (CK) via SPSS 20.0; $p < 0.05$ was considered significant.

3. Results

3.1. Effects of Cd and MPs on duckweed growth and photosynthesis

The growth rate of duckweed in the CK group was the highest, and the growth rate of the single stress group was significantly reduced. The largest decrease was in the Cd treatment group, where the lowest growth rate was 0.16 g d^{-1}, followed by 0.21 g d^{-1} in the PP and PE groups. Furthermore, the Cd/PE and Cd/PP combined treatments had significantly higher growth rates, which were 0.23 g d $^{-1}$ and 0.26 g·d^{-1}, respectively, than that in the Cd single stress group (Fig. 1a). As shown in Fig. 1b, there was no significant difference in the chlorophyll content among treatments. Duckweed showed strong resistance to Cd, PE, and PP, as there were no significant differences in external morphology either. Fig. 1c shows that the root length of duckweed in the Cd treatment group was 6.5 cm and that in the Cd/PE group was 7.7 cm, which was significantly higher than that in the Cd single treatment group. The root length of duckweed in the Cd/PP group was 7 cm, and there was no significant difference among the single stress groups. The accumulation of Cd by duckweed in the different treatment groups is shown in Fig. 1d and there was no significant difference among the Cd, Cd/PE, and Cd/PP groups. Under Cd stress, the duckweed accumulated 89.8 mg kg^{-1} Cd and duckweed in the combined Cd/PE and Cd/PP treatments accumulated 86 mg kg^{-1} and 120.9 mg kg^{-1}, respectively, with non-significant differences compared to the Cd single treatment. It can be seen that, under heavy metal stress, growth of duckweed were inhibited.

3.2. Effects of Cd and MPs on the antioxidant enzymes and membrane lipid peroxidation in duckweed

When duckweed is exposed to stress, it leads to the accumulation of reactive oxygen species (ROS). The excessive accumulation of ROS can lead to the apoptosis of various cells, which is harmful to plant growth. Antioxidant defense system of plants is stimulated and antioxidant enzymes are able to clear ROS (He et al., 2017). SOD is the first line of defense against oxidation in plants as this enzyme can remove the excess superoxide anions in cells (Gill et al., 2015). As shown in Fig. 2a, the SOD activity of the Cd stress group was significantly lower than that of the other groups. The SOD activity of the Cd/PE combined group was significantly higher than that of the single stress group, and the Cd/PP combined group was significantly higher than that of the other groups including the CK group.

CAT can disproportionate superoxide anions to H_2O (Tolmacheva and Nevinsky, 2022). The changes in CAT activity levels may be seen in Fig. 2b. The activity level of CAT under Cd stress was significantly lower than that of control group under Cd single stress and Cd/PP combined stress. Under stress conditions, the metabolic capacity of duckweed may be inhibited, resulting in decreased CAT activity levels under Cd stress.

POD can effectively remove excess free radicals that accumulate from damage, prevent peroxidative damage, and enhance plant stress resistance (Lukacova et al., 2021). The POD activity of duckweed under single stress and combined stress was not significantly different from CK group (Fig. 2c).

When plants are stressed by the external environment, this leads to an increase in ROS free radicals, which damages the membrane system and leads to membrane lipid peroxidation. The final stage of this damaging process is the production of MDA. MDA content can reflect the degree of lipid peroxidation and damage (Miller et al., 2010). As shown in Fig. 2d, the MDA content of duckweed under Cd/PP stress was higher than that under PP single stress, and the MDA content under Cd single stress was higher than that under PP and PE single stress.This indicates that the duckweed was severely damaged under heavy metal stress.

3.3. Effects of Cd and MPs on the ultracellular structure of duckweed

Fig. 3 shows the damage to duckweed mesophyll cells in each treatment. The CK group is shown in Fig. 3a, where the chloroplast membrane was transparent, the matrix was dense, the thylakoid sheets in the chloroplast were neatly stacked, and the ordered sheets were thicker and tightly arranged. There were no large starch granules in the chloroplast matrix, indicating that the photosynthetic end products in the duckweed chloroplasts were transported smoothly without accumulation. This indicates that the mitochondria and chloroplasts can cooperate closely, and continue to provide energy for the transportation and accumulation of photosynthetic substances. According to Fig. 3b–f, the damage to the cell wall was minor, and the thickness did not change significantly. However, as shown in Fig. 3b, the cell membrane was damaged, discontinuous, and was detached from the cell wall. In addition, some chloroplasts contracted from oval to ribbon. Chloroplasts were located along the edge of the plasma membrane near the cell wall, oval in shape. As shown in Fig. 3c–d, the chloroplast structure was severely damaged; the thylakoids were loosely structured, either stuck together or degraded, twisted, and arranged disorderly; and the mitochondria were atrophied. In Fig. 3e–f, the thylakoid in the chloroplast were thin, the mesophyll cell structure was not severely damaged, and the cells did not appear to be vacuolated. It can be seen from the TEM image that the order of the degree of chloroplast structural damage among treatments from most severe to least severe was PP > PE > Cd > Cd/PP > Cd/PE. The Cd/PE and Cd/PP groups exhibited less damage than the Cd, PE, and PP single stress groups. The chloroplasts were rich in starch granules and had many osmophilic granules of lipid substances. It has been shown that starch granules accumulate in duckweed when exposed to high light intensity, thus the large number of starch granules visible in each set of images may be the result of excessive light exposure (Wang et al., 2022).

