Journal of Hazardous Materials 190 (2011) 677–685

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Remediation of petroleum contaminated soils through composting and rhizosphere degradation

Zhenyu Wang[a], Ying Xu[a], Jian Zhao[a,b], Fengmin Li[a], Dongmei Gao[a], Baoshan Xing[b,*]

[a] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China
[b] Department of Plant, Soil and Insect Sciences, University of Massachusetts, Stockbridge Hall, Amherst, MA 01003, USA

ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 24 March 2011
Accepted 28 March 2011
Available online 4 April 2011

Keywords:
Petroleum hydrocarbon degradation
Composting
Rhizodegradation
Seepweed
Micropore

ABSTRACT

Composting along with rhizodegradation was used to remediate petroleum-contaminated soils in the Yellow River Delta, China. The average concentration of total petroleum hydrocarbons (TPH) in these soils was reduced from 7900–17,900 mg kg^{-1} to 1400–3700 mg kg^{-1} after field composting. The best volume ratio of amendment to contaminated soil was 2/1 and the best C/N ratio was 15/1. After composting, four local dominant plant species, Seepweed, Sealavander, Central Asia Saltbush and Reed, were selected and planted in composted soils for rhizodegradation in the field. After 90 days of cultivation, the highest net TPH degradation rate was over 40% for Seepweed, probably because of strong root system and active microbial community. In addition, Seepweed roots significantly reduced the surface and volume of soil micropores (which are able to sequestrate organic compounds inside), thus increasing the bioavailability of TPH. In sum, composting followed with planting Seepweed was most effective in remediating the contaminated soil in the Yellow River Delta.

1. Introduction

Soil pollution by petroleum hydrocarbons and their adverse effects create widespread environmental problems [1]. In past decades, bioremediation has been developed and improved to repair soils polluted by petroleum hydrocarbons [2,3]. A number of bioremediation technologies such as composting, land farming, bioventing, and bioreactor treatment have been used [4]. Composting has been confirmed to be one of the feasible methods to clean up petroleum hydrocarbon contaminated soils [5,6]. During composting, the addition of nutrients and amendments enhances the hydrocarbon degradation and ameliorates the structure of the soil [6]. However, some petroleum hydrocarbons and their metabolites remained in the soil after composting treatment, and the residual pollution levels still exceed stringent cleanup standard [7].

Phytoremediation is an emerging technology which uses various plants to extract, contain, degrade, and/or immobilize contaminants. There are various phytoremediation processes, including phytoextraction, rhizofiltration, phytostabilization, rhizodegradation, phytodegradation and phytovolatilization. Rhizodegradation is the breakdown of organic contaminants in soil through microbial activity which is enhanced by the presence of the root zone [8]. Thus, rhizodegradation of pollutants in soil becomes promising for the areas when plants can grow [9]. Earlier studies of rhizodegradation were mainly focused on the degradation of herbicides, pesticides and low concentrations of petroleum hydrocarbons contaminated soils [9,10]. In the severely polluted soil, high concentrations of total petroleum hydrocarbons (TPH) inhibited seed germination and create nutrient-deficient conditions, where the plants cannot survive [11]. Therefore, certain soil environmental conditions (e.g., oxygen permeability, water availability, nutrient sufficiency) need to be improved for effective rhizodegradation. Plant rhizospheres are the most biologically active microsites in soil. Plants provide nutrients, oxygen and favorable redox conditions to soil microorganisms, which in turn results in increased microbial community diversity and activity compared with non-rhizosphere soil [2,9]. Therefore plant species have to be selected according to their mutual interactions in the rhizosphere between these plants and microorganisms, which work in both direct and indirect ways for adsorption, extraction and/or degradation of organic pollutants [12].

However, previous studies have shown that after effective rhizodegradation of organic contaminants in soil, there were still about 10% of residual pollutants sequestrated in soil pores [9,13], especially in the micropores [14]; sequestration is likely to be an important mechanism for slow degradation. Hence, how to improve the bioavailability of remnant contaminants residing in soil micropores and how to enhance the rhizosphere decomposition are major issues which need further investigation.

The Yellow River Delta (YRD) is an important region of petroleum production in China. Over a long period of oil exploitation, spills, leaks, and other releases of crude oil, the soil is severely

* Corresponding author. Tel.: +1 413 545 5212; fax: +1 413 545 3958.
E-mail address: bx@pssci.umass.edu (B. Xing).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.03.103

contaminated. Only a few lab scale remediation techniques have shown to be effective for the contaminated soils from this region [11], but no field investigation has been conducted before. Therefore, the effective field remediation of highly petroleum polluted soils in the YRD is urgently needed.

The objective of this present research therefore was to develop a two-phase treatment technology combining bioremediation and phytoremediation together for remediation of the highly petroleum polluted soil in the YRD. Contaminated soils were composted first to reduce the TPH concentrations so that plants could grow. During the field composting, different amendments were used to study the effects of microorganisms, nutrient conditions and C/N ratios on the efficiency of petroleum hydrocarbon removal. Then, four local dominant species of plants, Seepweed (*Suaeda glauca*), Sealavander (*Limonium bicolor Kuntze*), Central Asia Saltbush (*Atriplex centralasiatica Iljin*) and Reed (*Phragmites communis Trin*) were selected for further degradation of TPH using the composted soil in the field. We also studied the rhizosphere effects (e.g., root morphology, microbial community diversity and activity) of the four plants and established an effective plant-microbial associated system. Furthermore, the soil micropores in the rhizosphere were examined to assess their change and the associated removal of TPH in the soils. This study will provide insights into the mechanisms of effective remediation of TPH contaminated soils.

