

Removal, distribution and plant uptake of perfluorooctane sulfonate (PFOS) in a simulated constructed wetland system

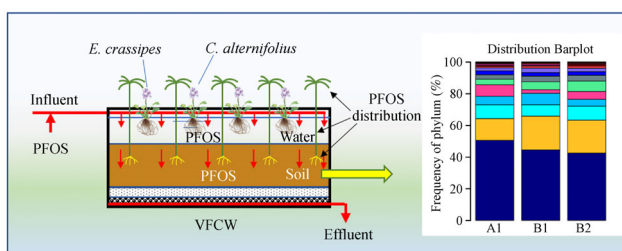
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HIGHLIGHTS

- PFOS was removed by soil adsorption and plant uptake in the VFCW.
- Uptake of PFOS by *E. crassipes* was more than that of *C. alternifolius*.
- PFOS in wastewater can inhibit the removal of nutrients.
- Dosing with PFOS changed the soil microbial community in the VFCW.

GRAPHIC ABSTRACT



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ABSTRACT

A vertical-flow constructed wetland (VFCW) was used to treat simulated domestic sewage containing perfluorooctane sulfonate (PFOS). The removal rate of PFOS in the domestic sewage was 93%–98%, through soil adsorption and plant uptake, suggesting that VFCWs can remove PFOS efficiently from wastewater. The removal of PFOS in the VFCW was dependent on soil adsorption and plant uptake; moreover, the percentage of soil adsorption was 61%–89%, and was higher than that of the plants uptake (5%–31%). The absorption capacity of *Eichhornia crassipes* (*E. crassipes*) (1186.71 mg/kg) was higher than that of *Cyperus alternifolius* (*C. alternifolius*) (162.77 mg/kg) under 10 mg/L PFOS, and the transfer factor of PFOS in *E. crassipes* and *C. alternifolius* was 0.04 and 0.58, respectively, indicating that PFOS is not easily translocated to leaves from roots of wetland plants; moreover, uptake of PFOS by *E. crassipes* was more than that of *C. alternifolius* because the biomass of *E. crassipes* was more than that of *C. alternifolius* and the roots of *E. crassipes* can take up PFOS directly from wastewater while *C. alternifolius* needs to do so via its roots in the soil. The concentration of 10 mg/L PFOS had an obvious inhibitory effect on the removal rate of total nitrogen, total phosphorus, chemical oxygen demand, and ammonia nitrogen in the VFCW, which decreased by 15%, 10%, 10% and 12%, respectively. Dosing with PFOS in the wastewater reduced the bacterial richness but increased the diversity in soil because PFOS stimulated the growth of PFOS-tolerant strains.

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1 Introduction

As a typical perfluorinated compound, perfluorooctane sulfonate (PFOS) has been classified as a new persistent organic pollutant (POP) (Wang et al., 2009). Owing to its high chemical stability, PFOS can not be degraded by

microorganisms and plants, thus causing serious pollution to the environment (Xiao, 2017; Mumtaz et al., 2019). Up to now, PFOS has been detected in waters. For instance, wastewater from a municipal plant and an airport industrial treatment plant that discharge to San Francisco Bay, USA, contained an average of 420 and 560 ng/L PFOS, respectively (Houtz et al., 2016). In China, very high concentrations of PFOS (sum concentrations of aqueous and particle phase) were found in effluents from a

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wastewater treatment pond at a per- and polyfluoroalkyl substances (PFASs) manufacturing facility, with a mean value of 1021 $\mu\text{g/L}$ (Yawei et al., 2010). Thus, removal of PFOS and reducing the associated risk to aquatic environments is an important and emerging topic.

Constructed wetlands have been widely used to treat wastewater because of the method's low financial and energy costs. The removal of pollutants in a constructed wetland system is mainly based on matrix adsorption, interception, plant uptake, enrichment, and microbial degradation. A constructed wetland can remove not only heavy metal ions but also emerging contaminants (Matamoros et al., 2008; Song et al., 2009; Yi et al., 2017). However, removal of perfluorinated compounds (PFCs) in constructed wetlands has been rarely studied. Chen et al. (2012) documented for a pilot-scale constructed wetland that the removal efficiencies for perfluorooctanoic acid (PFOA) and PFOS in liquid phase were 77%–82% and 90%–95%, respectively, with phytoextraction and soil sorption being the most significant removal mechanisms. Yin et al. (2017) reported that PFAAs were removed most efficiently in the reed bed (42%–49%), likely due to the combination of sorption to soils and sediments and plant uptake.

Plant uptake plays an important role on removal of pollutants in wetlands. Unlike the bioaccumulation mechanisms of PFASs in terrestrial plants, direct exchange between water and plant tissues is a key process for uptake and elimination of contaminants in wetland plants. Uptake into submerged macrophytes includes direct exchange with leaf surface, and uptake via pore water in the bed sediment. For free-floating plants, transpiration and air-leaf exchange are likely important uptake processes because of their root systems in the water (Pi et al., 2017). Mudumbi et al. (2014) determined the susceptibility to PFOA accumulation in 13 wetland plants from PFOA-contaminated riparian sediment, and found that the concentration of PFOA in the plants and/or reeds was in the range of 11.7 to 38 ng/g, with a bioaccumulation factor (BCF) range of 0.05 to 0.37. Pi et al. (2017) reported that all PFASs were readily accumulated in these aquatic macrophytes, and elimination rate constants and BCFs increased with increasing perfluoroalkyl chain length. Uptake of PFASs is similar in the different plant species. However, distribution of PFASs in different part of wetland plants is not clear, as well as the distribution in plants, soil and water in a wetland. Moreover, it was rarely reported the effect of PFASs on the removal capability of nutrients in wastewater by a wetland.