3.4. Effects of Cd and MPs stress on microorganisms in the duckweed rhizosphere

3.4.1. High-throughput sequencing results

Though high-throughput sequencing analysis, 855,901 valid sequences with 35,866,783,830 bases were sequenced in different treatments, and the sequence numbers of each sample ranged from 29,305 to 38,595 with an average sequence length of 419 bp. In total, 32 phyla, 69 classes, 190 orders, 314 families, 603 genera, 1088 species, and 2490 Operational Taxonomic Unit (OTUs) were detected in the six groups of samples. The dilution curves showed that the number of OTUs increased sharply and then levelled off as the amount of sequencing data increased. The coverage of all samples reached over 98 %, indicating that the sequencing results can truly reflect the species and number of microbial communities under the different treatments (Fig. 4).

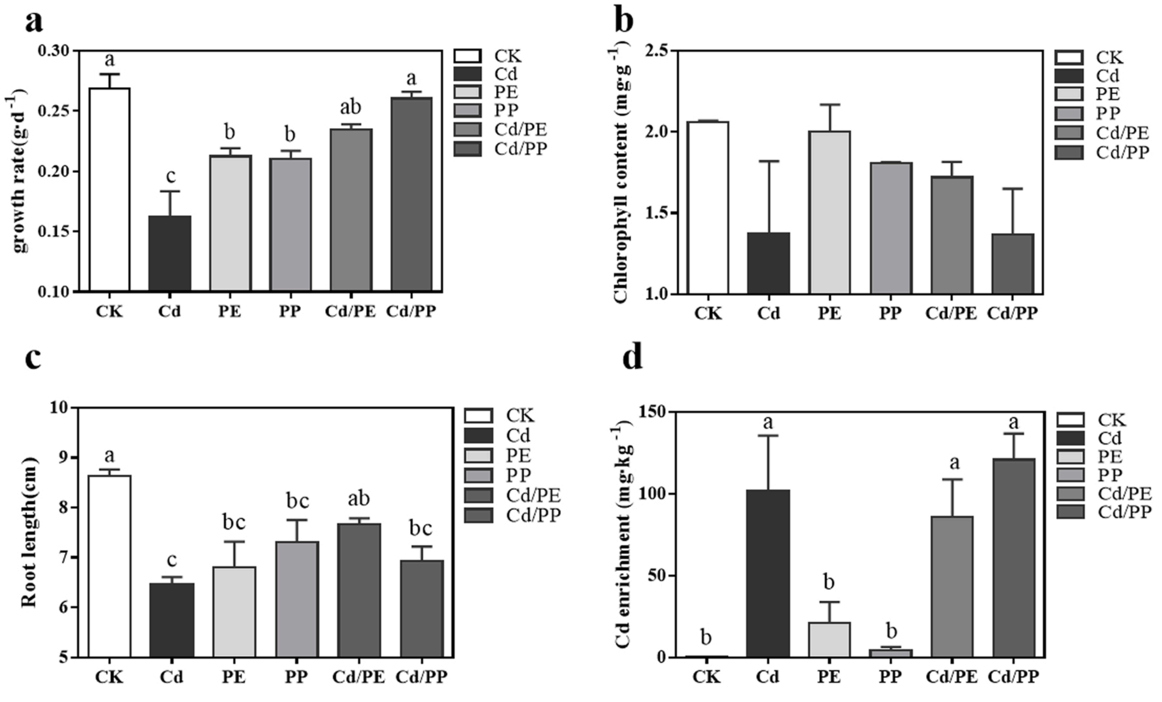

Fig. 1. Effects of different treatments on duckweed. (a) Effects of Cd and MPs single and combined stress on the growth rate of duckweed, (b) Effects of Cd and MPs single and combined stress on the chlorophyll content of duckweed, (c) Effects of Cd and MPs single and combined stress on the root length of duckweed, and (d) Effects of Cd and MPs single and combined stress on the accumulation of Cd in duckweed. The lowercase letters and corresponding error bars indicate significant differences ($p < 0.05$). Data are shown as the mean ± standard deviation.

