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Pig manure vermicompost (PMVC) can improve phytoremediation of Cd and PAHs co-contaminated soil by *Sedum alfredii*

Kai Wang · Jie Zhang · Zhiqiang Zhu · Huagang Huang · Tingqiang Li · Zhenli He · Xiaoe Yang · Ashok Alva

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Abstract
Purpose A major challenge to phytoremediation of co-contaminated soils is developing strategies for efficient and simultaneous removal of multiple pollutants. A pot experiment was conducted to investigate the potential for enhanced phytoextraction of cadmium (Cd) by *Sedum alfredii* and dissipation of polycyclic aromatic hydrocarbons (PAHs) in co-contaminated soil by application of pig manure vermicompost (PMVC).
Materials and methods Soil contaminated by Cd (5.53 mg kg^{-1} DW) was spiked with phenanthrene, anthracene, and pyrene together (250 mg kg^{-1} DW for each PAH). A pot experiment was conducted in a greenhouse with four treatments: (1) soil without plants and PMVC (Control), (2) soil planted with *S. alfredii* (Plant), (3) soil amended with PMVC at 5 % (*w/w*) (PMVC), and (4) treatment 2+3 (Plant+PMVC). After 90 days, shoot and root biomass of plants, Cd concentrations in plant and soil, and PAH concentrations in soil were determined. Abundance of PAH degraders in soil, soil bacterial community structure and diversity, and soil enzyme activities and microbial biomass carbon were measured.
Results and discussion Application of PMVC to co-contaminated soil increased the shoot and root dry biomass of *S. alfredii* by 2.27- and 3.93-fold, respectively, and simultaneously increased Cd phytoextraction without inhibiting soil microbial population and enzyme activities. The highest dissipation rate of PAHs was observed in Plant+PMVC treatment. However, neither *S. alfredii* nor PMVC enhanced PAH dissipation when applied separately. Abundance of PAH degraders in soil was not significantly related to PAH dissipation rate. Plant+PMVC treatment significantly influenced the bacterial community structure. Enhanced PAH dissipation in the Plant+PMVC treatment could be due to the improvement of plant root growth, which may result in increased root exudates, and subsequently change bacterial community structure to be favorable for PAH dissipation.
Conclusions This study demonstrated that remediation of Cd and PAHs co-contaminated soil by *S. alfredii* can be enhanced by simultaneous application of PMVC. Long-term evaluation of this strategy in co-contaminated field sites is needed.

Keywords Cadmium · Co-contamination · Hyperaccumulator · Organic fertilizer · Phytoremediation · Polycyclic aromatic hydrocarbons

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K. Wang · J. Zhang · Z. Zhu · H. Huang · T. Li (✉) · X. Yang (✉)
MOE Key Laboratory of Environment Remediation and Ecosystem Health, College of Environmental and Resources Science, Zijingang Campus, Zhejiang University, Hangzhou 310058, People's Republic of China
e-mail: litq@zju.edu.cn
e-mail: xyang571@yahoo.com

Z. Zhu
College of Agriculture, Hainan University, Renmin Road 58, Haikou 570228, People's Republic of China

Z. He
Indian River Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Fort Pierce, FL 34945, USA

A. Alva
Agricultural Research Service, U.S. Department of Agriculture, Prosser, WA 99350, USA

1 Introduction

Toxic metals and persistent organic pollutants are recognized as two major chemical constituents that cause soil pollution (Huang et al. 2011; Sun et al. 2011). Cadmium (Cd) and polycyclic aromatic hydrocarbons (PAHs) are of particular concern due to their persistence; potentially carcinogenic, mutagenic, and teratogenic properties; and their ubiquitous occurrence in the environment (Chaney et al. 2004; Lu and Zhu 2009; Murakami et al. 2009; Teng et al. 2011). Soil pollution by Cd and PAHs has been accelerated in China during the past decade because of rapid urbanization and industrialization (Sun et al. 2011). Co-contaminated sites by toxic metals and organic pollutants are common, and remediation of these soils is rather complicated since the chemical processes and remediation technologies are different for each group of pollutants (Sandrin and Maier 2003).

Phytoremediation is a promising and cost-effective strategy for removal of organic or inorganic pollutants from contaminated soils (McGrath and Zhao 2003; Lee et al. 2008; Sun et al. 2009). This technology has been evaluated for remediation of soils co-contaminated by toxic metals and organic pollutants (Lin et al. 2008; Zhang et al. 2009; Sun et al. 2011; Zhang et al. 2011). A major challenge to phytoremediation of co-contaminated soils is the simultaneous removal of multiple pollutants. Phytoextraction using hyperaccumulating plants to remove metals from contaminated soil has been widely studied (McGrath and Zhao 2003; Liu et al. 2008; Sun et al. 2011). The key limitation for application of phytoextraction is attributed to its low remediation efficiency because of slow growth and small biomass for most hyperaccumulators (Li et al. 2009). Many plant species could enhance PAH biodegradation in soils because plant root exudates increased the indigenous soil microbial population including PAH-degrading microorganisms (Gao and Zhu 2004; Lee et al. 2008). However, other reports indicated that some plants could inhibit the dissipation of PAHs in contaminated soil (Cofield et al. 2007; Smith et al. 2008). Competition for nutrients between plants and soil microorganisms may impede PAH dissipation in the rhizosphere (Fu et al. 2011). *Sedum alfredii* is a Cd hyperaccumulator native to China (Yang et al. 2004), which has been demonstrated to be effective to extract Cd from Cd-contaminated soil in the presence of phenanthrene (PHE) or pyrene (PYR) (Wang et al. 2012). However, dissipation of both PAHs was not influenced by *S. alfredii*. Therefore, additional strategies are needed to accomplish the simultaneous removal of Cd and PAHs from co-contaminated soils.