2. Materials and methods

2.1. Composting treatment

2.1.1. Physicochemical analysis of composting

To examine the effects of amendments on the degradation of crude oil, a 150 day composting was conducted in the field at a hydrocarbon contaminated site located in YRD wetlands, Shandong Province, China. The soil was naturally contaminated over a number of years during oil exploitation: the initial TPH concentration was approximately 18,000 mg kg^{-1}. Soil at the site was classified to be highly alkaline and saline. Physicochemical properties of the soil and organic amendments before composting are listed in Table S1. Maize straw and pine wood chips (4–8 cm × 2–4 cm) were added as bulking agents. Maize straw was collected from a farm near YRD. The volume ratios of these amendments to contaminated soil were 1/1, 2/1, and 3/1 (Table 1). The initial C/N ratio of the contaminated soil was approximately 30/1. The soils were also amended with soybean cake and NH_4HCO_3 to adjust the soil C/N ratio to 10/1, 15/1, and 20/1, respectively. Nine treatments were used for selecting the optimal C/N ratio and appropriate amount of amendments. One biopile without any amendment was used as control. The biopiles (about 4 m^3) were thoroughly mixed every month to maintain good aeration (Fig. S1), and water content was kept relatively constant by adding water every 5 days. The amount of water added was calculated to adjust the moisture content in the composing mixture to 50%.

Soil samples were collected from the ten soil biopiles for analysis and bioremediation monitoring after 30, 90, 120 and 150 days. Samples were taken from each biopile at 10–12 random locations in the intermediate layer and each set of these samples was mixed thoroughly before analysis. The composite samples from each biopile were broken into 3 portions to allow for triplicate analysis. Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ with a soil-to-solution ratio of 1:2.5. The organic carbon was determined using finely ground (<0.5 mm) samples by the Walkley and Black dichromate oxidation method [15]. The total nitrogen was estimated by the Kjeldahl method ($N_{kjeldahl}$) [16].

2.1.2. Microbial analysis

Petroleum hydrocarbon degraders were enumerated by the most-probable-number (MPN) technique [2]. A 96-well microtiter plate containing mineral salt medium (MSM) and crude oil was used to calculate the MPN of the hydrocarbon degraders and their number expressed as colony-forming units (CFU) kg^{-1} dry soil. Total microbial activity in soil was measured using fluorescein diacetate [3′, 6′-diacetylfluorescein (FDA)] hydrolysis rate [17]. The amount of FDA hydrolyzed was measured by absorbance at 490 nm (A_{490}). The value of A_{490} per gram of soil was referred to the microbial activity in the soil.

The metabolic profile of the microbial community was analyzed using a Biolog Microstation System (Biolog Inc., USA). Ten grams fresh soils were added to 100 mL of NaCl solution. Tenfold serial dilutions were made and the 10^{-3} dilution was added into the Biolog ECO plate. Then the plates were incubated at 28 °C and analyzed by a Microplate Reader at regular 24 h intervals. Community-Level Physiological Profiles (CLPP) were examined after 90 days of plant growth to assess the diversity of culturable portions of the soil microbial communities: Shannon–Weiner (H') diversity index, Simpson's index and Evenness were calculated according to Dunbar et al. [18]. These values were then subjected to principal component analysis (PCA) as described by Travis et al. [19].

2.2. Phytoremediation treatment

After composting the 10 treatments, the soil biopiles with the same ratio of amendments to contaminated soil were mixed thoroughly and spread in the field. To simplify the data presentation and enable comparison of the different fields, the 3 field plots were classified according to the added amendments. Specifically, the 3 treatment biopiles with the same amendment ratio of 1/1 were mixed together and assigned as plot 1 (made up of biopiles of No. 1, 2 and 3), the other two as plot 2 (made up of biopiles of No. 4, 5 and 6) and plot 3 (made up of biopiles of No. 7, 8 and 9). Then 4 local dominant plant species were selected for further rhizodegradation in the above three plots. The plants were Seepweed (*S. glauca*), Sealavander (*L. bicolor* Kuntze), Central Asia Saltbush (*A. centralasiatica* Iljin) and Reed (*P. communis* Trin). Plants were analyzed monthly after 90 days of plant growth. Plant samples were separated into shoots and roots, while the soils were divided into rhizosphere and non-rhizosphere soil by the hand shaking method [1]. The roots of the plants were scanned and analyzed to obtain the root parameters (Epson Scanning and WinRHIZO Pro. 2005).

2.3. TPH analysis

TPH was detected ultrasonically using a solvent extraction method with modification [13]. Briefly, air-dried soil samples (1 g) were mixed with 1 g anhydrous sodium sulphate, and extracted 3 times in 10 mL hexane with ultrasonication for 1 h each time. Then analysis was conducted with the method of Phillips et al. [1]. To simplify data presentation and comparison of the different soil samples, the degradation net rates of different composting biopiles were reported, i.e. the degradation rate of treatment biopiles minus that of the control. The percentage of TPH removal (%) was determined by the formula: TPH removal = $100 \times [(C_i - C_e)/C_i]$, where C_i stands for the initial soil TPH concentration, while C_e was the concentration in the tested soil samples of each month during composting remediation. The initial TPH concentrations in each biopile before composting are listed in Table S2.

Table 1
Ratio of added amendments to contaminated soil used for composting and the soil pH after 150 days of composting treatment.

Biopile No.	Mix ratio (v:v)	Mix ratio[a] (kg:kg)	C:N	pH value[b]
1	1:1	173:4330	10:1	8.08c
2	1:1	173:4330	15:1	7.86d
3	1:1	173:4330	20:1	7.91d
4	2:1	346:4150	15:1	8.23b
5	2:1	346:4150	20:1	7.90d
6	2:1	346:4150	10:1	8.22b
7	3:1	500:4000	20:1	7.92d
8	3:1	500:4000	10:1	7.92d
9	3:1	500:4000	15:1	7.92d
10[c]	0:1	0:4500	30:1	8.74a

a–d: In the table, values followed by different letters indicate significant differences at $p < 0.05$ level.
[a] Ratio of contaminated soil to organic amendments on a weight basis.
[b] Soil pH after 150 days of composting treatment.
[c] No. 10 pile is the control.