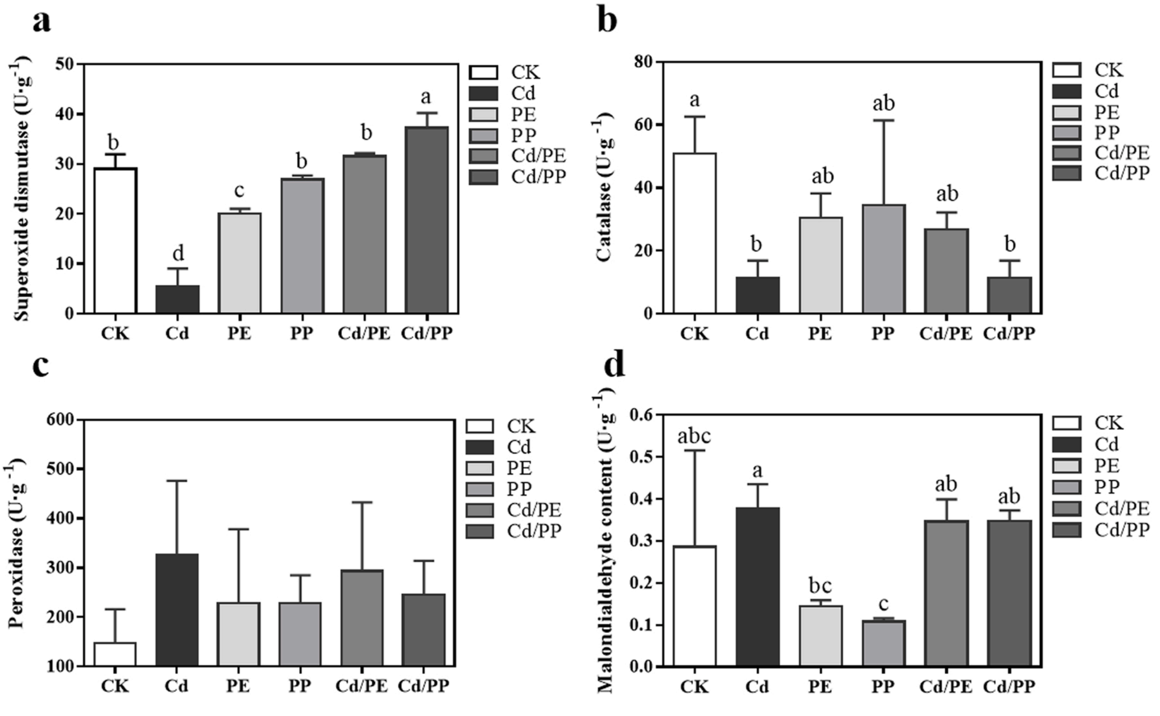

Fig. 2. Effects of Cd and MPs single and combined stress on antioxidant enzymes in duckweed. (a) Effects of Cd and MPs single and combined stress on SOD activity in duckweed, (b) Effects of Cd and MPs single and combined stress on CAT activity in duckweed, (c) Effects of Cd and MPs single and combined stress on POD activity in duckweed, and (d) Effects of Cd and MPs single and combined stress on MDA content in duckweed. The lowercase letters and corresponding error bars indicate significant differences ($p < 0.05$). Data are shown as the mean ± standard deviation.

3.4.2. Diversity analysis of microbial communities under different treatments

The alpha diversity refers to the diversity within a particular area or ecosystem. A higher diversity index indicates higher abundance and greater diversity of microbial communities. Commonly used metrics include the Chao, Shannon, ACE, and Simpson indices. The Shannon index reflects the diversity of species among communities; the higher Shannon index, the higher the species abundance and evenness in the