Application of organic fertilizers is a common approach for increasing soil quality and crop production (Yang 2002). Animal manure is commonly applied to agricultural soils in China (Yang 2002). Wei et al. (2010) reported that application of poultry manure increased the biomass of *Bidens tripartite* L., a Cd accumulator, thus enhancing extraction of Cd from contaminated soil. Dissipation of PHE and PYR in spiked soil planted with *Agropyron elongatum* was enhanced by application of pig manure compost (Cheng et al. 2008). Fu et al. (2011) reported that organic fertilizers could accelerate benzo[*a*]pyrene (BaP) dissipation in natural contaminated soil planted with alfalfa (*Medicago sativa*). However, the effects of organic fertilizers on phytoremediation of toxic metal and organic pollutants co-contaminated soil were rarely reported.

The main objectives of this study were to investigate the effects of pig manure vermicompost (PMVC) amendment to Cd and PAHs co-contaminated soil on: (1) Cd uptake by *S. alfredii*, (2) dissipation of PAHs in soil with or without *S. alfredii*, (3) soil bacterial abundance including PAH degraders, (4) soil bacterial community structure and diversity, and (5) soil microbial biomass carbon and enzyme activities as indicators of soil quality.

2 Materials and methods

2.1 Chemicals

Phenanthrene, anthracene (ANT), and PYR as representative PAHs were purchased from Sigma-Aldrich Chemical Co. with a purity >98%. Molecular weights (M_w) of the above PAHs were 178.23, 178.23, and 202.26 $g mol^{-1}$, respectively, while solubilities in water at 25°C (S_w) were 1.18, 0.04, and 0.12 mg L^{-1}, respectively (Yaws 1999).

2.2 Preparation of contaminated soil

Soil derived from marine sediments with background Cd concentration of 0.59 mg kg^{-1} DW was initially collected (0–20 cm depth) from Zhenhai, Ningbo City of Zhejiang Province, China and then transported to a trial field plot in Huajiachi campus, Zhejiang University prior to Cd spiking. The soil was spiked with aqueous solution of $Cd(NO_3)_2$ as a source of Cd (approximately 5 mg kg^{-1} DW) and aged for 2–3 years prior to the experiment. Selected physicochemical properties of the soil are presented in Table 1. The bulk soil was air-dried and sieved through a 5-mm sieve and then spiked with three PAHs together in acetone (250 mg kg^{-1} DW for each PAH). After acetone was evaporated, the treated soils were progressively mixed with PAH-unspiked soil and homogenized. The treated soils were equilibrated in a greenhouse for 1 month at 60% water holding capacity (WHC). At the end of incubation, triplicate samples were collected for analyses. The results showed that the PAHs were homogeneously distributed in the soil with concentrations of 174.4, 184.9, and 180.2 mg kg^{-1} DW for PHE, PYR, and ANT, respectively.

Table 1 Selected physicochemical properties of the soil (dry weight basis)

Parameters	Values
pH (soil/water, 1/5, *w/v*)	7.8±0.1
Organic matter (g kg^{-1})	29.8±2.3
CEC (cmol kg^{-1})	14.3±1.2
Total N (g kg^{-1})	1.9±0.1
Available N (mg kg^{-1})	73.7±5.9
Total P (g kg^{-1})	1.3±0.1
Available P (mg kg^{-1})	37.6±2.4
Clay (%)	31.6±0.2
Silt (%)	44.1±0.6
Sand (%)	24.3±0.4
Total metals (mg kg^{-1})	
Zn	121.6±2.6
Pb	36.6±1.3
Cd	5.5±0.1
PAHs (mg kg^{-1})	
Phenanthrene	ND
Pyrene	ND
Anthracene	ND

Values are mean±standard error (n=3)

ND not detected

2.3 Pig manure vermicompost

Pig manure vermicompost (PMVC) was obtained from a farm in Huajiachi campus, Zhejiang University. The physicochemical properties of PMVC were: pH=7.32 (1/2.5, PMVC/water); organic matter=394 gkg^{-1} DW; total nitrogen (N)=12.36 gkg^{-1} DW; available N=775 mg kg^{-1} DW; total phosphorus (P)=4.67 gkg^{-1} DW; and available P=1.44 gkg^{-1} DW. The total concentration of Cd was 0.57 mg kg^{-1} DW. The three PAHs (PHE, PYR, and ANT) were not detected in the PMVC.

2.4 Plant material

Healthy and uniform-sized shoots of *S. alfredii* were chosen from seedlings grown in Cd-free soil for 4 months and grown first in distilled water for initiation of new roots, then transferred to one fourth strength Hoagland solution for 4 weeks prior to transplanting (Wang et al. 2012).

2.5 Pot experiment and sample collection

The pot experiment was conducted in a greenhouse (Huajiachi campus, Zhejiang University) with natural light and day/night temperature of 30/24°C and humidity of 70/85 %. There were four treatments with three replications using a fully randomized design: (1) soil without plants and PMVC (Control), (2) soil planted with *S. alfredii* (Plant), (3) soil amended with 5 % (*w/w*, dry weight basis) PMVC (PMVC), and (4) soil amended with 5 % PMVC and planted with *S. alfredii* (Plant+PMVC). Contaminated soil (1 kg, dry weight basis) was placed in each pot. For the treatments of PMVC and Plant+PMVC, PMVC was applied to the soil and thoroughly mixed. After equilibration at 60 % WHC for 3 days, *S. alfredii* plants were transplanted (five plants per pot) to each pot for all treatments, except the control. During the experiment, soil moisture content in the pots was regularly adjusted to approximately 60 % WHC by weighing the pots.