2.4. Surface area and porosity measurements

Surface area and micropore volume of soil samples were determined with a Quantachrome Autosorb-1C automated gas sorption system using CO_2 as an adsorbate. All the samples were dried in an oven, then outgased at 105 °C for 24 h [20]. Micropore (<2 nm) porosity was determined according to the non-local density functional theory (NLDFT) method.

2.5. Statistical analysis

The results presented in the tables and figures were arithmetic means of three replicates. The substrate utilization data (absorbance in each Biolog ECO plate well minus the control) were subjected to multivariate analytical procedure, and PCA using the covariance matrix. The two principal components (PC1 and PC2) were extracted on the basis of a scatter plot. Statistical analyses were conducted using Excel 2003 and SPSS for Windows 12.0. The significance of the various parameters was tested by one-way analysis of variance (ANOVA) using the LSD test.

3. Results and discussion

3.1. Degradation of TPH by composting

After 150 days of composting, there was a distinct TPH decline in all treatment biopiles, and the net degradation rates were varied from 11.6% to 38.6% (Fig. 1A). A significant difference in TPH removal among treatments was observed during the first 30 days, suggesting that the initial period was critical for enhanced TPH biodegradation and composting amendments may act as a "booster" to the indigenous hydrocarbon degrading microorganisms at the start of bioremediation [21]. Most of the hydrocarbons (mainly saturated fractions) were easily degraded [22] at the beginning, while the degradation rates declined with time, especially after 120 days. This may be due to the length of degradation time and loss of beneficial degrading bacteria in the late degradation stage [5]. The remaining compounds were likely the recalcitrant fractions, which probably consisted of branched chain alkanes, multi-ring saturates and aromatics [5].

After 150 days of composting, the maximum TPH net degradation rate occurred with the C/N ratio of 15/1 in biopiles 2, 4 and 9, where it reached approximately 38.6%, 37.8% and 26.8%, respectively (Fig. 1A). However, the net degradation rates with the C/N ratio of 10/1 in biopiles 1, 6 and 8 were 20.1%, 29.3%, and 17.7%, respectively. The higher nitrogen ratio (such as C/N ratio of 10/1) could provide nutrients for microorganisms to use as an easily available carbon source instead of degrading TPH [3].

In this present study, treatments with the amendment ratio of 1/1 (biopiles 1, 2, and 3) and 2/1 (biopiles 4, 5, and 6) had better TPH removal efficacy, with the average degradation rates of 30.7% and 33.3%, respectively (Fig. 1A). For the biopiles with the amendments ratio of 3/1 (biopiles 7, 8 and 9), the net TPH degradation rates were between 11.6% and 26.8%. The maize straw and pine wood chips can supply easily available carbon resources to sustain degrading-microorganism growth, thus reducing the degradation rate of TPH. Therefore, an optimal amount of amendments to the composting biopiles will result in aeration of the composting biopiles and efficient degradation of TPH.

3.2. Microbial analysis during composting

The number of degrading microorganisms rapidly increased during the first 30 days in all treatment biopiles (Fig. 2A), especially in biopiles 2, 4 and 9 with the C/N ratio of 15/1, which had 7, 262 and 93 times more degrading microorganisms than that of control. In biopiles with C/N ratio of 20/1, the numbers of microorganisms were relatively low, probably because of the insufficient amount of nitrogen supply. As another critical factor for successful TPH degradation, microbial activities were remarkably increased for all treated soils, especially in biopiles 6 and 8 with the C/N ratio of 10/1, which were about 6 times the control biopile (Fig. 3A). The microbial activity sharply increased in the first month of composting, which matched the higher TPH degradation rates ($r = 0.715$, $p < 0.05$). After 30 days of treatments, microbial activity decreased till 120 days, then remained almost unchanged until the end.

The presence of large number of hydrocarbon degraders was a key to successful bioremediation. As composting continued, the gradually decreased microorganism counts were probably caused by limited nutrient supply [23], which led to little change of TPH degradation rate at the later stage (Fig. 1A). FDA was hydrolyzed by a number of different enzymes, such as proteases, lipases and esterase [22]. Thus, FDA hydrolysis rate is widely accepted as an accurate and simple method for measuring total microbial activity in soils [24]. In the present study, the markedly higher microbial activities in biopiles 6 and 8 (Fig. 3A) did not have a higher TPH degradation (Fig. 1A), which was probably due to the microbial community competition. Biopiles (No. 6 and 8) with abundant nutrients (C/N ratio of 10/1) and plentiful amendments provided more easily available substance for other types of microorganisms, which would outcompete the hydrocarbon degrading microbial community and reduce the TPH degradation rate. Thus, the optimal level (15/1) of the C/N ratio was important for an efficient degradation composting system.