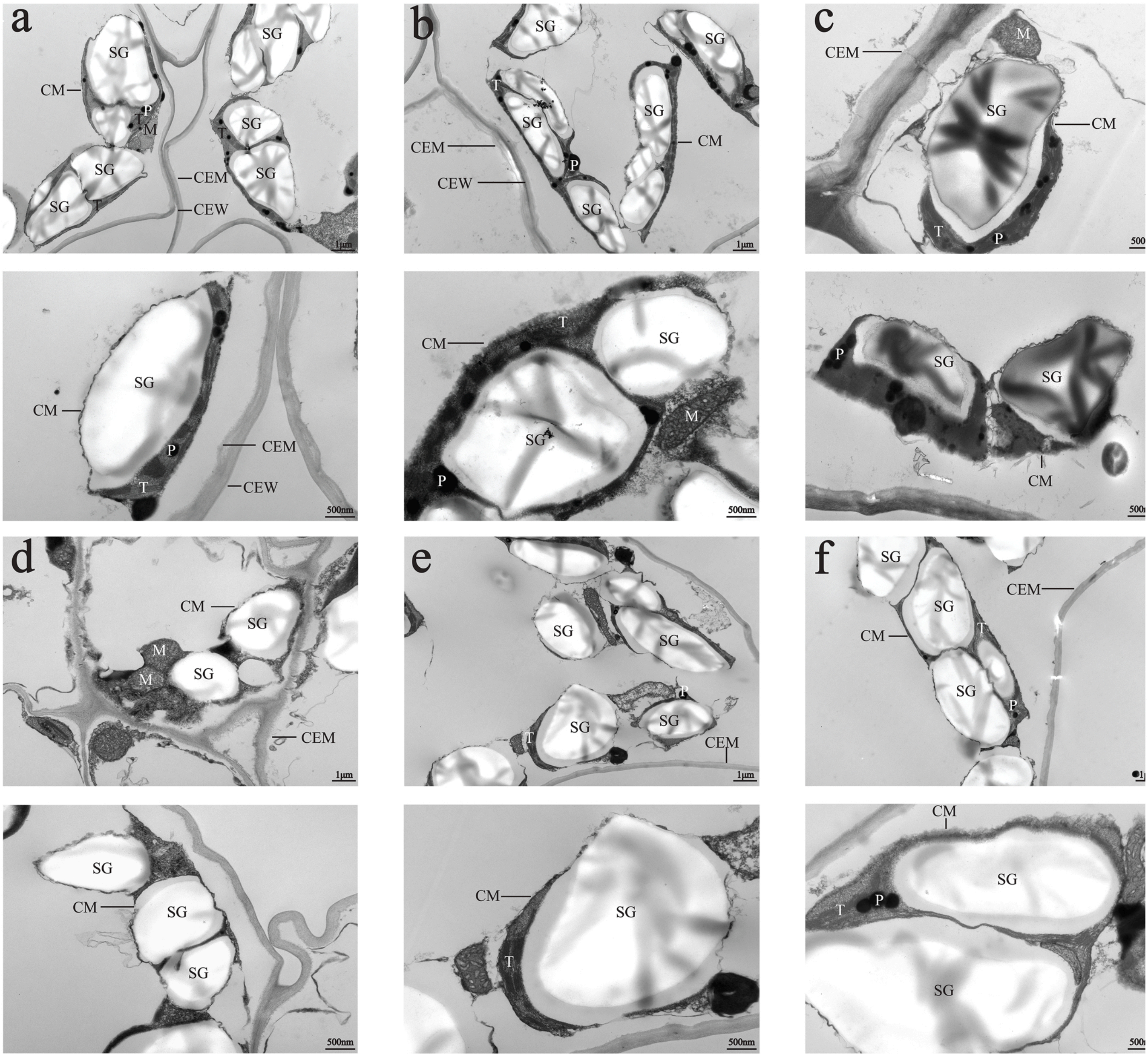

Fig. 3. Damage done to duckweed mesophyll cells under different treatments. Damage under the (a) CK group, (b) Cd treatment, (c) PE treatment, (d) PP treatment, (e) Cd/PE combined treatment, and (f) Cd/PP combined treatment. CM: chloroplast membrane, T: thylakoid, SG: starch granules, CEM: cell membrane, CEW: cell wall, M: mitochondria, P: plastoglobulus.

community. The Chao and ACE indices reflect the community abundance, and the Simpson index responds to community evenness. A higher coverage index indicates that the sequencing results can reflect the real situation and species abundance of the samples more accurately (Chen et al., 2019, 2021). The abundance and diversity of microbial communities under different treatments were determined (Table 3), and the results showed that the abundance of the microbial community in the duckweed rhizosphere decreased under the single and combined stress treatments of Cd, PE, and PP. Furthermore, the abundance in the Cd/PE and Cd/PP combined groups was higher than that of the Cd and PE single treatment groups, indicating that the microbial abundance in the rhizosphere of duckweed improved under combined stress compared with that under the Cd, PE, and PP single stress treatments. The Shannon index showed that the microbial community diversity was highest under the Cd/PP combined treatment, which was even higher than that under the CK group. Additionally, the diversity under the Cd/PE combined treatment was lower than that under the Cd and PE single treatments. The community abundance and diversity of the Cd/PP combined group were also higher than those of the Cd/PE combined group. Overall, the microbial diversity indices indicated that the diversity and abundance of the microbial community improved after the combined stress treatment compared to the single Cd stress.

3.4.3. Distribution of OTUs in microbial communities under different treatments

Venn diagrams can be used to count the number of species that are both common and unique to multiple groups or samples, thereby providing a visual representation of the similarity and overlap of species compositions in different environmental samples (Belibasakis and Manoil, 2021). To explore the microbial community in the rhizosphere of duckweed under different treatments, the 16 S rRNA sequence was used to study the root microbial communities of duckweed samples

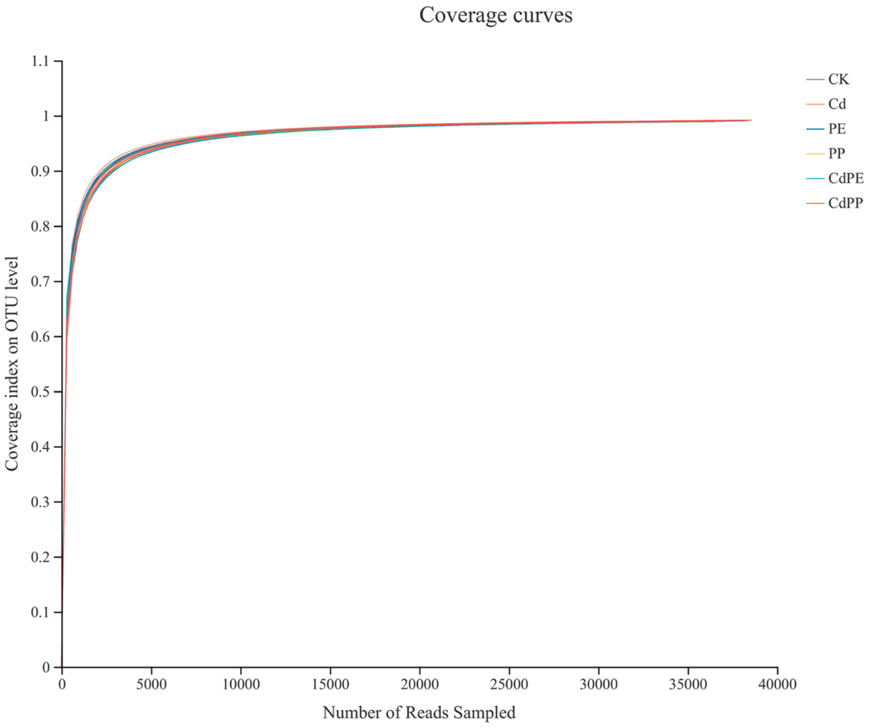

Fig. 4. Microbial dilution curves under different treatments.

Table 3
Microbial diversity index.