Soil and plants were sampled 90 days after transplanting. Plant shoots and roots, separated from soil, were carefully washed first with tap water, and then distilled water for several times. Shoots and roots were dried at 110°C for 10 min and then at 65°C in an oven until completely dry; dry biomass weights were recorded and ground to powder (< 0.25 mm) using a horizontal grinder (Retsch RS-100, Germany) prior to analysis of Cd.

The whole soil from each pot was well homogenized and divided into three subsamples. One subsample was stored at 4°C prior to analysis of microbial biomass carbon and enzyme activities. The second subsample was freeze-dried (Labconco, USA) and ground to powder (<0.25 mm) and stored in a deep freezer at −80°C prior to PAHs and Cd analyses. The third subsample was stored at −20°C prior to DNA extraction for real-time polymerase chain reaction (real-time PCR) and terminal restriction fragment length polymorphism (T-RFLP) assays.

2.6 Analysis of soil microbial biomass carbon and enzyme activities

The fumigation–extraction method described by Vance et al. (1987) was used to determine soil microbial biomass carbon. Soil dehydrogenase activity was determined by the reduction of 2, 3, 5-triphenylterazolium chloride to triphenyl formazane (TPF) as described by Lee et al. (2008). Soil polyphenol oxidase activity was measured according to the colorimetric method described by Chen et al. (2004) with minor modifications. Pyrogallic acid was employed as a substrate. The mixture of 1 g soil and 10 mL 1 % pyrogallic acid was incubated at 30°C for 2 h, and 4 mL citric–phosphoric acid buffer (pH=4.5) was added to the mixture. The purpurogallin produced was extracted with ether and then measured by a spectrophotometer (Perkin Elmer Lambda 35, USA) at 430 nm. Soil peroxidase activity was analyzed following the same procedure as polyphenol oxidase activity, except that 2 mL 0.5 % H_2O_2 together with 10 mL 1 % pyrogallic acid was used as a substrate.

2.7 Analysis of Cd and PAHs

Ground plant samples were digested in HNO_3-$HClO_4$, while the soil samples were digested in HF-HNO_3-$HClO_4$ for the

determination of total Cd. The concentrations of available Cd in soil samples were determined by Mehlich-3 extraction method (1:10 ratio of soil/Mehlich-3 solution (0.2 M CH_3COOH+0.25 M NH_4NO_3+0.015 M NH_4F+0.013 M HNO_3+0.001 M EDTA, pH=2.0) with 5-min reaction time, Mehlich 1984). Cadmium concentrations in the digested solution or Mehlich-3 extract were determined using an ICP-MS (Agilent 7500a, USA).

Translocation factor (TF) of Cd in the plant was calculated as:

$$\mathrm{TF} = \frac{\text{Cd concentration in shoot}}{\text{Cd concentration in root}}$$

The procedure reported by Wang et al. (2012) with some modifications was used for determining PAH concentrations in soil samples. Two grams of soil was weighed into a 25-mL glass tube; 10 mL dichloromethane and acetone mixture (1/1, *v/v*) were added, followed by ultrasonication for 1 h and centrifuged at 3,000 rpm for 10 min. Then, 2 mL of the supernatant was filtered through 2.5 g of silica gel column with 15 mL hexane and dichloromethane mixture (1/1, *v/v*). The solvent fraction was then evaporated using a rotary evaporator and dissolved in acetonitrile for a final volume of 5 mL. The above extract was filtered through a 0.22-μm filter, and PAH concentrations were determined using a HPLC (Agilent 1200, USA) with a 4.6×150-mm reverse phase XDB-C_{18} column and a UV detector. Chromatography was performed at 30°C with acetonitrile/water (85/15, *v/v*) as the mobile phase at a flow rate of 1 mL min^{-1}, and PHE, ANT, and PYR were detected at 254 nm.

PAH dissipation rate in soil was calculated as:

$$\text{PAH dissipation rate(percentage)} = 100 \times \frac{\text{initial PAH concentration in soil} - \text{PAH concentration in soil after 90 days}}{\text{initial PAH concentration in soil}}$$

2.8 Real-time PCR and T-RFLP assays

Total bacterial (16S rDNA) and PAH-ring hydroxylating dioxygenase (PAH-RHD_α) gene copies were measured by real-time PCR to identify soil bacterial abundance, especially for selective enrichment of PAH degraders. Gram-positive (GP) and Gram-negative (GN) PAH-RHD_α genes were selected as the model catabolic genes because they are responsible for PAH hydroxylation, which is the initial step of PAH metabolism (Cébron et al. 2008). Moreover, soil bacterial community structure and diversity were determined by T-RFLP analysis to characterize the likely mechanisms by which plant and PMVC treatments affect PAH dissipation.

Total DNA was extracted from soil samples with the E.Z.N.A. soil DNA kit (Omega, USA) according to the manufacturer's instructions. 16S rDNA and PAH-RHD_α gene copies were evaluated by a SYBR Green-based real-time PCR quantification using a realplex^2 Mastercycler (Eppendorf, Germany), and their primes and amplification protocols were specifically described by Cébron et al. (2008).

For bacterial T-RFLP analysis, soil DNA was amplified by PCR using the 6-carboxyfluorescein-labeled forward primer 27f and the unlabeled reverse primer 1492r (Moeseneder et al. 1999). The 50-μL reaction mixture contained 1 μL of DNA template (in 1/10 dilution of original extracts), 5 μL of 10× PCR buffer, 5 μL of 20 mM $MgCl_2$, 4 μL of 2.5 mM dNTP, 2 μL of 10 mg L^{-1} bovine serum albumin, 1 μL of each primer (10 mM), and 2.5 units of Taq DNA polymerase (Takara, Japan). The thermal profile for amplification was as described previously (Lu et al. 2010). The 6-carboxyfluorescein-labeled PCR products were purified using an agarose gel DNA extraction kit (Takara, Japan) and digested at 37°C overnight by a restriction endonuclease *HaeIII* (Takara, Japan), and the DNA fragments were size separated using a 3730XL genetic analyzer (Applied Biosystems, USA).

