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Effect of plant density on phytoremediation of polycyclic aromatic hydrocarbons contaminated sediments with *Vallisneria spiralis*

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ABSTRACTS

A 54-day-long study was conducted to investigate the effect of plant density (260, 780, 1300 and 2080 plants m^{-2}) of *Vallisneria spiralis* on the remediation of sediments contaminated by polycyclic aromatic hydrocarbons (PAHs). Dissipation ratios of phenanthrene and pyrene in sediments were initially the highest in treatment of 2080 plants m^{-2}. However, after a 54-day incubation, no statistical difference was observed in the dissipation ratios under different planting density treatments ($p > 0.05$) except lower dissipation ratio of phenanthrene in treatment of 780 plants m^{-2}. Compared with the unplanted sediments, the dissipation ratios of phenanthrene and pyrene in planted sediments increased by 15.2–21.5% and 9.1–12.7%. Considering the sustainability of the ecosystem, lower plant density (e.g., 260 plants m^{-2}) should be a better selection for phytoremediation of PAHs. Mass balance calculation indicated that plant accumulation accounted for less than 0.39% of the dissipation increment. Furthermore, dissipation ratio of PAHs was positively related to PAH-degrading bacterial population, suggesting that microbial degradation played a major role in the *V. spiralis*-promoted remediation. The redox potential, a signal of oxygen in sediments, was measured. Positive redox potentials were found in sediments with *V. spiralis* as a result of oxygen released by roots. Moreover, sediment redox potential positively correlated with PAH-degrading bacterial population. Considering high oxygen demand of PAHs catabolism and reduced conditions in unplanted sediments, it can be concluded that the enhanced dissipation of PAHs is mainly related to oxygen released by roots.

1. Introduction

As widespread organic contaminants, polycyclic aromatic hydrocarbons (PAHs) are byproducts mainly from anthropogenic activities such as coal combustion and automobile emissions (Ravindra et al., 2008). PAHs have been listed as priority pollutants for their toxic, mutagenic and carcinogenic properties, which could be a serious threat to the ecosystem and furthermore, human health (Franco et al., 2008). In the environment, PAHs can be released into water bodies through atmospheric deposition, surface run off and sewage discharge (Wu et al., 2012). PAHs could be easily adsorbed by the particles and accumulate in sediment due to their strong hydrophobicity, which make sediment as storage of PAHs (Chiou et al., 1998; Perelo, 2010). Owing to the limited supply of atmospheric oxygen into the flooded sediment and the contaminants' high chemical stability, the thorough oxidation and decomposition of PAHs were difficult.

Phytoremediation, the use of plants to enhance biodegradation and removal of pollutants, has been proved to be a cost-effective and eco-friendly method and also has been manifested for many years as a potential technology for restoration of contaminated soil (Perelo, 2010). Of all the species of aquatic plants, submerged macrophytes are the dominant aquatic plants, especially in shallow water bodies (Biernacki et al., 1997). Submerged hydrophytes are plants that are completely under the water and typically have their root systems in the bottom sediment. Recently, it has been reported that submerged macrophytes are capable of remediating contaminated sediments (Huesemann et al., 2009; Yan et al., 2011). In addition to releasing root exudates as terrestrial plants, submerged aquatic macrophytes are also capable of releasing oxygen to their rhizosphere to modify the anaerobic conditions and increase the availability of nutrients to the plants (Sand-Jensen et al., 1982; Perelo, 2010). PAHs are known to be susceptible to aerobic biodegradation (Huesemann and Truex, 1996). Therefore, the oxygen flux from plant roots into the anaerobic sediment might play an important role in increasing the

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removal rate of the contaminants. However, the specific mechanism by which submersed macrophytes enhance the remediation of PAHs-contaminated sediments has been not elucidated so far.