Samples	Shannon	Simpson	ACE	Chao	Coverage
CK	5.565760667	0.013379	1854.502035	1901.230871	0.98
Cd	5.338663667	0.020657667	1690.839287	1703.994711	0.98
PE	5.469303	0.015296333	1734.697632	1734.802676	0.98
PP	5.502885	0.016429667	1840.657847	1827.377177	0.98
Cd/PE	5.291464333	0.023553667	1763.686907	1756.123993	0.99
Cd/PP	5.641463	0.011884333	1749.974789	1774.596847	0.99

under different conditions. Fig. 5 shows the difference and proportion of microbial community OTU composition among the different treatments. The OTUs were classified based on a similarity level ≥ 97 %, and 1381 OTUs were obtained for species classification (Edgar, 2018). The microbial community-specific OTUs of the Cd, PE, and PP groups were 16, 22, and 18, respectively, and the microbial community-specific OTUs of the Cd/PE and Cd/PP groups were 8 and 14, respectively. The results show that the microbial community composition was different under the different treatments.

3.4.4. Microbial community composition under different treatments

Fig. 6a shows the differences in the distribution of microorganisms at the phylum level, with all samples having similar dominant flora but slightly different abundances. *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Myxococcota*, *Actinobacteriota,* and other dominant bacteria were the most common microorganisms. Among them, *Proteobacteria*, as the most tolerant of the microorganisms (Su et al., 2020; Wang et al., 2020a; Wang et al., 2020b), was the dominant phylum in all groups. The abundance percentage in the different groups was in the order of Cd/PE > CK > Cd > PE > PP > Cd/PP. At the family level, there were differences in the composition of microbes in the duckweed rhizosphere among the groups (Fig. 6b). In the control group, the three families with the highest abundance in the microbial communities were *Comamonadaceae*, *Rhodobacteraceae*, and *Saprospiraceae*. Under the three treatments of Cd, PP, and Cd/PP, the three families with the highest abundance of microbial communities were *Comamonadaceae*, *Rhodobacteraceae*, and *norank_o_Chloroplast*. Notably, under the Cd/PP treatment, the proportion of *Comamonadaceae* was significantly lower than that of the other groups.

3.4.5. Heat map of microbial communities at the phylum level under different treatments

Fig. 7 shows a heat map at the phylum level, which was used to compare the types and quantities of various symbiotic microorganisms. This method can be used to visually study the composition of the community. Changes in the abundance of different species in the sample were displayed through the color gradient of the color block. The values represented by the color gradient are shown on the right side of the figure. Red represents phyla with high abundance and blue represents phyla with low abundance (Chen et al., 2021a; Chen et al., 2021b). In the analysis of the microbial composition, 32 phyla were detected in the duckweed samples, with more microbial species and increased abundance seen in phyla such as *Verrucomicrobiota*, *Myxococcota*, *Actinobacteriota*, and *Acidobacteriota*. The abundances of *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* were all higher in the six treatments compared to the CK group. Among them, *Bacteroidetes* and *Cyanobacteria* were more abundant under Cd/PP stress than in the Cd single stress and CK groups. It was further confirmed that microorganisms can be present under the single or combined stresses of Cd, PE, and PP, and that Cd and MPs affect the microorganisms that live on the surface of duckweed.

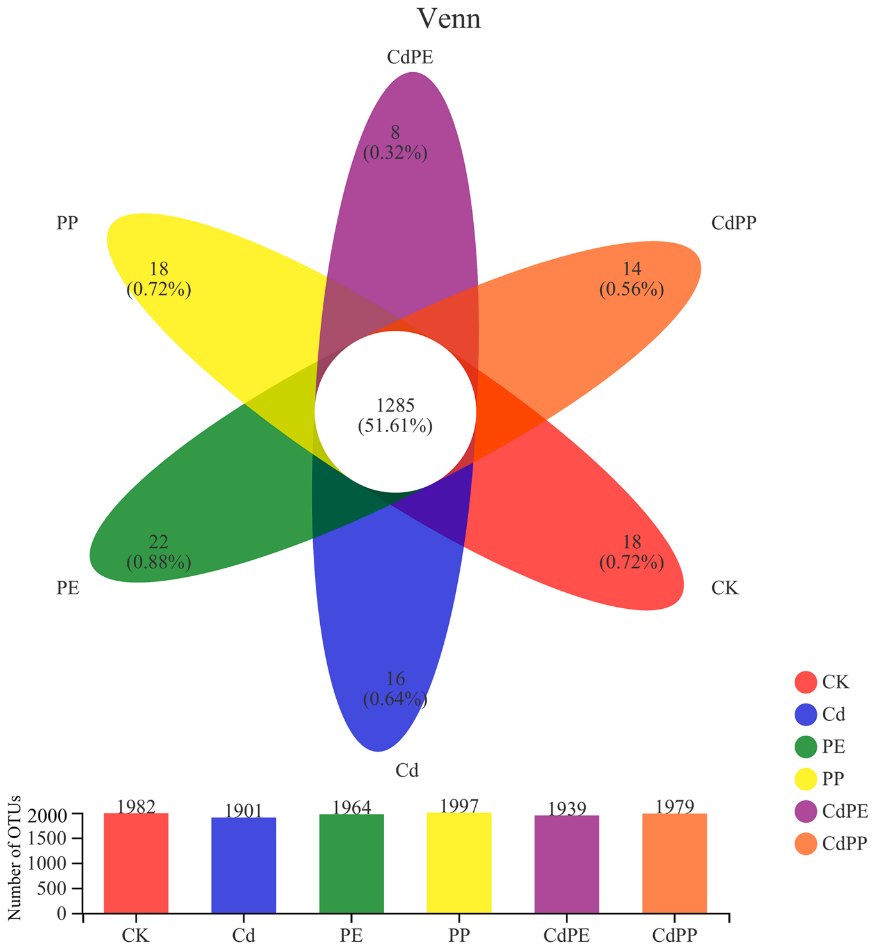

Fig. 5. Venn diagrams showing the level of OTUs of different taxa.