The microbial community structure of soil is an important factor which can affect the soil adsorption and degradation of pollutants. However, few studies have revealed the effects of PFASs on soil microbial community structure in wetlands. Zhang et al. (2020) reported that the bacterial diversity decreased upon PFASs exposure in aquatic plant-based systems, derived from a surface flow

constructed wetland (CW) planted with *Typha angustifolia*. Dosing with PFAAs at the high level led to a significant change of a soil bacterial community in terms of composition and structure in plant–soil–water systems (Zhang et al., 2019).

In this study, we constructed a pilot vertical-flow constructed wetland (VFCW) with a floating plant and an emerged plant. The objective was to evaluate the removal efficiency of PFOS from simulated wastewater in the wetland, and to further explore the distribution and translocation of PFOS in water, soil and plants in the wetland. In addition, the effects of PFOS on the water's properties, such as total nitrogen (TN), ammonia nitrogen ($\text{NH}_3\text{-N}$), total phosphorus (TP) and chemical oxygen demand (COD), as well as the soil microbial community, were also investigated.

2 Materials and methods

2.1 Chemicals

The detailed information of all chemicals, standards, materials and plants is shown in Table S1. The soil samples were collected from unpolluted forest land of Nanjing Forestry University (China), after which they were air-dried and picking out residual branches and leaves, and then stored at 4°C until analysis. The characteristics of the soil were: pH, 7.13; TN, 1.75 g/kg; and TP, 0.179 mg/kg.

2.2 Treatment of wastewater PFOS by the VFCW

Figure S1 shows the layout and structure of the experimental VFCW. Eight plants of *Cyperus alternifolius* (*C. alternifolius*) and *Eichhornia crassipes* (*E. crassipes*) (planting density of 6–16 clumps/m²) were planted together at intervals in each tank. The study was conducted in autumn, and the average water temperature ranged from 9°C to 29°C each day. The initial weights of *E. crassipes* and *C. alternifolius* were 935±10 g and 430±5 g, respectively, controlled in the VFCW.

The wastewater flowed uniformly into the VFCW from its top, and discharged from its bottom. The wastewater was simulated domestic sewage, and was added at concentrations of 1 $\mu\text{g/L}$, 100 $\mu\text{g/L}$ and 10 mg/L. The characteristics of the wastewater are shown in Table S2. The influent mode was continuous water inflow, the residence time was 2 d, and the wetland was run for 42 d. Each experiment had three replicates, including blank controls. The blank control is the original wastewater without dosage of PFOS. The original properties of the wastewater have been shown in Table S2.

2.3 Determination of water quality

During the experiment, water samples were collected from

the influent and effluent of all the VFCWs every three days. The water's properties, including pH, COD, TN, TP and NH₃-N were analyzed. All analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

2.4 Determination of PFOS

The pretreatment and determination of PFOS methods can be referred to in a previous study (Mudumbi et al., 2014). Briefly, the collected water samples were enriched and purified by solid phase extraction (SPE) after being added to the internal standard. The extraction was concentrated to 0.5 mL by nitrogen blowing concentrators (Doprah, Shanghai, China) and then filtered through a 0.22 μm nylon filter membrane for subsequent determination. The soil/plant sample (0.5 g) was mixed with 4 mL of methanol and sonicated in for 20 min before the supernatant was collected after centrifugation (5000 × g). The appropriate amount of extract (0.01–20 mL) was diluted with ultrapure water and then extracted by an SPE cartridge (PWAX, 150 mg/6 mL, Agela, China). The extraction was eluted with 4 mL of methanol and 4 mL of 0.5% NH₄OH in methanol in turn. The eluent was then concentrated to 250 μL by the nitrogen blowing concentrator at 40°C before filtering the sample through a 0.22 μm nylon filter membrane for LC-MS/MS analysis. The detailed method of PFOS determination is described in Supplementary Materials. Information on other MS parameters is shown in Table S3. The method detect limit (MDL) and (method quantification limit (MQL) data of the extraction method are shown in Table S4.

2.5 Analysis of the soil microbial community in the VFCW

Soil samples were collected from control and 10 mg/L PFOS groups after 42 d of VFCW operation, and original soil stored at –80°C without PFOS treatment was used to analyze the microbial communities.

The microbial community in the VFCW was determined according to the method reported by Qiao et al. (2018). Genomic DNA was extracted from the soil samples according to the manufacturer's instructions (Mag-Bind Soil DNA Kit, Omega Bio-Tek Co., USA). An agarose gel image of the extracted genomic DNA is shown in Fig. S2. A forward primer, 341F (5' -CCCTACAC-GACGCTCTCCGATCTGCCTACGGGNGGCWG-CAG-3'), and a reverse primer, 806R (5' -GACTGGAGTTCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC- 3') were used for polymerase chain reaction (PCR) (Herlemann et al., 2011). The purified PCR products were sequenced using an Illumina® HiSeq 2000 system (Illumina, USA). The analysis of the microbial community was conducted using Vegan 2.5–6 in R package, UPARSE and Quantitative Insight of Microecology (QIIME) (Caporaso et al.,

2010; Hamady et al., 2010; Edgar, 2013).