Plant density is an important parameter for phytoremediation. According to previous studies, plant density could affect the growth and quality of plant in many ways (Sangoi, 2001). Søndergaard reported that the nutrient availability of macrophyte might depend on both species and density of plants (Søndergaard, 1988). Loadesa et al. found that plant density had an influence on fibrous root reinforcement of soils (Loadesa et al., 2010). The density had a significant effect on the reproduction, biomass accumulation and root morphology due to competition among plants (Xie et al., 2006; Jiang et al., 2008). However, currently, little information is available on the effect of plant density of submerged macrophytes on the remediation of contaminated sediments.

In this study, the perennial *Vallisneria spiralis* (*V. spiralis*) was selected because this species is ubiquitous in freshwater environments and has a high adaptive capability (Wang and Yu, 2007). Effect of plant density of *V. spiralis* on the dissipation of phenanthrene and pyrene in sediments was investigated. Plant growth, root morphology, sediment redox potential and PAH-degrading bacterial population were monitored, and the remediation mechanisms were discussed.

2. Experimental

2.1. Materials

A total of 153 seedlings of *V. spiralis* with similar size (3–4 leaves, 0.5 cm root, 17 ± 2 cm shoot) were prepared, which were obtained by seed culture for 2 months before the experiment.

Surface sediments samples (top 10 cm, pH 7.47, 2.70% organic matter) were collected from the mainstream of the Haihe River (39° 07′17″N, 117° 13′14″E), Tianjin, China. After air-dried, the sediments were sieved to remove rocks and roots by a 2 mm screen. 1/6 of the sediments were then spiked with a mixture of phenanthrene and pyrene (1:1) in acetone. When acetone evaporated off, the spiked sediments were progressively mixed with the remaining non-spiked sediments to ensure the homogeneity of the sediment samples. The sediments were then sieved again through a 2 mm mesh to produce final concentrations of about 15 mg kg^{-1} of each PAH.

2.2. Experimental design

Sediment samples of 100 g each (dry weight) were placed in glass pots (diameter 6.3 cm). *V. spiralis* were transplanted in the glass pots, which were taken as planted treatments and consisted of four plant densities (1, 3, 5 and 8 plants per pot, namely 260, 780, 1300 and 2080 plants m^{-2}). At the same time, unplanted and sterilized (0.2% w/w NaN_3) treatments were also constructed as controls. Nine pots were prepared for each treatment. The pots were then placed into glass tanks (60 cm long × 30 cm wide × 40 cm high) filled with 50 L of tap water. White fluorescence light (2200 ± 100 Lux) was installed on the top of the tanks; and the ratio of light:dark was 12:12. The water in the tank was gently stirred with a submerged pump during the experiment to achieve homogeneity and kept at the temperature of 22 ± 1 °C. The experiment lasted for 54 days and 3 replicate pots of each treatment were removed every 18 days.

After carefully removing the overlying water in the pots, sediment redox potential was measured immediately with a platinum redox electrode coupled to a portable pH/mV meter. The platinum redox electrode was placed in the sediments at the central point and about 2 cm depth for 15 min. Mean value of three parallel pot sediments was calculated. Then plants in the pots were carefully rinsed with tap water to remove any sediment particles and dried with filter paper. After measuring the heights and weights, plant samples were placed into polytetrafluoroethylene bags and stored at 4 °C for further analysis. Sediment samples were stored at −20 °C after freeze drying for 24 h.

2.3. Analytical methods

2.3.1. Root morphology

Roots were manually separated from the plants and scatted over a clear tray that was filled with deionized water. Root images were obtained by an STD-1600 EPSON scanner and were used to measure root diameter, root length and surface area with the WinRhizov. 4.0b software. Scanned roots were cleaned for root biomass assessment.