3.4.6. *Analysis of the difference in microbial community structure under different treatments*

Fig. 8 shows the analysis of the differences in the microbial community structure. The duckweed samples under different treatments and the CK group samples were separated by a long distance in the picture, indicating that the root microbial community of duckweed was significantly different under the varying treatments. The microbial communities were clearly distinguishable from those under the single and combined stresses of Cd, PE, and PP (Wang et al., 2016).

4. Discussion

Some studies have found that heavy metals and microplastic are released into the environment due to human activities and accumulate in the environment (Abbas et al., 2022; Khalil et al., 2022). Heavy metal in the environment can significantly reduce the content of photosynthetic pigments in plants, mainly by affecting electron transfer in plant photosynthesis, and destroying the integrity of photosynthetic systems, such as chloroplasts and chlorophyll (Zhang et al., 2020a). Heavy metals and microplastics entering the plant first affect the cell membrane, which acts as the medium and barrier for material exchange and information transfer between plant cells and the external environment. It is mainly composed of phospholipid molecules and proteins, with a large number of unsaturated fatty acids (Keyster et al., 2020). When duckweed suffers from stress, it produces a large number of reactive oxygen radicals that can attack the cell membrane, and damage the unsaturated fatty acids and proteins in the cell membrane. This results in a loose cell membrane structure, increased membrane permeability, and reduced enzyme activity, making it easier for pollutants to enter the cell. Additionally, the intracellular enzyme concentrations become out of balance, and some soluble components in the cell are more likely to seep out, causing serious damage (Zhang et al., 2017). When the heavy metals and microplastics entered the duckweed, the damage to the organelles such as the nucleus, chloroplasts, and mitochondria became intensified. This damage was manifested in chromatin condensation and the partial rupture of nuclear membranes. In addition to this, the chloroplast membrane and part of the vesicle disappeared, resulting in serious damage to the chloroplast. There was also damage to some of the mitochondrial membranes, and vacuoles appeared.

In the study of *Triticum aestivum L.* and *Chlorella vulgaris*, the addition of MPs alleviated the toxic effect of Cd on plants, which is consistent with the results of this study (Wang et al., 2021; Zong et al., 2021). The reason why combined treatment of MPs and Cd can reduce the toxicity of Cd is worth exploring. Many studies have shown that MPs can be used as a carrier to adsorb heavy metal ions in the environment (Gao et al., 2019; Guo and Wang, 2021). MPs have negative surface charge due to aging, abrasion and photo-oxidation, and thus can bind to heavy metal ions with cations. In addition, the main factors affecting the adsorption of MPs with heavy metal ions are the particle size, aging, environmental pH and concentration of MPs. the smaller the particle size of MPs, the larger the specific surface area, which provides more spatial locations for heavy metal ions to be adsorbed. The more aged or worn MPs have a larger surface area, providing negatively charged active sites capable of adsorbing more heavy metal ions (Wang et al., 2021). The smaller the pH, the more other cations present in the environment compete with heavy metal cations for binding sites on MPs (Khalid et al., 2021). Some scholars believe that, it may be that the adsorption of Cd by MPs leads to a decrease in the concentration of Cd in the solution, thereby reducing

Fig. 6. Community abundance at different levels. (a) The percentage of community abundance at the phylum level, and (b) the percentage of community abundance at the family level.

the toxicity to plant (Zhou et al., 2019). However, The effect of the complexes formed by MPs and Cd is more complex. The complexes are usually not transferred into plant tissues due to their considerable molecular weight and may adsorb to the plant root surface. However, MPs with small particle size (less than 1 μm) may carry heavy metal ions into plants through apoplastic and symplastic pathways, accumulate in plant roots, or transfer to aerial parts of plants by transpiration flow through the xylem (Kumar et al., 2022). The toxicity of the complex to plants is mainly due to the effects of MPs on plants and oxidative stress caused by Cd, as well as the possible combined toxicity of both. Studies have shown that MPs attached to plant root surfaces may interfere with nutrient and water uptake by plant roots due to their hydrophobicity, and also affect Cd uptake by plants (Wang et al., 2020a; Wang et al., 2020b). And MPs with smaller particle size absorbed by plants may block root channels, interfere with water and nutrient transport and cause oxidative stress (Zong et al., 2021). The negative effects caused by MPs and oxidative stress induced by Cd may form a combined toxicity to plants, affecting plant biomass, photosynthetic activity, chlorophyll content and root length (Kumar et al., 2022). Hyperaccumulators such as duckweed have a strong mechanism of tolerance and detoxification of heavy metals, which can reduce the toxicity of Cd through chelation and compartmentalization (Yang et al., 2021a, 2021b). The mechanism by which the formed complexes are taken up by plants, the distribution in plants, and the reasons for the reduced toxicity of Cd need further study.