Soil pH, an important factor in evaluating the composting microbial metabolism, was reduced at the end of composting treatments (Table 1). After 150 days of composting, there was a significant

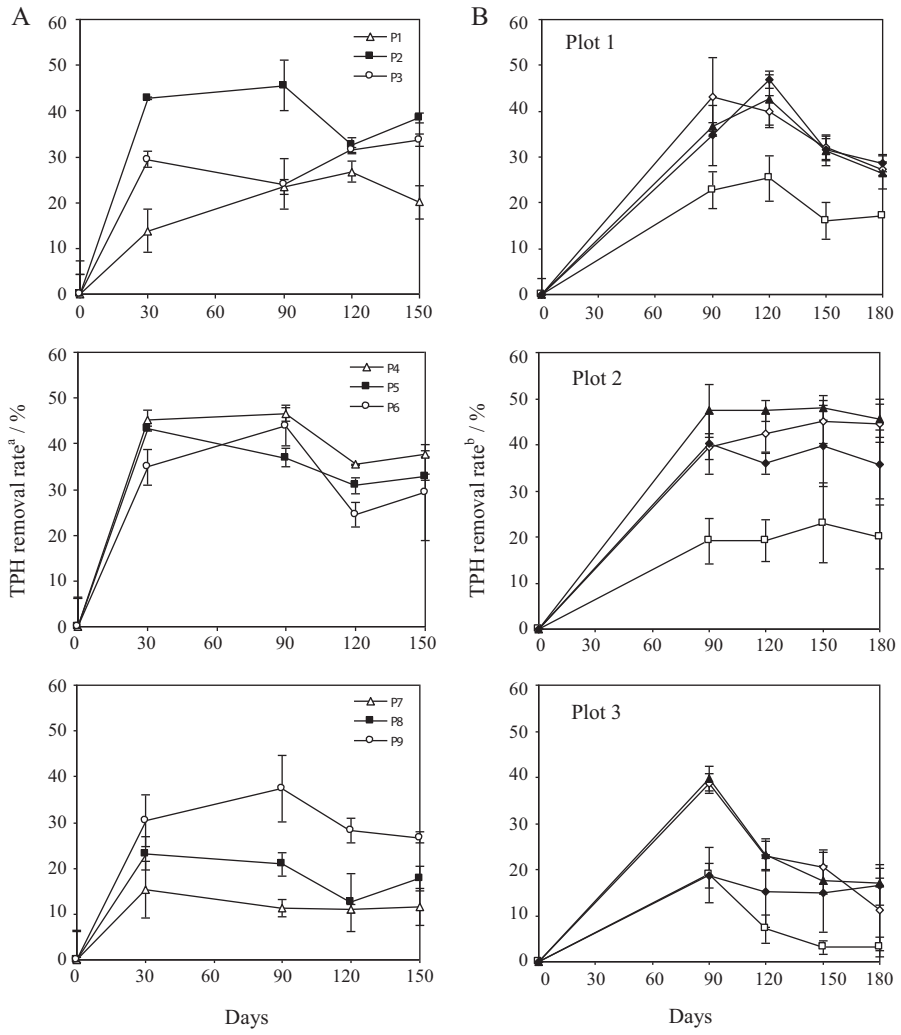

Fig. 1. (A) Net removal rate of total petroleum hydrocarbon (TPH) during composting. [a]Net% loss = % loss in TPH (soil + organic amendment) – % loss in TPH (control biopile). Error bars indicate the standard deviation ($n = 3$). (B) Net removal rate of TPH after rhizodegradation. [b]Net% loss = % loss in TPH (rhizosphere) – % loss in TPH (non-rhizosphere). Symbols in (B) stand for Seepweed (◊), Sealavander (♦), Central Asia Saltbush (▲) and Reed (□). Error bars indicate the standard deviation ($n = 3$).

decline of the soil pH in all treatment biopiles, measuring between 7.68 and 8.23, compared to the control biopile at pH of 8.74. This decrease was mainly due to the accumulation of acidic metabolites such as aliphatic acids produced during alkane degradation [3]. Furthermore, these lower pH values could also be explained by the formation of channelizations facilitated with the addition of amendments, which promoted aerobic biodegradation and subsequent CO_2 production [25]. We conclude that decreased pH close to 7 from composting soils enhances plant growth at the rhizodegradation stage.

3.3. Rhizodegradation of TPH in the composted soil

The composting process significantly decreased TPH concentration in soils, from 7900–17,900 mg kg^{-1} to 1400–3700 mg kg^{-1}, but this concentration still exceeds the soil safety standards [26]. Therefore, four local dominant plant species were selected for further remediation of the composted soil in the field. Net removal rate of TPH (rhizosphere–non-rhizosphere%) was measured in the rhizosphere monthly after 90 days of planting (Fig. 1B). All plants had significant rhizosphere TPH degradation, the highest net degradation rate of TPH was about 40.5% in the rhizosphere of Seepweed during the first 90 days. After 180 days of planting, in plot 1 that was made up of biopiles 1, 2 and 3, the net degradation rate was 28.7%, 27.4%, and 26.6% in the rhizosphere of Sealavander, Seepweed and Central Asia Saltbush ($p < 0.05$), respectively, while Reed had a lowest degradation rate of 17.1%. In plot 2 (made up of biopiles 4, 5 and 6), the highest degradation rate of TPH was observed in the rhizosphere of Seepweed and Central Asia Saltbush (44.6–45.7%), Sealavander had the degradation rate of 35.6%, and Reed (27.5%) was the least effective ($p < 0.05$). In plot 3 (made up of biopiles 7, 8 and 9), all the net degradation rates were low and Reed had the lowest rate of 3%. These data indicate that the plant rhizosphere facilitate the removal of TPH from petroleum contaminated soil [27]. The plant rhizosphere creates an active ecosystem where plant, microorganisms and other organisms could interact with each other for the effective degradation of contaminants [12], which leads to higher rhizodegradation rate compared to the non-rhizosphere (Fig. 1B). The net degradation rates of TPH were also different among these 3 plots (Fig. 1B). In general, plants had the higher TPH removal rates in plot 2, which was made up of the 3 biopiles with amendment ratio of 2/1. During the composting period, the TPH removal of these 3 biopiles was also better than the others (Fig. 1A). As a result, the degrading microorganisms in these composted soils might be one possible reason for the efficient degradation of TPH in the rhizosphere (Fig. 1B).