2.3.2. Analysis of PAHs in sediments and plants

The analysis of PAHs in sediment was conducted according to standard analytical method (USEPA, 1996). In brief, samples of freeze-dried sediments were ground and passed through an 80-mesh sieve. Sediment samples (5.0 g) were Soxhlet extracted using 45 mL dichloromethane for 24 h. The extracts were concentrated to 1 mL by a rotary evaporator at a 38 °C water bath. The condensed extracts were concentrated to 1.0 mL again after adding 4 mL n-hexane. The concentrated extract was cleaned up with a self-packed chromatographic column (1 cm sodium sulfate, 7 cm neutral chlorine dioxide and 14 cm silica gel from up to down). N-hexane was used to transfer the concentrated extracts from the rotary evaporator to the cleanup column, and the extracts were eluted with 25 mL mixture of pentane and dichloromethane (2:3, v/v) at a rate of 2 mL min^{-1}. The eluent collected was reduced to 1 mL. Internal standard (hexamethyl benzene) was added before GC–MS analysis.

The plants were separated into roots and shoots before analysis. One gram of fresh plant sample was extracted in a histoid grinding tube with 5 mL dichloromethane at 200 g for 5 min. The solution was centrifuged at 1600 *g* for 5 min. After removal of the water phase, the dichloromethane layer was reduced to 1 mL and purified by the procedure as sediment samples. The final volume was reduced to 0.5 mL. Internal standard (hexamethyl benzene) was added before GC–MS analysis.

PAHs in the extracts were quantified by GC–MS in a selected ion monitoring mode (SIM). The GC–MS system consists of an Agilent 6890N gas chromatograph equipped with a fused-silica capillary column (HP-5, 30.0 m × 250 μm × 0.25 μm) and coupled to a mass spectrometer detector (5975C). The temperatures of injector and detector were both set at 250 °C. Helium was used as a carrier gas at a flow rate of 1.0 mL min^{-1}. The initial oven temperature was 100 °C, and was raised to 280 °C at 20 °C min^{-1} with holding time of 2 min. The total time was 11 min. The MS conditions for EI ionization were as follows: the ion energy was 70 eV and the ion source temperature was 230 °C.

Strict quality control procedures were applied to all data obtained. None of the target compounds was detected in solvent blanks and procedure blanks for plants and sediments. Using internal standard calibration, good linearities of phenanthrene and pyrene were observed with correlation coefficients above 0.9998. The limits of detection (LOD) (S/N = 3) of phenanthrene and pyrene were both 6 μg kg^{-1} for sediment and 15 μg kg^{-1} for plant. Average recoveries of phenanthrene and pyrene were 85.0 ± 3.2% and 93.7 ± 3.0% in sediments, 95.3 ± 4.0% and 96.1 ± 3.6% in plants.

2.3.3. PAH-degrading bacterial population

Sediment samples were processed immediately for PAH-degrading bacteria population analysis by the plate-count techniques as colony forming units (CFU) per gram of dry

sediment. The PAH-degrading bacteria were cultured with Minimum Medium (MM; 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.02 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.4 g L^{-1} KH_2PO_4, 0.6 g L^{-1} $NaHPO_4$, 0.02 g L^{-1} $MnSO_4 \cdot H_2O$, 1 g L^{-1} NH_4NO_3, and 18 g L^{-1} agar; pH 7.2) containing 50 mg L^{-1} of phenanthrene and 50 mg L^{-1} of pyrene (in crystal form) as the sole carbon source. Aqueous suspensions of the microbial population of 1.0 g sediment sample were serially diluted. Then 0.2 mL sample from each dilution level was spread on the medium surface. Three replicates were conducted for each dilution. After incubating at 30 °C for 5 days, colonies which were capable of growing on the PAH-containing MM were counted as PAH-degrading bacteria.

2.3.4. Statistical analysis

Treatment effects were tested by one-way ANOVA. Comparisons of means were carried out using Duncan's test. The significance value was $p < 0.05$. All statistical analyses were performed using the software Statistical Package for Social Sciences (SPSS 13.0 for Windows).