Duckweed is composed of fronds and roots. A large number of previous studies have focused on the interaction between duckweed leaves and microorganisms, indicating that duckweed water purification may be associated with its leaves. Recent research has revealed the diversity

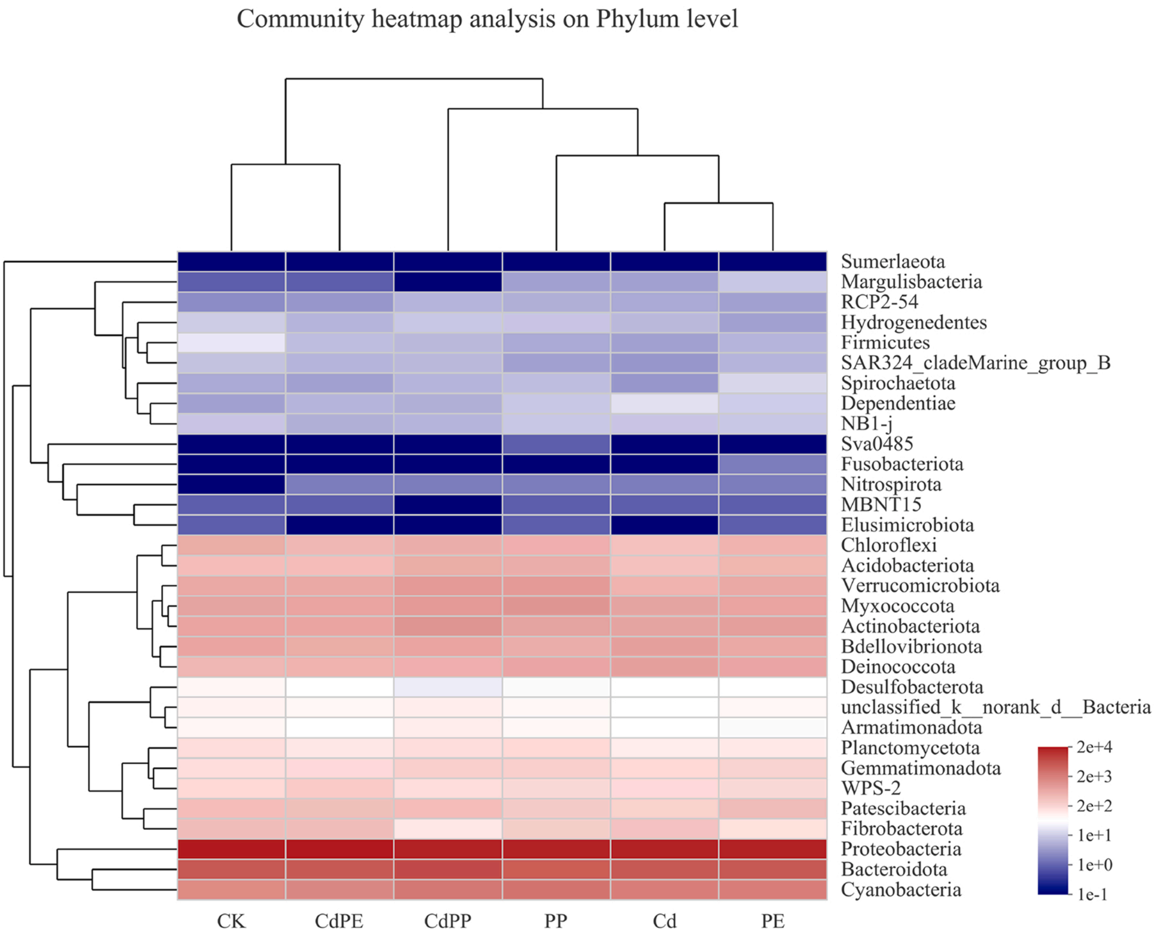

Fig. 7. Heat map of microorganisms at the phylum level under different treatments.

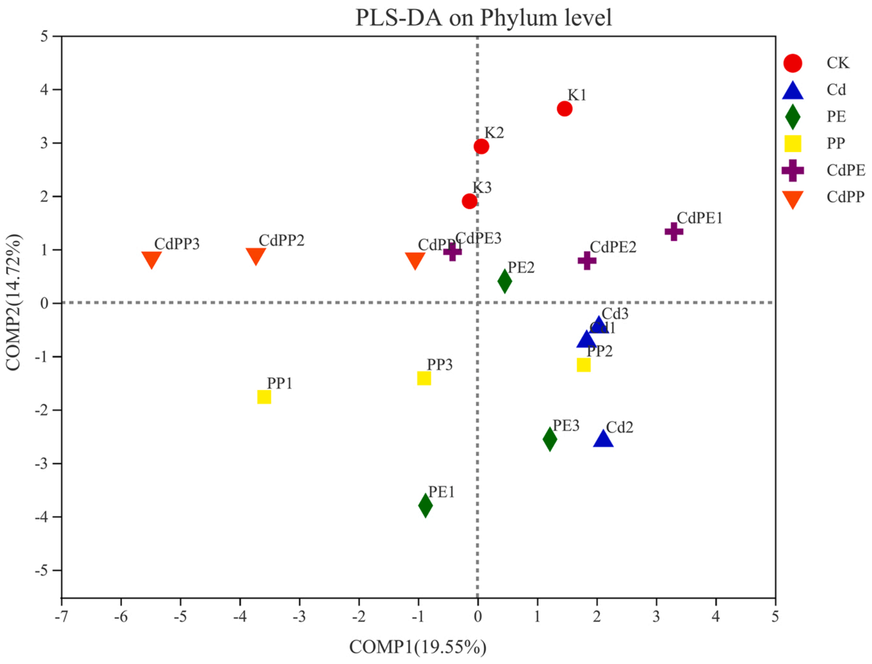

Fig. 8. Differences in microbial community structure (PLS-DA analysis).