In order to further assess rhizosphere effects of different plants on TPH removal, root biomass, shoot biomass and the ratio between

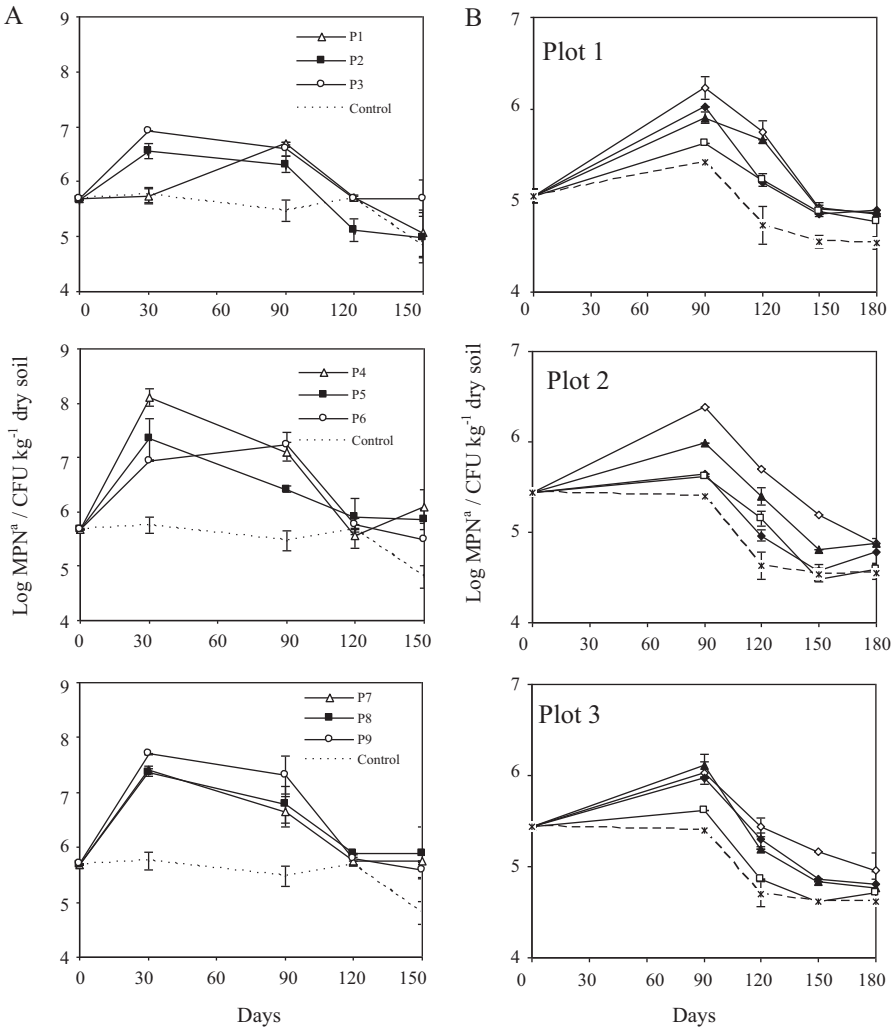

Fig. 2. (A) Changes in the number of hydrocarbon-degrading microorganisms during composting and (B) The number of hydrocarbon-degrading bacteria in rhizosphere and non-rhizosphere. Symbols in (B) stand for non-rhizosphere (✱), Seepweed (◊), Sealavander (♦), Central Asia Saltbush (▲) and Reed (□). Error bars indicate the standard deviation ($n = 3$). [a]MPN indicates the most-probable-number of microorganisms.

them were measured (Table 2). After 180 days of growth, the biomass of Seepweed and Central Asia Saltbush were significantly larger than the others, and the Reed had the smallest biomass. Root growth was necessary for remediation, and therefore higher levels of root biomass could stimulate degradation of TPH [13]. In plot 3 (consisting of the biopiles 7, 8, and 9 with amendment ratio of 3/1), the negative effect of petroleum contamination on the growth was pronounced. All plants biomass values were smaller than those of plots 1 and 2. This could be due to the relatively higher concentration of residual TPH in the soil (Fig. 1A). The parameters of root morphology such as root length, root surface area, root volume, and the number of root tips showed obvious differences among the

Table 2
Root and shoot biomass and the parameters of root morphology of the four plants.

Plot	Plant species	Root biomass (g)	Shoot biomass (g)	Ratio between root and shoot	Root length (m)	Root surface area (mm^2)	Root volume (mm^3)	Number of root tips
P1	Seepweed	3.23b	53.8b	0.06b	25.6b	38,200b	4590b	13,500b
	Sealavander	0.23a	29.0ab	0.01a	4.81a	7180a	897a	4890a
	Central Asia Saltbush	0.78a	40.0b	0.02a	4.66a	5900a	605a	3920a
	Reed	0.19a	7.58a	0.02a	5.75a	8130a	932a	3390a
P2	Seepweed	1.11ab	13.5ab	0.08a	12.6a	16,600a	1850a	8150a
	Sealavander	1.22ab	28.9b	0.04a	13.0a	19,700a	2410a	8100a
	Central Asia Saltbush	3.04b	49.6c	0.06a	10.8a	15,000a	1670a	6340a
	Reed	0.11a	2.55a	0.04a	6.22a	8410a	905a	2960a
P3	Seepweed	0.61a	4.30a	0.14ab	9.21a	11,100a	1060a	8080a
	Sealavander	0.20a	12.3b	0.02a	10.2a	12,300a	1220a	7610a
	Central Asia Saltbush	0.44a	1.65a	0.27b	5.29a	6600a	658a	7060a
	Reed	0.11a	11.4b	0.01a	7.54a	9220a	904a	5560a

a–b: in the table, values followed by different letters in the same column for the same plot treatment indicate significant differences at $p < 0.05$ level by LSD test.