The read number was normalized before computing bacterial richness and diversity according to a criteria protocol. Briefly, the primer splice sequences were first removed, and then the paired reads were merged into a sequence according to the overlap relationship between paired reads. The sample data were obtained after the samples were identified and distinguished according to the barcode tag sequence. Finally, the quality of each sample data was filtered to get the effective reads.

2.6 Data and statistical analysis

The transfer factor (TF) of PFOS in plants was calculated as follows:

$$TF = \frac{\text{PFOS concentration in aerial parts (mg/kg)}}{\text{PFOS concentration in roots (mg/kg)}} \quad (1)$$

An analysis of variance (ANOVA) was used to test the significance of the results using software SPSS 19.0, IBM, USA. A *p*-value of *p* < 0.05 was considered as statistically significant.

3 Results and discussion

3.1 Removal and distribution of PFOS in the VFCW

The removal efficiency and distribution of PFOS in the VFCW were obtained by measuring the concentration of PFOS in the influent, effluent, soil and plants in the system. The removal rate of PFOS in wastewater reached 93%–98%, as shown in Fig. 1(a), indicating that VFCWs can remove PFOS effectively from wastewater. The results were similar to the study by Chen et al. (2012). Figure 1(b) shows that the percentage of soil adsorption was 61%–89%, and was higher than that of the PFOS content in plants (5%–31%). The removal of PFOS in the VFCW was dependent on soil adsorption and plant uptake, which was consistent with Chen et al. (2012). Moreover, PFOS can not be removed effectively by volatilization, photolysis, hydrolysis, biodegradation and plant degradation (Natarajan et al., 2010). In addition, the amount of soil was much higher than that of plant biomass, so the total PFOS absorption amount of the soil was much higher than the uptake amount of plants.

With increasing PFOS concentration, the proportion of PFOS in water increased while that in plants and soil decreased, and the uptake of plants also decreased. When the PFOS concentration was low (10⁻³ mg/L), the proportion of PFOS in plants and soil was obviously higher than under treatment with high-concentration PFOS. In addition, when the absorption amount of soil and the uptake amount of plants for PFOS in the VFCW exceeded the highest loading rates of soil and plants, the

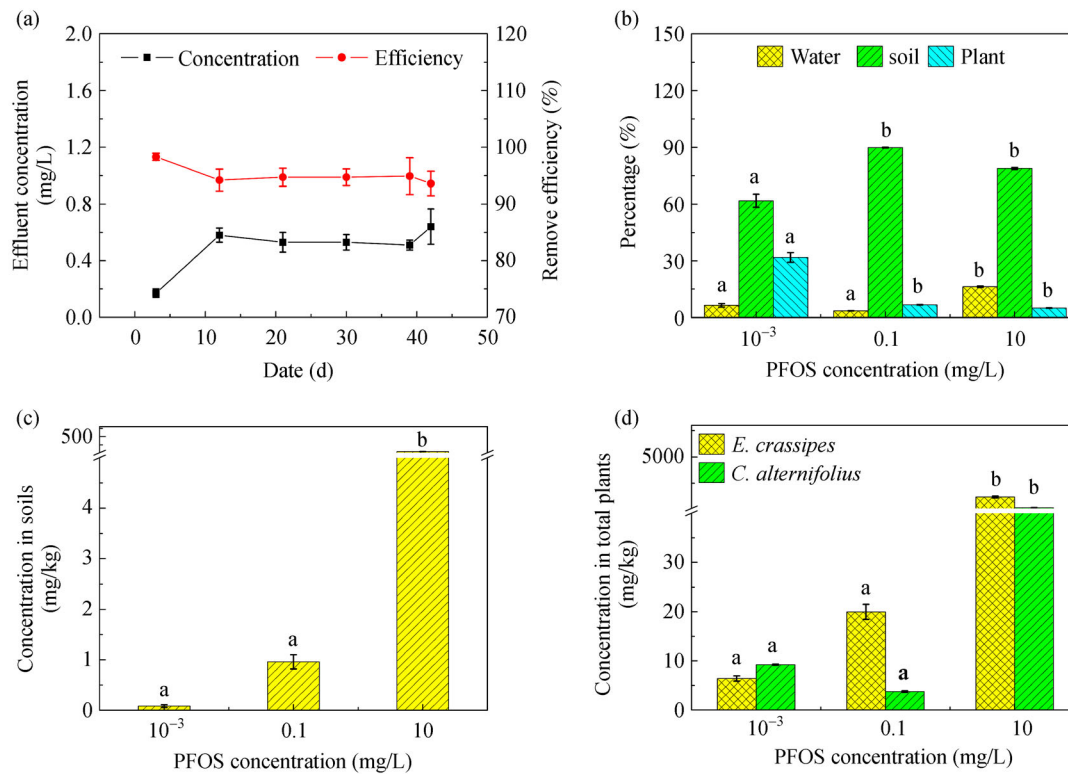


Fig. 1 Removal (a), distribution (b) and concentration of PFOS in the VFCW's soil (c) and plants (d). Values with different letters differ significantly ($p < 0.05$). Error bars represent standard deviations ($n = 3$).

accumulation of PFOS did not change obviously with increasing PFOS concentration in the effluent wastewater.