and composition of microbial communities throughout the whole plant and roots of duckweed. They found that microorganisms inhabiting the roots play a key role in the degradation of pollutants (Zhao et al., 2015). Microorganisms associated with duckweed are involved in the removal and detoxification of contaminants, which can increase the growth rate of duckweed and facilitate contaminant removal. Therefore, the presence of microorganisms in the rhizosphere of duckweed may alter its remediation potential. Under the combined stress of microplastics and heavy metal Cd, rhizosphere microorganisms can effectively maintain the growth, development and physiological metabolism of duckweed, alleviate the damage of chloroplast in mesophyll cells of duckweed, and reduce the toxic effect of Cd on duckweed (O'Brien et al., 2020a, 2020b).

Rhizosphere microorganisms are the first line of defense against the contaminants that come in contact with duckweed, and they play an important role in enhancing the plant's tolerance. *Proteobacteria* are the microorganisms that are most tolerant to heavy metals. High concentrations of heavy metals and toxic substances serve as a source of energy and nutrition for *Proteobacteria*; as a result, these microbes can tolerate or use pollutants to promote the growth of duckweed. *Proteobacteria* encode multiple heavy metal oxidase genes that are involved in heavy metal resistance, thereby offering the possibility of using duckweed phytoremediation efforts (Altimira et al., 2012; Drzewiecka, 2016). *Bacteroidetes* can promote nutrient turnover under high Cd concentrations, and *Bacteroidetes* are especially abundant in the rhizosphere of hyperaccumulators (Hou et al., 2018). It is important to mention that the species and abundance of *Proteobacteria* and *Bacteroidetes* were high in all treatment groups, with *Proteobacteria* being the dominant phylum under all treatment conditions, followed by *Bacteroidetes*. The dominance of Proteobacteria under Cd/PE combined treatment was the largest, which was greater than that of Cd and PE single stress group, and also greater than that of CK group. In addition, the abundance of Bacteroidetes and cyanobacteria in the Cd/PP composite treatment was greater than that in the Cd single stress group and the CK group.This indicates that the resistance of duckweed to Cd may be improved under the combined stress of heavy metals and microplastics.

There were positive and negative effect between duckweed and rhizosphere microorganisms. One positive effect was in the rhizosphere microorganisms promoting host growth, whereas negative effect included the rhizosphere microorganisms competing with hosts for nutrients, and infesting hosts to cause diseases (Bais et al., 2006). Plant roots provide nutrients for the growth and reproduction of rhizosphere microorganisms, which in turn have an important impact on the diversity and structure of rhizosphere microbial communities. The activities and metabolites of plant rhizosphere microorganisms can also directly or indirectly affect the absorption of nutrients by the roots, thereby affecting their growth and development (Mendes et al., 2013).

5. Conclusions

In this study, the growth rate of duckweed decreased to 0.16 g d^{-1} after 15 days of 0.01 mg L^{-1} Cd treatment, which was much lower than the normal growth rate of 0.27 g d^{-1}, indicating that Cd inhibited the growth of duckweed. After the addition of 50 mg L^{-1} polyethylene or polypropylene to form a combined stress, the growth rate recovered to 0.23 g d^{-1} and 0.26 g d^{-1}, respectively. There was no significant difference in chlorophyll content among the groups, and the root length of the stress group was lower than that in the control group. There was no difference in the accumulated Cd content between the Cd single stress group and the combined stress group. Superoxide dismutase activity was higher in the combined treatment group than in the single stress group, malondialdehyde content was higher under combined stress of Cd and polypropylene than under polypropylene single stress, and there was no difference in catalase activity and peroxidase activity. It could be seen by scanning electron microscopy that chloroplasts were less damaged under the combined stress than Cd single stress, indicating that the combined stress of microplastic and Cd may alleviate the toxic effects of Cd on duckweed. Microbial community analysis showed that the diversity and abundance of microbial communities were improved after combined stress treatment compared to single Cd stress. The abundance of *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*, which are heavy metal-tolerant microbial communities, was higher than that of the Cd single stress group. Studying the effect of Cd, polyethylene, polypropylen and their complexes on duckweed, and their effect on the duckweed rhizosphere Microbial community may not only improve the adaptability of duckweed in polluted water, but also provide new insights for the more efficient use of duckweed for environmental remediation.

CRediT authorship contribution statement

Gui-Li Yang: Conceptualization, Methodology, Writing – review & editing. **Meng-Meng Zheng:** Investigation, Methodology, Writing – original draft. **Hai-Min Liao:** Investigation, Methodology. **Ai-Juan Tan:** Supervision. **Dan Feng:** Investigation, Methodology, Writing – original draft. **Shi-Ming Lv:** Methodology, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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