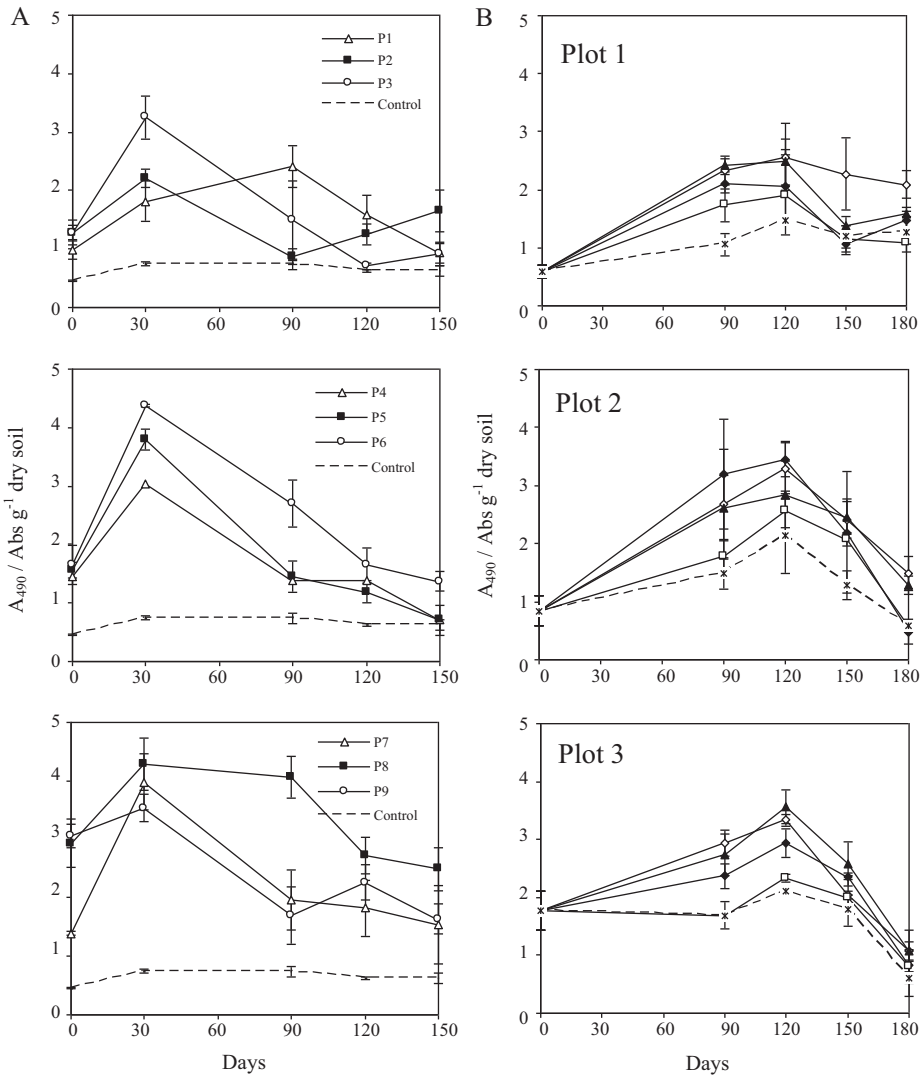

Fig. 3. (A) Changes of FDA activity in the composted soil and (B) (right): the FDA activity in rhizosphere and non-rhizosphere of plants grown in composted soils. Symbols in (B) stand for non-rhizosphere (✱), Seepweed (◊), Sealavander (♦), Central Asia Saltbush (▲) and Reed (□). Error bars indicate the standard deviation ($n = 3$).

four plants. In plot 1, compared to other plant species, Seepweed had significantly higher values in all the parameters of root morphology, and its root length, surface area and volume were about 4 times larger than Reed, which had no obvious difference from Sealavander and Central Asia Saltbush. In plots 2 and 3, Reed was significantly different from the other plant species ($p < 0.05$). The number of root tips had the same trend as the root length and root surface. All these parameters of root morphology show that Seepweed had the strongest root system among the four plants.

Phillips et al. reported that hydrocarbon uptake in roots occurs primarily in the root branching zones and increased hydrocarbon uptakes in the roots were due to the augmented soil volume colonization [1], but no exact data of root parameters were presented. In our study, the Seepweed showed the potential for high rhizodegradation of TPH in the soil, which could be due to its fibrous root system providing a large specific surface area and root volume and a high number of root tips to interplay with microorganisms in the rhizosphere (Table 2). Root growth promote soil aeration, which could enhance oxidative degradation of organic compounds, and this may also widen the way for "trapped" contaminants to become accessible to degrading bacteria [28]. Overall, the current findings suggest that strong root systems led to higher TPH degradation as demonstrated, in particular by the Seepweed rhizosphere (Fig. 1B).

Root morphology parameters also impacted microbial community composition and the number of degrading bacteria, which were related to the pollutants degradation [1]. More hydrocarbon-degrading microorganisms were observed in the rhizosphere than in the non-rhizosphere (Figs. 2B and 3B), consistent with the greater TPH degradation rate in rhizosphere (Fig. 1B). In plot 2, the bacteria count in Seepweed rhizosphere was 1.1×10^6 CFU kg^{-1} dry soil, significantly higher than the non-rhizosphere count of 2.5×10^5 CFU kg^{-1} dry soil (Fig. 2B). In addition, the number of degrading bacteria increased up to 90 days of plant growth, then decreased afterwards gradually, but always remained above the non-rhizosphere values. In all 3 plots, the bacteria count of Seepweed, Central Asia Saltbush and Sealavander was higher than that of Reed (Fig. 2B). There was also a marked difference between the microbial activities in the rhizosphere and those in the non-rhizosphere (Fig. 3B). In the rhizosphere, many plant exudates attract and stimulate microbial communities (e.g., pseudomonads). The introduction of plants to a contaminated soil promotes the growth and activity of indigenous alkane degraders, which results in substantial increases in specific *alkB* genotypes and higher degradation [29]. Thus, more hydrocarbon-degrading microorganisms (Fig. 2B) and higher microbial activity (Fig. 3B) in the Seepweed rhizosphere had a positive correlation to the TPH degradation (Fig. 1B).