3. Results and discussion

3.1. Plant growth and root morphology

As shown in Table 1, the biomasses of roots and shoots of *V. spiralis* in sediments increased significantly over time for all the four treatments. However, the growth rate per plant, which was calculated by dividing the total increment of plant fresh weight between two samplings of 18 days by the number of *V. spiralis* in the pot, was quite different among different treatments (Table S1). During the first 18 days, there was no statistical difference in growth rate per plant among different plant densities ($p > 0.05$). But thereafter, the growth rate was higher in lower plant density treatment than in higher plant density treatment. At the end of the experiment, the growth rate was in the order of 260 plants m^{-2} > 780 plants m^{-2} > 1300 plants m^{-2} > 2080 plants m^{-2}, and total biomass increased by 3.9, 2.7, 1.4 and 1.3 times, respectively, indicating that plant growth was negatively related to plant density. The decline in plant growth in higher density treatment might be due to the competition for resources and spaces among plants (Jiang et al., 2008).

Root parameters, such as diameter, length, and surface area, are important attributes involved in nutrient uptake (Casper and Jackson, 1997). Variation of root length, surface area per plant, and diameter in different plant densities are shown in Fig. 1. The average root length and surface area per plant increased from 2.83–4.00 cm to 6.33–7.57 cm and from 1.93–4.51 cm^2 to 8.23–22.89 cm^2 over time, whereas the root diameter decreased from 0.61–0.84 mm to 0.38–0.59 mm. With the increase of plant density, the average root length and diameter increased, whereas the average root surface area per plant decreased. For example, the roots of *V. spiralis* were thinner and had larger surface area in treatment of 260 plants m^{-2}. This might be related to the high efficiency in nutrient acquisition (Xie and Yu, 2003; Xie et al., 2005), leading to a faster plant growth in treatment of 260 plants m^{-2}.

Fig. 1. Variations of root length, root surface area per plant, and root diameter of *V. spiralis* in sediments with different plant densities. Letters A–C and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

Table 1
Biomasses of roots and shoots of *V. spiralis* (g/pot, fresh weight) with different plant densities (plants m^{-2}).

Sampling time	Biomass of roots				Biomass of shoots			
	260	780	1300	2080	260	780	1300	2080
Day 0	0.01 ± 0.00	0.05 ± 0.01	0.08 ± 0.02	0.15 ± 0.04	0.60 ± 0.04	1.82 ± 0.06	3.31 ± 0.08	4.50 ± 0.12
Day 18	0.02 ± 0.01	0.07 ± 0.02	0.17 ± 0.06	0.26 ± 0.05	0.69 ± 0.07	2.55 ± 0.46	3.87 ± 0.42	5.42 ± 0.65
Day 36	0.06 ± 0.04	0.23 ± 0.02	0.29 ± 0.03	0.49 ± 0.03	1.39 ± 0.61	4.51 ± 0.35	5.11 ± 1.20	7.36 ± 1.03
Day 54	0.20 ± 0.09	0.49 ± 0.18	0.81 ± 0.28	0.93 ± 0.20	2.74 ± 0.50	6.41 ± 0.94	7.36 ± 1.61	9.60 ± 1.55

Data are expressed as mean ± SE ($n = 3$).

Table 2
Concentrations of phenanthrene and pyrene in sediments (mg kg^{-1}, dry weight) with different plant densities.

Plant density	Phenanthrene			Pyrene		
	18th day	36th day	54th day	18th day	36th day	54th day
Unplanted control	7.58 Db	7.42 Cab	6.84 Ca	6.53 Ba	6.54 Ca	6.53 Ba
260 plants m^{-2}	7.24 Cc	5.80 Bb	4.47 Aa	6.47 Bb	5.30 Ba	5.10 Aa
780 plants m^{-2}	6.72 Bb	6.04 Bb	5.16 Ba	6.27 ABb	5.68 Ba	5.40 Aa
1300 plants m^{-2}	6.95 BCc	5.44 Bb	4.79 ABa	6.26 ABb	5.76 Ba	5.38 Aa
2080 plants m^{-2}	5.98 Ab	4.05 Aa	4.49 Aa	5.91 Ab	4.62 Aa	5.02 Aa

Letters A–C and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

3.2. Dissipation of PAHs in sediments

Concentrations of phenanthrene and pyrene in both planted and unplanted sediments are shown in Table 2. Concentrations of phenanthrene and pyrene in unplanted sediments decreased rapidly during the first 18 days and then declined slightly during the remaining 36 days, whereas the concentrations in planted sediments decreased more significantly after the first 18 days. During the experiment, PAHs concentrations in planted sediments were always lower than those in unplanted sediments.