Data were processed to peak relative fluorescence with subsequent removal of peaks representing less than 1 % of total fluorescence in each sample. The dominant terminal restriction fragments (T-RFs), which contribute more than 80 % of relative fluorescence to the total fluorescence of T-RFs, were used to assess microbial diversity and composition (Liu et al. 1997; Kim et al. 2008). The Shannon diversity index (H') (Shannon 1948) was calculated as follows:

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

where pi is the proportion of peak height of the ith T-RF from the total peak height of dominant T-RFs, and species richness (S) is the total number of dominant T-RFs in T-RFLP profiles. Evenness (E, calculated as H' divided by ln S) was also calculated.

2.9 Statistical analyses

One-way or two-way analysis of variance (ANOVA) was performed using SPSS 16.0, and least significant difference (LSD) was applied to test for significance at $P<0.05$ between the means.

3 Results

3.1 Plant growth and biomass

After 90 days of growth, no visible symptoms of contaminants toxicities were observed in *S. alfredii,* regardless of PMVC amendment. The growth of plant was significantly improved by application of PMVC (Fig. 1). The dry biomass of shoot and root in PMVC treatment was greater by 2.27- and 3.93-fold, respectively, as compared to those of plants grown in the soil without PMVC.

3.2 Cd concentration and accumulation in plant

Cadmium concentration in the shoot or root slightly decreased with the addition of PMVC as compared to that of plants in the unamended soil; however, this difference was only statistically significant in root ($P<0.05$; Table 2). Nevertheless, total extracted Cd by *S. alfredii* shoot was greater by 1.97-fold in PMVC-amended soil as compared to that in the unamended soil. The TF of Cd was not significantly different between treatments with and without PMVC. The concentration of extractable Cd in PMVC-amended soil was lower by 7.5 and 11.4 %, respectively, before planting and after 90 days of plant growth, as compared to that in the unamended soil.

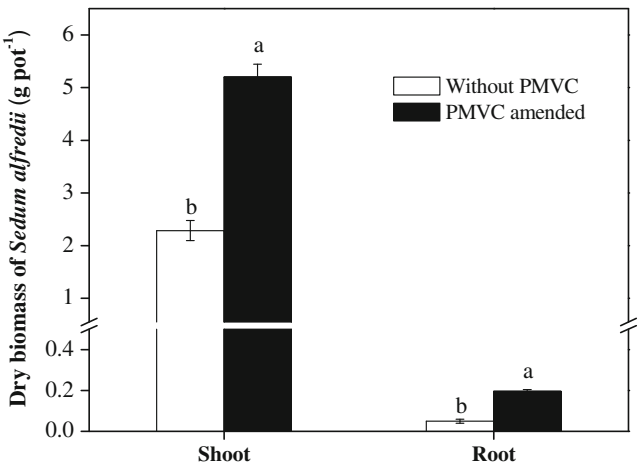


Fig. 1 Shoot and root biomass of *S. alfredii* after 90 days of growth. Means followed by different letters by shoot and root are significantly different at $P<0.05$ (based on LSD). *Error bars* show standard error ($n=3$). *PMVC* pig manure vermicompost

3.3 Soil microbial biomass carbon and enzyme activities

Soil microbial biomass carbon and enzyme activities are good indicators of soil quality. Soil microbial biomass carbon was greater in the soil with PMVC amendment, regardless of bare soil or soil planted with *S. alfredii*, as compared to that in unamended soil (Table 3). The activities of dehydrogenase, polyphenol oxidase, and peroxidase were not significantly influenced by any treatment.

3.4 PAH dissipation in soil

After 90 days of experiment duration, the dissipation rates of three PAHs decreased in the order: PHE>PYR>ANT. Dissipation rates were in the ranges of 98.63 to 99.12 %, 91.16 to 97.00 %, and 67.46 to 74.61 % for PHE, PYR, and ANT, respectively (Fig. 2). The dissipation rates of all three PAHs were significantly greater in the Plant+PMVC treatment as compared to those in the rest of the treatments. Plant or PMVC treatment alone showed no significant enhancement in the dissipation of PAHs. The results of two-way ANOVA showed that the dissipation of PHE, PYR, and ANT was significantly influenced by the interactions between *S. alfredii* and PMVC ($P<0.01$).

3.5 Quantification of the 16S rDNA and PAH-RHD$_\alpha$ genes

The number of 16S rDNA gene copies was greater than 10^9 g^{-1} DW in all the treatments (Fig. 3). The number of 16S rDNA gene copies was significantly lower in the control soil than the other treatments ($P<0.05$). However, no significant difference was observed within Plant, PMVC, and Plant+PMVC treatments. The number of PAH-RHD$_\alpha$ gene copies from Gram-positive degraders dominated over that of Gram-negative degraders. The number of Gram-positive PAH-RHD$_\alpha$ gene copy changed in the order: Plant>Plant+PMVC=Control>PMVC. The number of Gram-negative PAH-RHD$_\alpha$ gene copies showed no significant difference across all the treatments.