Table 3
Diversity indices of rhizosphere and non-rhizosphere soils.

Plot	Plant species	Shannon (H')	Evenness (E)	Simpson (D)
P1	Non-rhizosphere	2.36a	0.94a	0.87a
	Seepweed	2.11ab	0.96a	0.79a
	Sealavander	2.87ab	0.94a	0.93a
	Central Asia Saltbush	3.13b	0.96a	0.95a
	Reed	2.19a	1.09a	0.80a
P2	Non-rhizosphere	2.19a	1.09a	0.84a
	Seepweed	2.68b	0.97a	0.92b
	Sealavander	2.78b	0.93b	0.92b
	Central Asia Saltbush	3.00b	0.94b	0.94b
	Reed	2.80b	0.94b	0.93b
P3	Non-rhizosphere	2.38ab	0.89a	0.89ab
	Seepweed	2.93bc	0.94a	0.94bc
	Sealavander	2.96c	0.95a	0.94c
	Central Asia Saltbush	2.34a	0.94a	0.88a
	Reed	2.80bc	0.93a	0.93bc

a–c: In the table, values followed by different letters in the same column for the same plot treatment indicate significant differences at $p < 0.05$ level by LSD test.

The longer roots of Seepweed (Table 2) could also help bacteria to spread through the soil and thereby penetrate deeper into soil layers, leading to much more extensive TPH degradation. Our previous field study in YRD also showed that total microbial numbers, activity, and diversity were significantly higher in Seepweed rhizosphere compared to non-rhizosphere soils. Reed, another naturally occurring plant in the YRD wetland, had few change of microbial activities and diversity in its rhizosphere compared to non-rhizosphere soils [30]. Lin et al. also reported that Seepweed increased the amount of organic matter and total nitrogen in the soil, helping to stimulate the survival and action of the rhizosphere microorganisms [31].

Soil microbial community diversity indices were analyzed using the Biolog ECO plate data (Table 3). In soil plot 1, the 3 diversity indices showed no significant difference between the non-rhizosphere and rhizosphere except for the Shannon index of Central Asia Saltbush. In soil plot 2, most of the plant species had clear influences on the microbial diversity ($p < 0.05$). The Shannon indices were 2.19, 2.68, 2.78, 2.80 and 3.00 for non-rhizosphere soil and rhizosphere soils planted with Seepweed, Sealavander, Reed and Central Asia Saltbush, respectively. In plot 3, the 3 diversity indices in the rhizosphere were larger than in the non-rhizosphere (Table 3). The Shannon index was influenced by microbial species richness, Simpson index reflected the most common species in the microbial community, and Evenness index was a measure of uniformity [18]. Therefore, after 90 days of rhizodegradation, with the decreasing TPH, the initial complex degradation products (usually carboxylic acids) appear to convert into much simpler and less toxic forms [21], allowing many more types of microbial populations to survive and flourish, increasing microbial community richness. The uniformity of the microorganism community decreased with decreasing TPH concentration in plot 2, but no significant difference in the Evenness index was observed in plots 1 and 3.

To further interpret the complex patterns produced by the Biolog ECO plate data, the multivariate statistical tool of PCA was employed to obtain a more detailed insight into the patterns of carbon utilization across the different plant species. The data presented in Fig. 4 shows the functional ability and diversity of rhizosphere/non-rhizosphere soil–bacterial community. All the data in non-rhizosphere and rhizosphere soils exhibited distinctly different profiles based on the plots of PC1 vs. PC2. In plot 1, the first and second principal components (PC1 and PC2) explained 33.9% and 13.2% of the variance in the data. In plot 2, the PC1 and PC2 explained 39.1% and 10.4%, respectively. In plot 3, these two values were 57.1% and 8.8%, respectively. The variations in principal components were mainly associated with plant-specific factors. The flux of substrates is a governing factor for the composition of microbial communities. In the present study, the increased growth and activity of hydrocarbon-degrading microorganisms in the rhizosphere (Figs. 2B and 3B) could utilize much more petroleum hydrocarbons as energy. With the decline of TPH concentration, the diversity of rhizobacteria increased (Table 3), subsequently altering the structure and functioning of microbial populations in the rhizosphere (Fig. 4). In these 3 soil plots, there was a trend for PC1 and PC2, the plant rhizosphere had relatively positive effects on microbial community composition (Fig. 4) and consequently promoted the microbial activities of the hydrocarbon contaminated soil. These changes could be induced by complex factors, such as the tropic condition [19], the concentration of pollutants [32], plant root exudates [9], as well as the possible induction of degrading enzymes through secretion of compounds which cause co-metabolism of hydrocarbons [22]. All of these complicated inter-

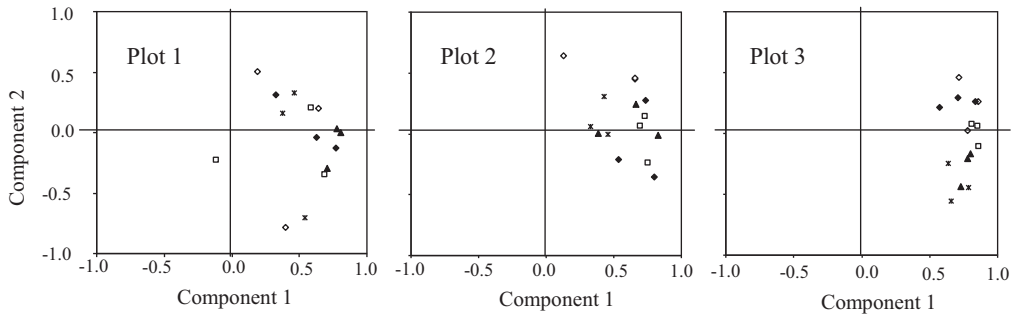

Fig. 4. Comparison of the culturable bacterial community in the rhizosphere and non-rhizosphere soils. Orthogonal plot of principal components 1 and 2 for carbon substrate utilization analysis with plant species represented as non-rhizosphere (✱), Seepweed (◊), Sealavander (♦), Central Asia Saltbush (▲) and Reed (□).