The dissipation ratios of PAHs in sediments with different plant densities are shown in Fig. 2. After 18 days of experiment, the PAHs dissipation ratios were in the order of 2080 plants m^{-2} > 780 plants m^{-2} > 260 and 1300 plants m^{-2} for phenanthrene, and 2080 plants m^{-2} > 260, 780 and 1300 plants m^{-2} for pyrene. However, after 36 days of experiment, there was no statistical difference in PAHs dissipation ratios among different plant densities ($p > 0.05$) except higher dissipation ratio in treatment of 2080 plants m^{-2}. At the end of the experiment, there was no statistical difference in PAH dissipation ratios among different plant densities ($p > 0.05$) except lower dissipation ratio of phenanthrene in treatment of 780 plants m^{-2}, and the dissipation ratios of phenanthrene and pyrene were 53.3–59.6% and 50.0–53.6% in planted sediments, and 38.1% and 40.9% in unplanted sediments. Besides, phenanthrene was more readily degradable and the dissipation ratio was clearly higher than that of pyrene, indicating that PAHs with higher molecular weight and more benzene rings were more recalcitrant in sediments, which was also confirmed by the previous findings (Derudi et al., 2007; Ling et al., 2010). After 54 days of incubation, the total dissipation increments were 15.2–21.5% for phenanthrene and 9.1–12.7% for pyrene.

3.3. Bioaccumulation of PAHs in plants

Table 3 shows the concentrations of phenanthrene and pyrene in roots and shoots of *V. spiralis*. The concentrations of total PAHs in roots (1.01–3.65 mg kg^{-1}) were obviously higher than those in shoots (0.11–0.51 mg kg^{-1}) and the quantities of pyrene stored in both roots and shoots were much higher than those of phenanthrene, which was similar to the previous findings (Gao and Zhu, 2004; Yan et al., 2011). This reveals that translocation of PAHs from roots to the shoots was limited. During the later period of the experiment, PAHs concentrations in shoots and roots generally decreased. This might be due to degradation in *V. spiralis* and reduced plant uptake with decreasing PAHs concentration in sediments. Release of PAHs from plant to water should be unimportant because of their hydrophobic properties and low concentrations in shoots.

At the initial period, PAHs concentration in roots generally increased with increasing plant density, and no significant difference could be found among different plant densities as a result of more rapid plant growth in low density treatment. Furthermore, there was no significant difference in the concentrations of PAHs in shoots among different treatments. Dissolved PAHs can be absorbed by plant roots and translocated throughout the body of plants. Faster plant growth and larger root surface area facilitated the absorption of PAHs by plants. Variation of PAHs concentrations in roots was found to be similar to that of total root surface area.

Compared with the unplanted sediments, dissipation ratios of the PAHs in planted sediments were found to be significantly improved. From the sterilization control test, abiotic losses of phenanthrene and pyrene were 11.0% and 8.2%, which were low