3.6 Soil bacterial community structure and diversity

Relative peak height of each T-RF has been considered as a measure of the relative abundance of each bacterial operational taxonomic unit (OTU) among the total bacterial community (Lu et al. 2010). Despite the large amount of OTUs in the soil samples, the most abundance of 30 OTUs explained more than 80 % of relative fluorescence of total active soil bacteria (Fig. 4a). Species richness (S) in soil bacterial community decreased in the order: Plant+PMVC>PMVC>Plant>Control (Table 4). And Shannon diversity index (H') changed in the order: Plant+PMVC=PMVC>Plant>Control. However, evenness (E) was only significantly different between the

Table 2 Effects of pig manure vermicompost (PMVC) on Cd phytoextraction by *S. alfredii*

Treatments	Shoot Cd (mg kg^{-1})	Root Cd (mg kg^{-1})	TF[a]	Shoot Cd uptake (μg pot^{-1})	Soil extractable Cd (mg kg^{-1})	
					Initial	Harvest
Without PMVC	54.87±8.49 a	24.87±1.98 a	2.23±0.35 a	122.3±7.66 b	3.61±0.05 a	3.67±0.14 a
PMVC amended	46.14±5.10 a	18.34±0.77 b	2.60±0.18 a	240.7±8.00 a	3.34±0.12 b	3.25±0.06 b

Mean values±standard error (n=3) followed by different lowercase letters within the same column indicate significant differences at $P<0.05$ (based on LSD)

[a] TF (translocation factor)=Cd concentration in shoot/Cd concentration in root

Control and Plant treatments. Principal component analysis (PCA) of bacterial T-RFLP profiles shows the first principal component (PC1 presented 64.39 % of the variation) was significantly influenced by the addition of PMVC, and the second principal component (PC2 presented 14.56 % of the variation) was significantly influenced by plant and PMVC×plant interactions (Fig. 4b). The four treatments were separated clearly within both the first and second components. The overall bacterial community structure in soil planted with *S. alfredii* alone was similar to that in the control soil. However, addition of PMVC and the interactions between *S. alfredii* and PMVC significantly influenced the bacterial community structure.

4 Discussion

4.1 Effects of PMVC on plant growth and Cd removal

In this study, Cd concentration in shoot or root was lower than that of *S. alfredii* in the soil (6.38 mg Cd kg^{-1} DW) in the previous study (Wang et al. 2012). The soil used in this study was spiked with Cd and aged for 2–3 years; therefore, the available Cd for plant could be much lower than that in the freshly spiked soil with similar total Cd concentration in the previous study. Moreover, Cd uptake by Cd hyperaccumulator was not only affected by Cd availability but also by soil characteristics, especially pH (Yanai et al. 2006). The soil used in this study had a higher pH and thus might decrease the mobilization of Cd in soil, consequently reducing Cd uptake by *S. alfredii*. This was in agreement with the results of the study of Huang et al. (2012) using a natural Cd-contaminated soil with a similar total Cd concentration (6.45 mg kg^{-1} DW) and pH (7.8). Another possible explanation was the negative effects of PHE or PYR on Cd uptake by *S. alfredii* in the soil (6.38 mg Cd kg^{-1} DW, Wang et al. 2012). In this study, the negative effects of PAHs on Cd uptake by *S. alfredii* were expected to be greater because of increased concentrations of PAHs applied.

Application of PMVC (5 %) significantly increased the shoot biomass of *S. alfredii* and simultaneously increased Cd phytoextraction. The application of animal manure improves soil fertility (Fu et al. 2011). As reported by Wei et al. (2010, 2011), poultry manure can improve Cd extraction by *Bidens tripartite* L. and *Rorippa globosa* (Turcz.) by increasing the

Table 3 Soil microbial biomass carbon (C), and enzyme activities after 90 days

Treatment	Microbial biomass C (μg C g^{-1} dry soil)	Dehydrogenase (μg TPF g^{-1} dry soil)	Polyphenol oxidase (μg purpurogallin $2h^{-1}$ g^{-1} dry soil)	Peroxidase (μg purpurogallin $2h^{-1}$ g^{-1} dry soil)
Control	124.3±4.0 b	84.0±5.8 a	100.3±12.4 a	978.3±140.5 a
Plant	122.1±3.5 b	77.5±7.9 a	84.3±1.8 a	1091.7±86.8 a
PMVC	222.0±13.5 a	85.1±10.3 a	97.3±12.0 a	840.4±21.9 a
Plant+PMVC	237.9±12.1 a	93.1±5.7 a	92.4±15.9 a	1045.9±59.5 a
ANOVA				
Plant	n.s.	n.s.	n.s.	n.s.
PMVC	**	n.s.	n.s.	n.s.
Plant× PMVC	n.s.	n.s.	n.s.	n.s.

Mean values±standard error (n=3) followed by different lowercase letters within the same column indicate significant differences at $P<0.05$ (based on LSD). The results of two-way analysis of variance (ANOVA) are shown, **$P<0.01$ and *n.s.* not significant

Control soil without plants and pig manure vermicompost (PMVC), *Plant* soil planted with *S. alfredii*, *PMVC* soil amended with 5 % PMVC, *Plant + PMVC* soil amended with 5 % PMVC and planted with *S. alfredii*

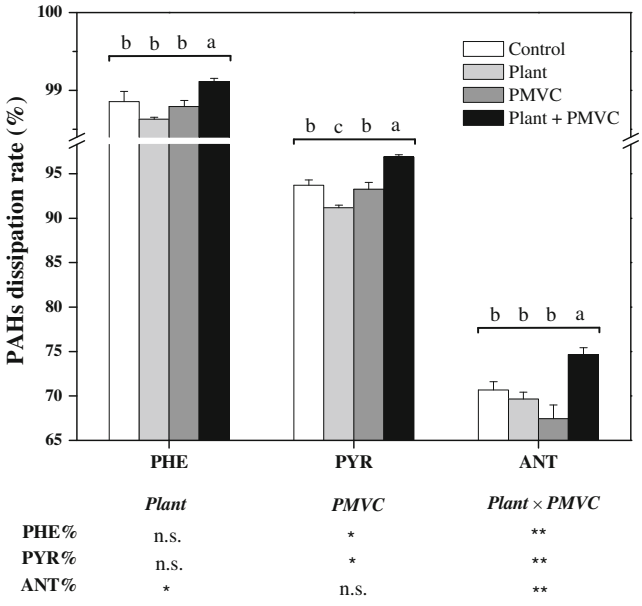


Fig. 2 PAH dissipation rate in different treatments after 90 days. Means followed by different letters, within each PAH, are significantly different at $P<0.05$ (based on LSD). *Error bars* show standard error ($n=3$). The results of two-way analysis of variance (ANOVA) are shown below the graph, $*P<0.05$, $**P<0.01$, and *n.s.* not significant. *Control* soil without plants and PMVC, *Plant* soil planted with *S. alfredii*, *PMVC* soil amended with 5 % PMVC, *Plant+PMVC* soil amended with 5 % PMVC and planted with *S. alfredii*