3.2 Accumulation of PFOS in different media of the VFCW

The concentration distribution of PFOS in the VFCW's soil and plants is shown in Figs. 1(c) and 1(d). The adsorption amounts of soil for PFOS at concentrations of 10⁻³, 0.1 and 10 mg/L were 0.083, 0.96 and 56.07 mg/kg, respectively, after 42 d of running the VFCW. The uptake capacity of *E. crassipes* at high concentrations was greater than that of *C. alternifolius*. The uptake rates of *E. crassipes* for PFOS at concentrations of 10⁻³, 0.1 and 10 mg/L were 6.39, 19.97 and 1186.71 mg/kg, respectively, while those of *C. alternifolius* were 9.21, 3.74 and 162.77 mg/kg. The soil adsorption to PFOS in wetlands is a physical process, which is faster than plant uptake. So, under low concentration (1 µg/L) of PFOS, PFOS was first accumulated in soil prior to uptake of *E. crassipes*, and then *C. alternifolius* takes up PFOS via its roots in the soil, whereas *E. crassipes* can take up less PFOS from water in the wetland than *C. alternifolius*.

The difference in absorption between the two plants would have been due to many factors, including their different protein contents, different root compositions, and surface areas (Wen et al., 2016). Inorganic and organic

matter in wetlands will affect the fate of PFOS, but the interaction between PFOS and the organic fraction may be more important (Johnson et al., 2007). In this study, the roots of *E. crassipes* were developed, and the surface area was bigger compared with that of *C. alternifolius*. Therefore, the uptake capability of *E. crassipes* was stronger than that of *C. alternifolius*. Moreover, as a floating plant, the roots of *E. crassipes* can take up PFOS directly from wastewater, whereas the roots of *C. alternifolius* need to do this from the soil. The mass transfer process might inhibit the accumulation of PFOS in plants.

The significant difference between soil adsorption and plant uptake is related to the high organic content in plants. The whole operational process of the VFCW was mainly in the neutral pH range. Under this condition, the charged property of PFOS can basically be described as a negative charge shell around a positive charge core, which makes adsorption to the negatively charged soil difficult (Johnson et al., 2007). However, the absorption and adsorption of organic matter are also affected by many factors, such as carbon content, the surface area of the matrix surface, and functional groups (Enevoldsen and Juhler, 2010). Therefore, further studies should be carried out to determine the absorption and adsorption mechanisms of PFOS in constructed wetland systems, especially the uptake mechanism of PFOS in the two different plants.

3.3 Distribution of PFOS in different parts of the plants

The difference in the mass distribution of PFOS between the aerial parts of the plants and their roots is shown in Fig. 2. The content of PFOS in the roots of *E. crassipes* was larger than that in the aerial parts; moreover, the proportion of PFOS in roots increased with increasing PFOS concentration. The content of PFOS in the roots of *C. alternifolius* was lower than that in the aerial parts under PFOS exposure. However, there was no significant difference between exposures to different PFOS concentrations ($p > 0.05$). The proportion of PFOS in the roots of *E. crassipes* was 58.65%–89.1%, which was higher than that of *C. alternifolius* (27.59%–38.66%), because the roots of *E. crassipes* can take up PFOS directly from wastewater while *C. alternifolius* needs to do so via its roots in the soil; moreover, the mass of root and aerial parts of *E. crassipes* than that of *C. alternifolius* as shown in Table S5.

The distribution of PFOS in roots of *E. crassipes*

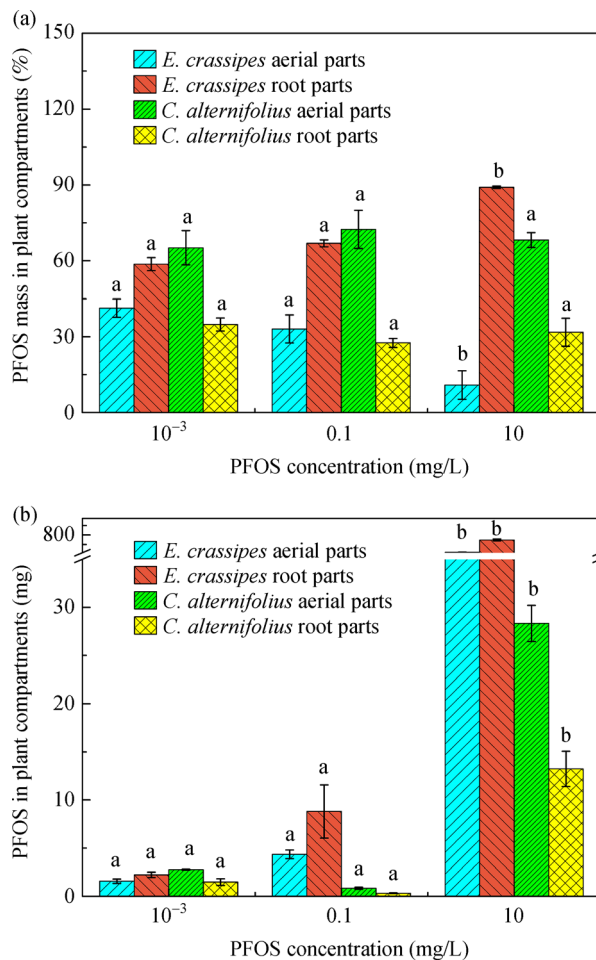


Fig. 2 Mass of PFOS in each compartment normalized to the total mass of PFOS in the whole plant. Values with different letters differ significantly ($p < 0.05$). Error bars represent standard deviations ($n = 3$).