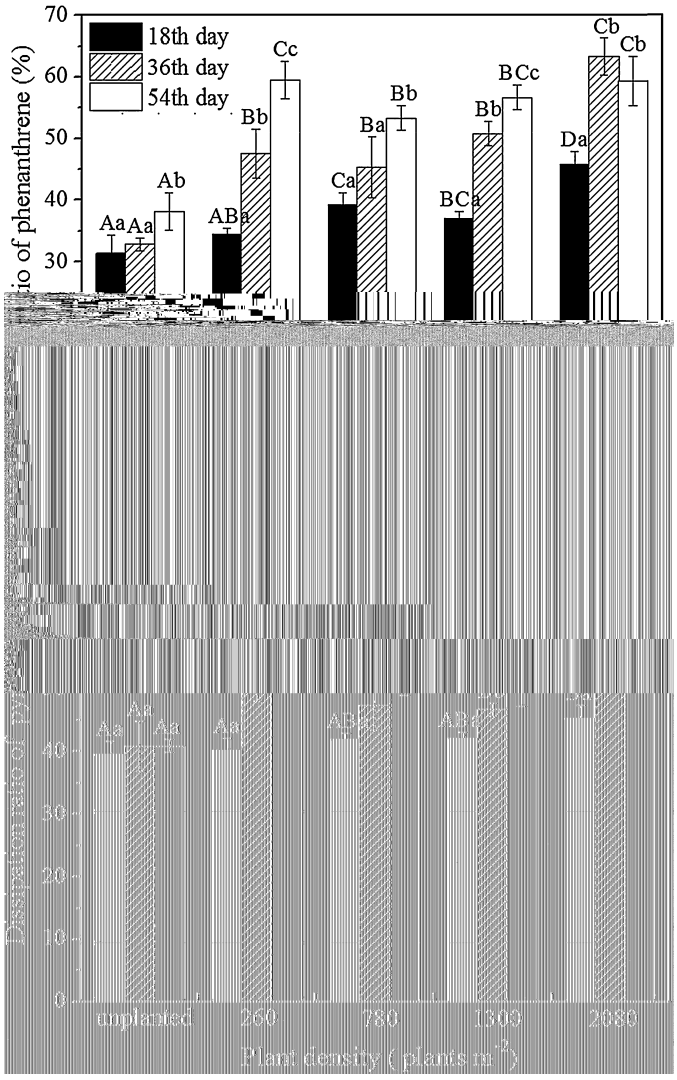

Fig. 2. Dissipation ratios of phenanthrene and pyrene in sediments with different plant densities. Letters A–C and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

Table 3
Concentrations of phenanthrene and pyrene in roots and shoots of *V. spiralis* (mg kg^{-1}, wet weight).

Plant density	Phenanthrene in roots			Pyrene in roots			Phenanthrene in shoots			Pyrene in shoots		
	18th day	36th day	54th day	18th day	36th day	54th day	18th day	36th day	54th day	18th day	36th day	54th day
260 plants m^{-2}	0.25 Aa	0.82 Ac	0.52 Ab	1.02 Aa	2.83 Bb	1.13 Aa	0.05 Aa	0.07 Aa	nd	0.25 Aa	0.44 Ab	0.17 Aa
780 plants m^{-2}	0.40 Ba	0.81 Ab	0.24 Aa	1.97 Bb	2.39 Bc	0.77 Aa	0.05 Aa	0.07 Aa	nd	0.26 Ab	0.23 Ab	0.11 Aa
1300 plants m^{-2}	0.43 Ba	0.96 Ab	0.89 Bb	3.04 Cc	2.44 Bb	1.12 Aa	0.05 Aa	0.05 Aa	nd	0.33 Ab	0.29 ABb	0.09 Aa
2080 plants m^{-2}	0.76 Ca	0.75 Aa	0.79 Ba	3.08 Cc	1.60 Ab	1.22 Aa	0.08 Ba	0.07 Aa	nd	0.27 Ab	0.33 ABb	0.09 Aa

Letters A–C and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

compared with biotic processes. Therefore, it can be considered that biotic processes played a major role in the dissipation of PAHs in sediments, and the enhanced dissipation of PAHs in plant-grown sediments might result from two paths, plant uptake and plant-promoted degradation. Plant uptake may decrease the PAHs quantity in sediments and accumulate the chemicals in plant body. Despite the fact that phenanthrene and pyrene accumulated in the roots and shoots of *V. spiralis*, mass balance calculation showed that plant accumulation accounted for less than 0.39% of the dissipation increment in sediments during the 54-day experimental period. This suggests that microbial degradation rather than plant accumulation played a major role in the *V. spiralis*-promoted remediation.