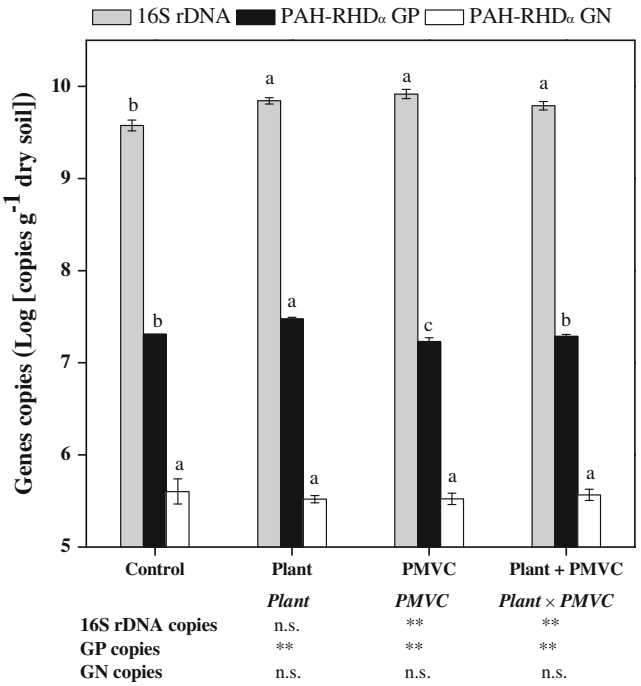


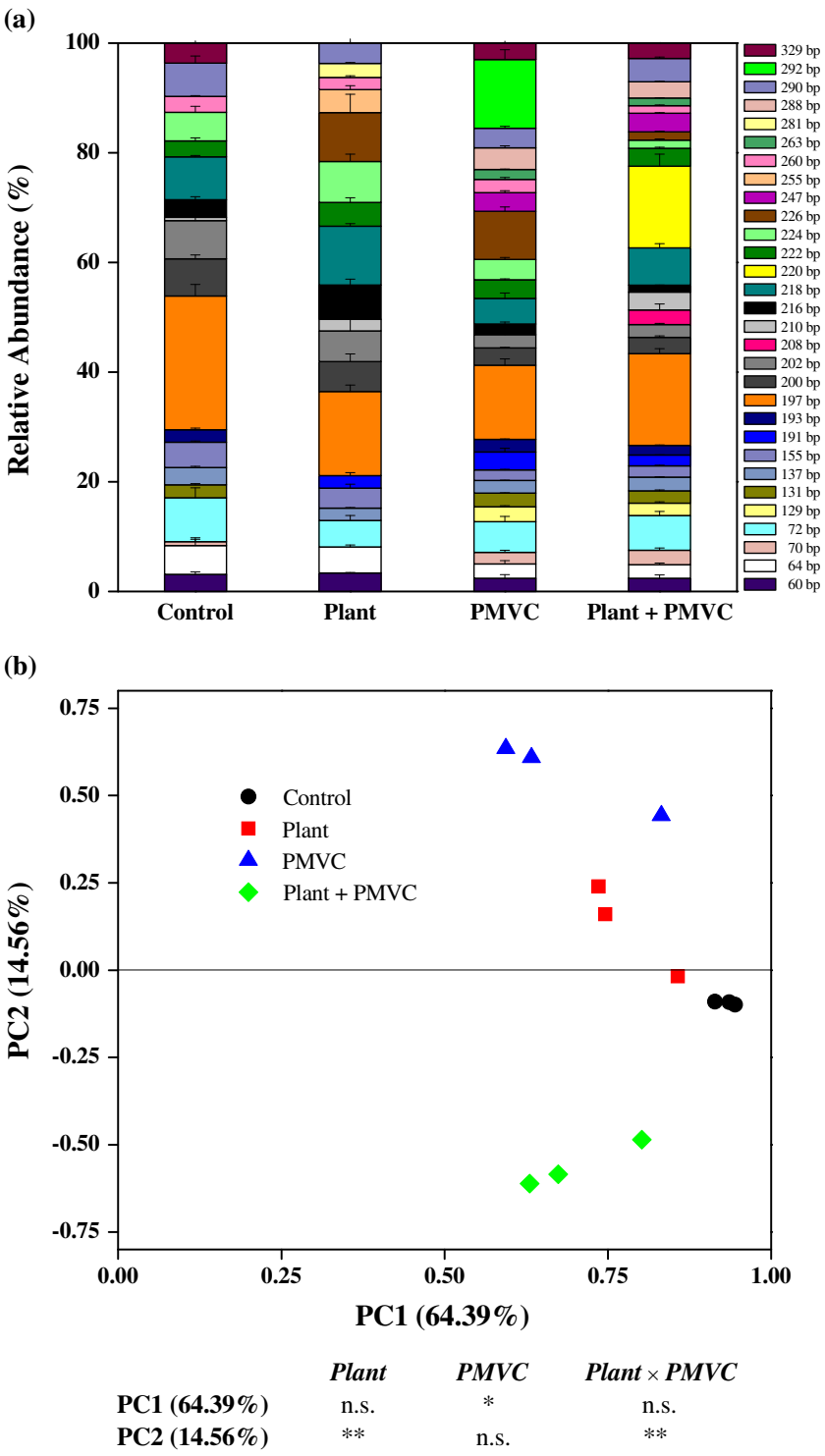
Fig. 3 Real-time PCR quantification results of 16S rDNA, PAH-RHD$_\alpha$ GP, and PAH-RHD$_\alpha$ GN gene copies in soils after 90 days. Means followed by different letters within each gene are significantly different at $P<0.05$ (based on LSD). *Error bars* show standard error ($n=3$). The results of two-way analysis of variance (ANOVA) are shown below the graph, $**P<0.01$, and *n.s.* not significant. Refer to Fig. 2 legend for explanation of treatment notations