showed some differences under different PFOS concentrations. Long-chain PFASs mainly existed in plant roots due to the resistance of the plants' casparian strip, although plant roots have high adsorption ability for long-chain PFASs (Felizeter et al., 2012). The distribution of PFOS in *C. alternifolius* was inconsistent with that of plants planted hydroponically or in soil culture (Blaine et al., 2014; Wen et al., 2014; Bizkarguenaga et al., 2016; Zhao et al., 2018). *C. alternifolius* is an emerged plant; the main source of PFOS in the roots is the PFOS adsorbed by the soil. Because the water level of the VFCW was higher than that of the soil, the stem part of the plant was directly exposed to the sewage, so the PFOS in the aerial part was absorbed directly from the sewage, and the other part comes from the transfer of the roots. Therefore, the PFOS content in the roots of *C. alternifolius* was lower than that in the aerial part at any concentration, which was similar to the findings of Pi et al. (2017). Specifically, their study showed that PFASs accumulate more effectively in the leaf tissue of *E. horemanii* than of *E. crassipes*, due to the nature of its submergence. *E. crassipes* is a floating plant whose leaves are not directly exposed to water, meaning nutrients need to be transported from the root to the leaf. To study the distribution of PFOS in plants, the TF was calculated. As shown in Fig. 3, the TF of *E. crassipes* was 0.04–0.18, and that of *C. alternifolius* was 0.58–0.82, indicating that PFOS is not easily transferred from the roots of *E. crassipes* and *C. alternifolius* to the aerial parts, but the transfer factor of *C. alternifolius* was larger than that of *E. crassipes*.

Two absorption mechanisms of PFASs in roots have been reported (Felizeter et al., 2012). First, PFASs are absorbed to root surface tissue between the root surface and the casparian strip, which is the dominant process. Secondly, the transpiration stream provides the roots a power of PFAAs absorption (Sebastian et al., 2014). PFOS is a long-chain PFC, and the absorptive capacity of plants

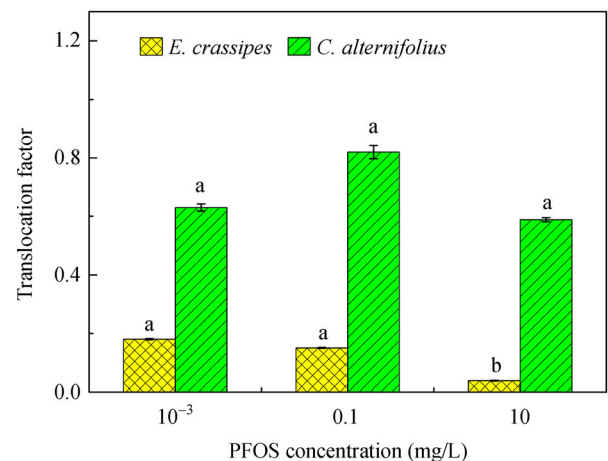


Fig. 3 The translocation factor (TF) of PFOS in *E. crassipes* and *C. alternifolius*. Values with different letters differ significantly ($p < 0.05$). Error bars represent standard deviations ($n = 3$).

is closely related to its root surface area. The accumulation of PFOS in *E. crassipes* was much higher than that in *C. alternifolius*, because of its large root surface area. In addition, absorption and translocation of pollutants by plants are dependent on the hydrophobic nature of pollutants (Krippner et al., 2014). The octanol-water partition coefficient ($\log K_{ow} = 4.13$) of PFOS indicates that PFOS is sufficiently lipophilic to move through the lipid bilayers of plant membranes and has adequate water solubility for transportation (Lechner and Knapp, 2011; Zhao et al., 2018). PFOS was not easily translocated in *E. crassipes* and *C. alternifolius*, but it was mainly absorbed and accumulated in plant roots. A previous study has shown that the TF decreases regularly with chain length (< 1 , PFOA and PFCAs, chain length $> C8$), indicating that these chemicals are mainly retained by roots, such as PFOA and PFOS in maize plants reported by Wen et al. (2013).

3.4 Influence of PFOS on the performance of the VFCW

A previous experimental study has shown that VFCWs have a good sewage treatment effect, but there is little in the literature on the effect of adding PFOS to wetlands (Yin et al., 2019). In this study, the VFCW was used to treat simulated domestic sewage, and the impacts of PFOS on effluent quality were observed. As shown in Fig. S3, the effluent pH value of each VFCW varied from 6.91 to 7.64

in the neutral range, indicating that the system operated normally.

After adding PFOS to the VFCW, the COD, TN, TP and $\text{NH}_3\text{-N}$ removal efficiencies fluctuated greatly in the first 21 d of operation, and then the fluctuation range gradually decreased thereafter, with the removal effect becoming basically stable. ANOVA analysis showed that the degradation rates of COD, TN, TP and $\text{NH}_3\text{-N}$ at 10 mg/L PFOS in the VFCW were 50%, 55%, 56% and 53%, respectively. Compared with the control group, these removal rates were significantly inhibited ($p < 0.05$), decreasing by 10%, 15%, 10% and 12%, respectively (Fig. 4). However, low-concentration (10^{-3} mg/L) PFOS showed no significant impact on wetland performance (see Table S6 for an analysis of the difference in removal efficiency). At the beginning of the experiment, because the root system of plants was in its growth stage but not yet fully grown, root branching had not developed and there was no capacity for large numbers of microorganisms to grow and attach. As a result, the water treatment effect in the early stage was basically declining, and the wetland operation was unstable. The reason why high-concentration (10 mg/L) PFOS inhibits the water quality of constructed wetlands is that it has certain toxicity to microbial activity and community structure. Bao et al. (2018) showed that higher levels of PFOS have a negative impact on bacterial abundance and diversity. Similarly, Qiao et al. (2018) showed that low concentrations of PFOS