3.4. PAH-degrading bacterial population in sediments

Plants were reported to have a positive effect on the number of cultivable PAHs degraders in soils, which is related to the promoting impact of plants on PAHs dissipation (Ma et al., 2010). In this study, PAH-degrading bacterial populations in sediments under different treatments are shown in Fig. 3. PAH-degrading bacterial population increased with increasing plant density at the initial period (i.e., the first 18 days), but thereafter, variation of PAH-degrading bacterial population was observed. In the treatment of 260 and 780 plants m^{-2}, PAH-degrading bacterial population generally increased during the remaining 36 days, whereas opposite trend could be found in the treatment of 2080 plants m^{-2}. At the end of the experiment, the difference of PAH-degrading bacterial population among different plant densities was not significant ($p > 0.05$) except lower PAH-degrading bacterial population in the treatment of 260 plants m^{-2}.

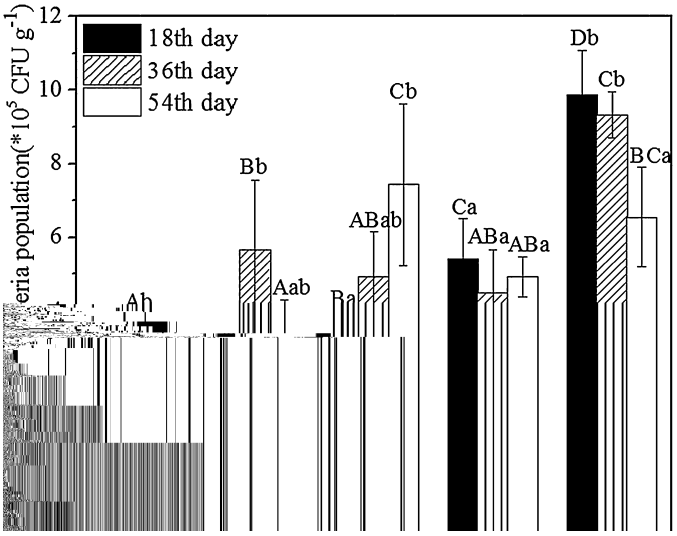

Fig. 3. The PAH-degrading bacteria population in sediments with different plant densities. Letters A–C and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

Besides, PAH-degrading bacterial population was generally higher in planted sediments than in unplanted sediments. This is similar to the change trend of dissipation ratio of PAHs to some extent, and a positive correlation between PAH-degrading bacterial population and the dissipation ratio of PAHs (for phenanthrene $r = 0.595$, $n = 45$, $p = 0.019$; for pyrene $r = 0.617$, $n = 45$, $p = 0.014$; Fig. S1) was obtained.

Compared with the bulk soil and sediment, microbes are abundant in the rhizosphere because of the root activities. The number of PAHs degraders is, however, affected by root exudates in two opposing ways. On one hand, the root exudates promoted the activity of microbes, including the degraders in the rhizosphere (Cofield et al., 2008; Lee et al., 2008). On the other hand, root exudates may facilitate flourishing of microbes that may compete with, and thus inhibit, PAHs degraders (Jones et al., 2004; Rentz et al., 2005). In this study, the decrease of PAH-degrading bacterial population in the treatment of 2080 plants m^{-2} during the remaining 36 days might be due to the inhibition effect of root exudates.

3.5. Change of sediment redox potential

Previous studies have indicated that the biodegradation rates under aerobic conditions are significantly higher than those under anoxic conditions (Reddy et al., 1989; Louvel et al., 2011). The first step of aerobic biodegradation of PAHs involves the incorporation of molecular oxygen into the ring. Hence, high amount of oxygen is necessary. However, oxygen contents are often low in the submerged sediments and thus do not provide favorable conditions for the aerobic microbial biodegradation of PAHs. Therefore, biodegradation in sediments might be limited due to low oxygen supply.