shoot biomass. However, Cd concentrations in shoot of both species decreased with addition of manure mainly due to a decrease in extractable Cd in the soil. In this study, extractable Cd in soil was estimated by Mehlich-3 method, which is commonly used to measure labile Cd in soil, including water-soluble, exchangeable, and a variable portion of soil Cd bound to organic matter, oxides, or acid-soluble fraction (Mehlich 1984). These fractions of Cd are readily dissolved in the mild acidic/chelating conditions like the rhizosphere. Murakami et al. (2009) reported that Mehlich-3 extractable Cd in the soil was strongly correlated to Cd concentration in rice grain. A slight decrease of Mehlich-3 extractable Cd was observed both at the beginning and end of the experiment in the *S. alfredii*-planted soil with PMVC amendment as compared to that of the soil without PMVC. This could be attributed to the Cd bound by organic ligands in PMVC (Wei et al. 2011). At the beginning of the experiment, the decrease in soil extractable Cd can also be due to the dilution effect of PMVC addition. However, the decrease in extractable Cd in PMVC-amended soil at the end of the experiment could be partly explained by the extra Cd uptake by plants. Cadmium concentration was significantly lower in the roots in PMVC-amended treatment as compared to that of the plants in unamended soil. However, the TF of Cd was not significantly influenced by the above treatments. The results indicated that *S. alfredii* could extract Cd from the soil with relatively low Cd availability.

4.2 Effects of plant, PMVC, and their interactions on dissipation of PAHs

Dissipation of PAHs in planted soil is mainly through the following pathways: (1) plant uptake or accumulation, (2) degradation by soil microorganisms, and (3) abiotic loss such as volatilization. The sum of (2) and (3) explains the removal of PAHs in unplanted soil. As reported in our previous study (Wang et al. 2012), uptake of PAHs by *S. alfredii* was rather insignificant. Therefore, under the conditions of this experiment, plant uptake of PAHs was not a factor for a reduction in soil PAH concentrations following 90 days of *S. alfredii* growth.

The percent of biotic loss predominantly via microbial degradation (Cheng et al. 2008; Fu et al. 2011) was reported to be associated with the chemical nature of different species of PAHs (Sun et al. 2010). It is of interest that ANT was the most stable PAH, and PYR was more readily degraded, which may be due to the lowest water solubility of ANT and consequently the lowest bioavailability in soil. Various effects of organic fertilizers on dissipation of PAHs in unplanted soil were previously reported. For example, vermicompost contained a large amount of organic material, nutrients such as N and P, and microorganisms, which might accelerate PAH dissipation (Marinari et al. 2000). However,



	Plant	*PMVC*	*Plant* × *PMVC*
PC1 (64.39%)	n.s.	*	n.s.
PC2 (14.56%)	**	n.s.	**

Fig. 4 Soil bacterial community structure (**a**) and PCA of bacterial terminal restriction fragment length polymorphism (T-RFLP) profiles (**b**) in different treatments after 90 days. The graph (**a**) shows top 30 terminal restriction fragments (T-RFs) of the relative abundances used as a measure of the composition of the bacterial community. Data are means±standard error (n=3). The results of two-way analysis of variance (ANOVA) in the graph (**b**) are shown below the graph, *P<0.05, **P<0.01, and *n.s.* not significant. Refer to Fig. 2 legend for explanation of treatment notations

in this study, PAH dissipation was not enhanced in the soil amended with PMVC alone. Similar reports by Wellman et al. (2001) and Alvarez-Bernal et al. (2006) indicated that addition of organic fertilizers had only a limited and transient effect on PAH dissipation in soil. Application of vermicompost increased microbial activity as indicated by an increase in CO_2 production, but this had only a small effect on PAH dissipation (Wellman et al. 2001). As reported by Fu et al. (2011), degradation of BaP in natural contaminated soil was related to soil inorganic N level. However, elevated inorganic N levels in soil amended with organic fertilizer did not enhance PAH dissipation (Alvarez-Bernal et al. 2006). Kastner and Mahro (1996) reported that addition of vermicompost enhanced the dissipation of PHE, ANT, and

Table 4 Shannon's diversity index (*H'*), species richness (*S*), and species evenness (*E*) determined from the dominant T-RFs in bacterial T-RFLP profiles

Treatment	Bacterial diversity indices		
	H'	*S*	*E*
Control	2.59 c	18 d	0.89 b
Plant	2.73 b	19 c	0.93 a
PMVC	2.95 a	25 b	0.92 ab
Plant+PMVC	2.96 a	27 a	0.90 ab
ANOVA			
Plant	n.s.	**	n.s.
PMVC	**	**	n.s.
Plant× PMVC	n.s.	**	*

Means followed by different lowercase letters within the same column indicate significant differences at $P<0.05$ ($n=3$, based on LSD). The results of two-way analysis of variance (ANOVA) are shown, *$P<0.05$, **$P<0.01$, and *n.s.* not significant

Control soil without plants and pig manure vermicompost (PMVC), *Plant* soil planted with *S. alfredii*, *PMVC* soil amended with 5 % PMVC, *Plant+PMVC* soil amended with 5 % PMVC and planted with *S. alfredii*

BaP within initial 25 days, but it was not due to the microorganisms found in the compost, and the residual rates of PAHs in the soils were similar at the end of the experiment. This study demonstrated greater PAH dissipation in the Plant+PMVC treatment as compared to that with single plant or PMVC treatment. Similar result was reported by Cheng et al. (2008), as evidence from significantly increased growth of *A. elongatum* and enhanced PAH dissipation in contaminated soil amended with pig manure compost.