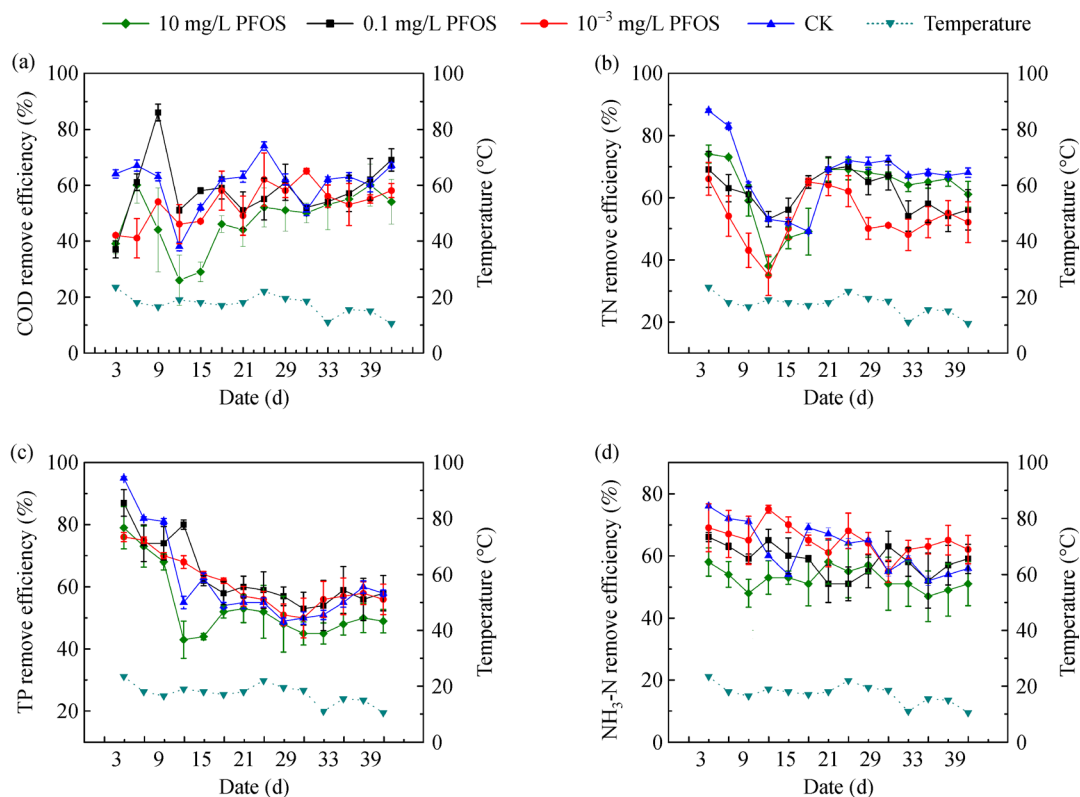


Fig. 4 COD (a), TN (b), TP (c) and $\text{NH}_3\text{-N}$ (d) removal rates in the VFCW. (see Table S5 for an analysis of the difference in removal efficiency).

led to a positive effect on the soil bacterial community, which was stronger than the toxic effect. In contrast, at high concentrations (10 mg/L) of PFOS, the toxic effects were stronger.

3.5 Bacterial community richness and diversity in the VFCW's soil

The raw Illumina sequence of VFCW's soil was deposited into the NCBI's Sequence Read Archive (SRA) database with accession number: PRJNA630683. The richness and diversity of the bacterial communities in different soil samples are presented in Table 1. The microbial richness was evaluated by Chao1 and ACE index, and the Shannon and Simpson indexes were used to evaluate the diversity. The readings in the library ranged from 45719 to 60502, and different OTUs ranged from 8456 to 13384. The species richness represented by Chao1 varied between 30915 and 79893; ACE in the soil samples ranged from 56322 to 18233. The uniformity calculated by the Simpson index ranged from 0.0028 to 0.01, and the range of Shannon index estimates was between 7.13 and 7.60. All samples had a high Shannon index; and after the sequencing number reached 30000, the Shannon index curves of all samples tended to flatten and the reliability of the sequence data were high (Fig. S4). The richness and diversity of soil microorganisms in the VFCW decreased after 42 d of operation with no addition of PFOS; whereas, dosing with PFOS resulted in increased microbial richness and diversity when compared with the unamended control after 42 d. The results were inconsistent with the previous study (Qiao et al., 2018). As a surfactant, PFOS increases the transport of organics from the soil matrix into the aqueous phase, resulting in increased bioavailability to microorganisms, which increases the richness and diversity of soil (Wolf et al., 2019). In addition, there was uniformity in the microbial community in soil treated with PFOS, indicating the abundance of microorganisms with strong tolerance to PFOS increased in the soil of the VFCW.