In this study, sediment redox potential was measured (shown in Table 4). During the experiment, the redox potential was always higher in planted sediments than that in unplanted sediments. The redox potential in unplanted sediments was relatively stable and below zero. Negative redox potentials were also encountered in planted sediments at the initial stage except that in treatment of 2080 plants m^{-2}. At the initial period (i.e., the first 18 days), redox potentials increased with increasing plant density, and then increased more rapidly with decreasing plant density during the remaining 36 days. There was no statistical difference in sediment redox potentials among four plant densities at the end, and the sediment redox potential was in the range of 235–295 mV.

Several studies have proved wetland plants can release oxygen into the root zone, occupying for as much as 90% of the total oxygen in substrate (Reddy et al., 1989; Bodelier et al., 1996; Allen et al., 2002). In our experiment, sediment redox potential was positively related to the total root surface area ($r = 0.763$, $n = 36$, $p = 0.006$; Fig. S2), implying that the oxygen released by roots could contribute a lot to enhance sediment redox potential. Therefore, redox potential around the rhizosphere could be an important signal of the oxygen in the sediment. Moreover, a positive correlation between sediment redox potential and PAH-degrading bacterial population ($r = 0.621$, $n = 45$, $p = 0.013$) was also obtained, suggesting that the oxygen released by roots boosted the growth of

Table 4
Redox potential (mV) in sediments with different plant densities.

Sampling time	Unplanted	260 plants m^{-2}	780 plants m^{-2}	1300 plants m^{-2}	2080 plants m^{-2}
18th day	−229 Aa	−193 Aa	−204 Aa	−29 Ba	167 Ca
36th day	−259 Aa	−149 Ba	−132 Ba	189Cb	253 Cab
54th day	−135 Ab	257 Bb	235 Bb	277 Bc	295 Bb

Letters A–D and a–c indicate significant difference ($p < 0.05$) among different density treatments on the same sampling day and among the three sampling days of the same plant treatment, respectively.

microorganism. Considering high oxygen demand of PAHs catabolism and reduced conditions in unplanted sediments, it can be concluded that the enhanced dissipation of PAHs is mainly related to oxygen released by roots.

4. Conclusions

Accelerated removal of phenanthrene and pyrene in sediments was observed in the presence of submersed macrophyte *V. spiralis*. During the 54-day incubation, the dissipation ratios of phenanthrene and pyrene were up to 53.3–59.6% and 50.0–53.6% in planted sediments, 38.1% and 40.9% in unplanted sediments. The total increments were 15.2–21.5% for phenanthrene and 9.1–2.7% for pyrene. Moreover, the dissipation ratio of PAHs was found to be positively related to PAH-degrading bacterial population in sediments, suggesting that microbial degradation played an important role in the *V. spiralis*-promoted remediation.

Plant growth and root morphology, such as diameter, length, and surface area, were significantly related to plant density. Faster plant growth and larger total root surface area facilitated the dissipation of PAHs in sediments. At the end of the experiment, there was no significant difference in PAH dissipation ratio among different plant densities. Considering the sustainability of ecosystem, lower plant density (e.g., 260 plants m^{-2}) should be a better selection for phytoremediation of PAHs.

As a signal of oxygen in the sediments, the redox potential was measured. Sediment redox potential was found to increase linearly with total root surface area and PAH-degrading bacterial population. Considering susceptibility of PAHs to aerobic biodegradation and reduced conditions in unplanted sediments, the enhanced dissipation of PAHs by *V. spiralis* should be mainly attributed to oxygen release by roots. This study provides additional information for a better understanding of phytoremediation mechanisms of PAHs-contaminated sediments by submerged macrophytes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2014.09.084.

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