The copy numbers of PAH-RHD$_\alpha$ genes were reported as good indicators of PAH degraders in soil (Cébron et al. 2008; Meng and Zhu 2011). In this study, the number of Gram-positive PAH-RHD$_\alpha$ gene copies was not the highest in the Plant+PMVC treatment. The dissipation rates of PAHs were not only affected by the number of PAH degraders but also by PAH bioavailability in the soil (Meng and Zhu 2011). The interactions between *S. alfredii* and PMVC could facilitate the desorption of PAHs from the soil particles into the aqueous phase due to combined effects of plant root exudates stimulated by growth enhancement of plant and the hydrophobic dissolved organic matter from the added PMVC, which could be released into the soil–water system (Cheng et al. 2008). On the other hand, relative abundance of PAH-RHD$_\alpha$ genes in 16S rDNA gene showed that PAH degraders were not the dominant group of bacteria (below 1 %, data not presented) in this study. Polycyclic aromatic hydrocarbons could be degraded not only by bacteria utilizing PAHs as sole carbon source but also by co-metabolism by rhizosphere microorganism in the presence of plant root exudates (Yoshitomi and Shann 2001; Rentz et al. 2005). For example, the non-PAH carbon source utilization ability of the soil microbial community was strongly correlated to PAH dissipation (Teng et al. 2011). As reported by Yoshitomi and Shann (2001), PYR degradation in the rhizosphere can be due to changes in the microbial community. Bacterial community analysis showed significant differences in the dominant groups of bacteria in the Plant+PMVC treatment as compared to that of the other treatments. Moreover, the most diverse composition was also found in this treatment. Previous studies have shown that composition and diversity of bacterial community affect degradation of organic contaminants in soil (Yoshitomi and Shann 2001; Louvel et al. 2011; Teng et al. 2011). In this study, the bacterial community composition in the Plant+PMVC treatment may be the most favorable for PAH dissipation. Plant root exudates stimulated by root growth enhancement might also accelerate the co-metabolism of PAHs.

Cheng et al. (2008) reported that the abiotic losses of PHE or PYR in the freshly spiked soil with PAH contamination history were less than 3 %. In contrast, the contribution of abiotic losses in the freshly spiked soil without PAH contamination history was high both for PHE (83.4 %) and PYR (57.2 %; Sun et al. 2010). Soils with PAH contamination history had greater potential for PAH biodegradation because of the selective richness of PAH degraders (Cébron et al. 2008). This partly explains the high contribution of biodegradation in PAH dissipation and the strong correlation between abundance of PAH degraders and PAH dissipation in the study of Cheng et al. (2008). Therefore, the abiotic losses of PAHs in this study may also be high. Moreover, evaluation of phytoremediation of PAHs in freshly spiked soil could cover up the potential of plants in enhancing PAH biodegradation (Smith et al. 2011). Thus, future investigation is needed to validate the remediation efficiency by planting *S. alfredii* associated with PMVC amendment in natural Cd and PAHs co-contaminated soils.

4.3 Utilization of PMVC for phytoremediation of Cd and PAHs co-contaminated soil

Several studies have shown that many chemical chelating agents like EDTA, DTPA, and EDDS can improve phytoextraction of metals by increasing their bioavailability (Wu et al. 2004; Lopez et al. 2005; Liu et al. 2008; Wang et al. 2009). However, chemical chelating agents also showed negative effects on soil environment such as leaching risk (Wu et al. 2004) and inhibiton of soil microbial activity (Mühlbachová et al. 2011). For PAHs, amendment of surfactant could effectively enhance dissipation of PAHs in rhizosphere due to increased bioavailability of PAHs in soil (Cheng et al. 2008). However, application of surfactants

such as Tween 80 to remediate PAH-contaminated soil may be cost-prohibitive. In addition, the potential negative effects of surfactants on the soil environment are not fully understood; hence, future investigation is needed (Lu and Zhu 2009). This study demonstrated that application of PMVC significantly enhanced remediation of Cd and PAHs co-contaminated soil by *S. alfredii* without inhibiting soil microbial population and enzyme activities. Animal manure is one of the main solid wastes in agricultural production in China (Yang 2002). Application of manure compost for phytoremediation of contaminated soils can also be an alternate effective technique for beneficial use of this by-product.

5 Conclusions

Application of PMVC to Cd and PAHs co-contaminated soil significantly improved Cd phytoextraction efficiency by *S. alfredii* due to an increase in biomass production. Simultaneous planting of *S. alfredii* and amendment of PMVC significantly enhanced PAH dissipation in the soil. The above practice had no negative effects on the soil microbial population or enzyme activities. Enhanced dissipation of PAHs in the *S. alfredii*-planted soil amended with PMVC could be due to the improvement of plant root growth leading to increased root exudates, and subsequently resulted in bacterial community structure more favorable for PAH dissipation. Further studies are needed to understand the mechanism of root exudate-induced abundance of PAH degraders and microbial community in Cd and PAHs co-contaminated soils. Field recommendation on the rate of PMVC application for phytoremediation of co-contaminated sites can be estimated based on long-term field trials to evaluate various responses to PMVC application, including contaminant removal efficiency, fate of contaminants, and loading and offsite losses of nutrients.

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