3.6 Bacterial community structure of the VFCW's soil

At the phylum level, soil bacteria were mainly composed of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* (Fig. 5(a)). *Proteobacteria* and *Acidobacteria* were the dominant bacteria, but decreased by 2.1% and 0.51% when compared with the control

running for 42 d (Fig. 5(b)). *Proteobacteria* play an important role in $\text{NH}_3\text{-N}$ removal (Wang and Zhu, 2014), which may be the main reason why the removal rate of $\text{NH}_3\text{-N}$ in the VFCW treated with PFOS was lower than that in the control. Previous studies have found that beta-proteobacteria play an important role in carbon and nitrogen cycling (Chen et al., 2016); plus, they are conducive to phosphorus removal (Bucci et al., 2012). *Acidobacteria* comprise many genera that lead to the degradation of organic matter, and was the second-most dominant bacteria in the soil dosed with PFOS. Thus, the reduction in *Acidobacteria* with dosing of PFOS in wastewater results in the inhibition of COD removal. *Actinobacteria* accounted for 5.39% and 4.31% in the initial soil and PFOS treatments, respectively. PFOS stress decreased its abundance, resulting in the decline of the organic matter removal rate under PFOS stress, which is in accordance with results reported by Liu et al. (2017). The results also explain that the removal rates of COD, TN, $\text{NH}_3\text{-N}$ and TP in the effluent of the high-concentration PFOS treatment group were lower than those of the control group.

At the genus level, *Acinetobacter*, *Gp6*, *Sphingomonas* and *Cloacibacterium* were the dominant genera (Fig. 6(a)). The proportions of *Gemmatimonas*, *Gp6* and *Sphingomonas* in soils treated with PFOS and the control after 42 d were higher than those in the initial soil, while those of *Acinetobacter* and *Cloacibacterium* decreased by 9.74% and 2.32%, respectively (Fig. 6(b)). PFOS stress increased the abundance of *Sphingomonas* and *Gemmatimonas*, which have the capacity to resist harsh environments and degrade pollutants (Wang et al., 2018). However, the abundance of these two bacteria decreased under PFOS stress. In addition, as shown in Fig. S5, the abundance of genes controlling energy production and conversion, amino acid transport and metabolism, carbohydrate transport and metabolism, transcription, inorganic ion transport and metabolism, and signal transduction mechanisms in the PFOS-contaminated soil and the control after 42 d, were higher than in the original soil.

4 Conclusions

This study highlights the potential for PFOS removal in VFCWs. The removal mechanism of PFOS in the VFCW was mainly soil adsorption and plant uptake, with the role of the former being more important than that of the latter.

Table 1 Bacterial community richness and diversity index of soil samples. A1: initial soil sample; B1: soil samples of the control group after 42 d; B2: soil samples after adding 10mg/L PFOS for 42 d

Sample	Reads (after Trim)	OTUs	Shannon	Chao1	ACE	Simpson
A1	60502	13384	7.17	79893	182193	0.01
B1	45719	8456	7.13	30915	56322	0.006
B2	52194	12759	7.60	76711	169450	0.0028

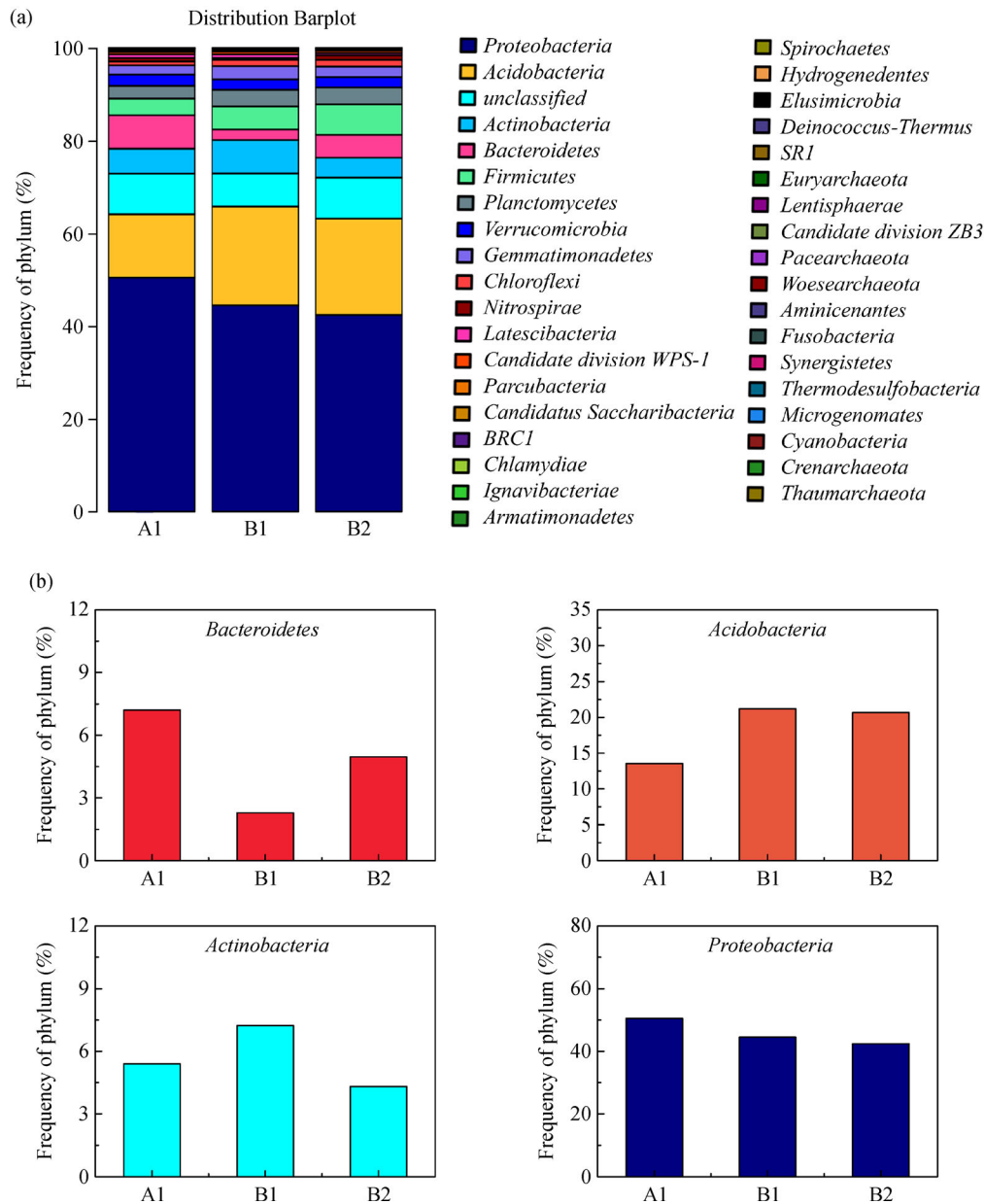


Fig. 5 Bacterial community structures (a) and the main variations of the samples (b) at phylum level. A1: original soil sample; B1: soil samples of the control group after 42 d; B2: soil samples after adding 10 mg/L PFOS for 42 d.

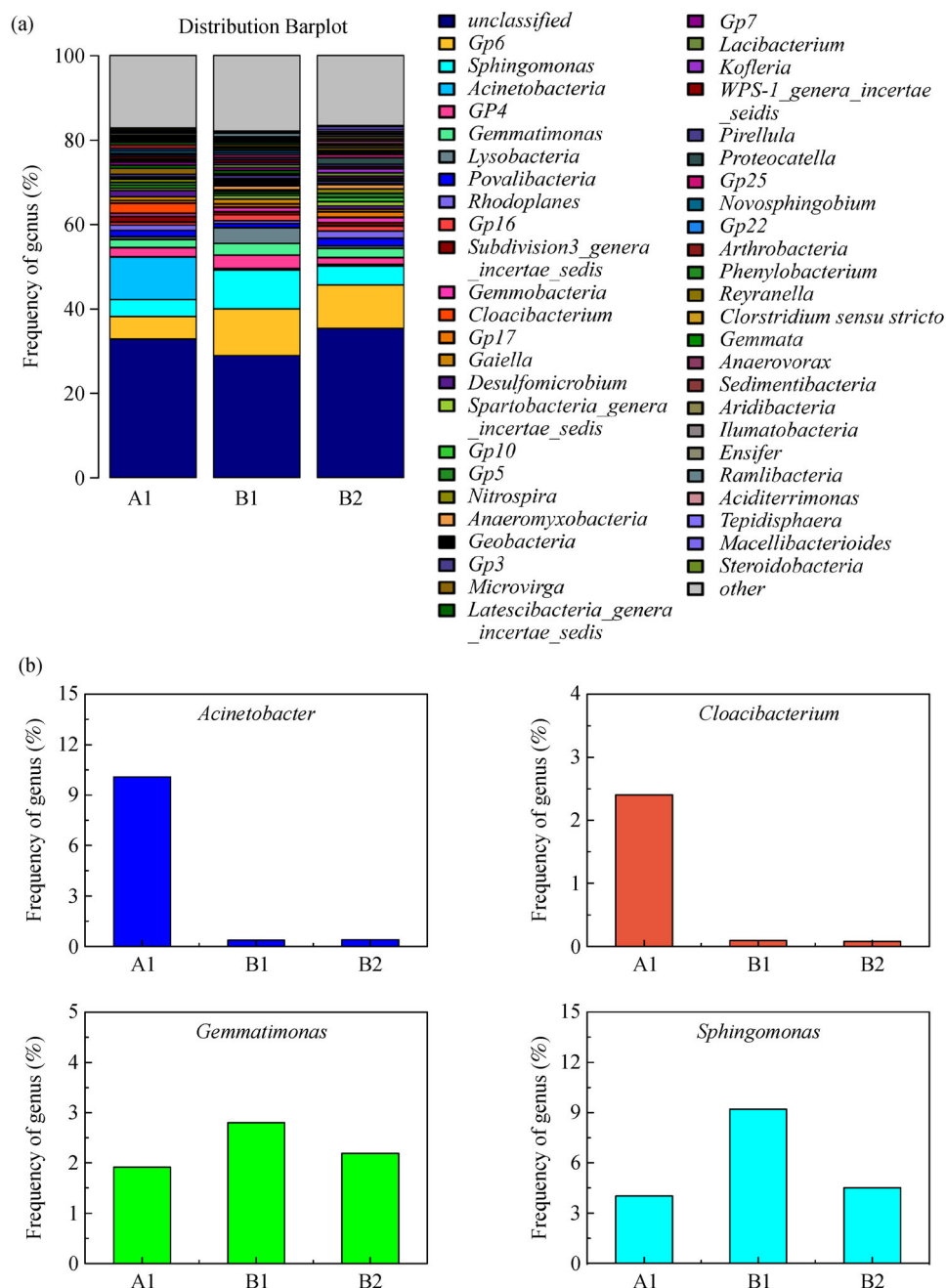


Fig. 6 Bacterial community structures (a) and the main variations of the samples (b) at genus level. A1: original soil sample; B1: soil samples of the control group after 42 d; B2: soil samples after adding 10 mg/L PFOS for 42 d.

The capacity of *E. crassipes* to take up PFOS was stronger than that of *C. alternifolius*. Moreover, the study confirms the effect of PFOS on properties of wastewater during treatment.

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Conflict of Interest Statement Authors declare that they have no competing interests.

Electronic Supplementary Material Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s11783-020-1312-3> and is accessible for authorized users.

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