Table 4
Soil micropore analysis.

	Surface area ($m^2\ g^{-1}$)	Micropore volume ($mm^3\ g^{-1}$)	Micropore size (nm)
Non-rhizosphere	6.31a	2.25a	0.57a
Seepweed rhizosphere	5.03b	1.72b	0.57a

a–b: In the table, values followed by different letters in the same column indicate significant differences at $p < 0.05$ level by LSD test.

actions between the plants and the biological regime appear to create a more ecologically robust and stable system for rhizodegradation.

The surface area and volume of soil micropores in the non-rhizosphere and rhizosphere after planting were measured. In plot 2, the micropore surface and volume for the Seepweed rhizosphere soil were significantly reduced from 6.31 $m^2\ g^{-1}$ and 2.25 $mm^3\ g^{-1}$ to 5.03 $m^2\ g^{-1}$ and 1.72 $mm^3\ g^{-1}$, respectively after 180 days of planting (Table 4). The decrease of soil micropore surface area and volume was probably due to the organic acids released from plant roots. Seepweed could exudate organic acids, dominated by oxalic acid [33]. These low molecular weight organic acids (LMWOA) can chelate soil cations, resulting in localized soil aggregate destruction which subsequently accelerates pollutant desorption and increases its solubility, mobility and bioavailability [34,35]. This may explain why the TPH degradation rate in the Seepweed rhizosphere was higher than that for other 3 plants. Previous research has shown that root acid exudates can increase desorption of pollutants [34]. This present work is the first to directly relate increased degradation to the reduction of soil micropore surface area and volume. Exploiting this relationship to obtain the utmost TPH rhizodegradation would benefit soil remediation. After 180 days of rhizodegradation, the TPH concentration in Seepweed rhizosphere soil was reduced to 383 mg kg^{-1}, much lower than, for example, the Chinese government-required standard (<500 mg kg^{-1}) [26].

4. Conclusions

Planting after composting seems promising to remediate heavily TPH-polluted soils. Composting with optimal organic amendment amounts (2/1) and C/N ratio (15/1) effectively reduced TPH concentration and allowed dominant plant species to grow in the soil. Seepweed with its strong root system and active microbial community was the best of the four dominant plants tested at degrading residual TPH after composting. Also, the fact that micropore surface and volume decreased significantly in Seepweed rhizosphere soil, could be critical for microbes to have access to the sequestrated contaminants by breaking apart soil aggregates with root-released LMWOA. The exact relationship between residual TPH and soil micropore warrants further investigation in the future.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 41073067), Foundation for Key Program of the Education Ministry, China (No. 308016), and the National Major Special Technological Program Concerning Water Pollution Control and Management, China (No. 2008ZX07010-008). The authors are grateful to Song Xiuli, Farhana Maqbool, Luo Ye and Xu Zhenhua of the Ocean University of China for their technical assistance and Mark Eugene Reynolds of the University of Massachusetts for editing the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.03.103.

References

[1] L.A. Phillips, C.W. Greer, R.E. Farrell, J.J. Germida, Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments, Appl. Soil Ecol. 42 (2009) 9–17.
[2] J.L. Kirk, J.N. Klironomos, H. Lee, J.T. Trevors, The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil, Environ. Pollut. 133 (2005) 455–465.
[3] J. Aislabie, D.J. Saul, J.M. Foght, Bioremediation of hydrocarbon-contaminated polar soils, Extremophiles 10 (2006) 171–179.
[4] R. Boopathy, Factors limiting bioremediation technologies, Bioresour. Technol. 74 (2000) 63–67.
[5] K.S. Jørgensen, J. Puustinen, A.M. Suortti, Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles, Environ. Pollut. 107 (2000) 245–254.
[6] K. Van Gestel, J. Mergaert, J. Swings, J. Coosemans, J. Ryckeboer, Bioremediation of diesel oil-contaminated soil by composting with biowaste, Environ. Pollut. 125 (2003) 361–368.
[7] M. Lu, Z. Zhang, W. Qiao, X. Wei, Y. Guan, Q. Ma, Y. Guan, Remediation of petroleum-contaminated soil after composting by sequential treatment with Fenton-like oxidation and biodegradation, Bioresour. Technol. 101 (2010) 2106–2113.
[8] EPA (Environmental Protection Agency): Introduction to Phytoremediation. EPA-report EPA/600/R-99/107 (2000). Mai 2003 at http://clu-in.org/techpubs.htm.
[9] D.L. Korade, M.H. Fulekar, Rhizosphere remediation of chlorpyrifos in mycorrhizospheric soil using ryegrass, J. Hazard. Mater. 172 (2009) 1344–1350.
[10] T.A. Anderson, E.L. Kruger, J.R. Coats, Enhanced degradation of a mixture of three herbicides in the rhizosphere of a herbicide-tolerant plant, Chemosphere 28 (1994) 1551–1557.
[11] S. Peng, Q. Zhou, Z. Cai, Z. Zhang, Phytoremediation of petroleum contaminated soils by *Mirabilis Jalapa* L. in a greenhouse plot experiment, J. Hazard. Mater. 168 (2009) 1490–1496.
[12] V. Bianchi, G. Masciandaro, B. Ceccanti, S. Doni, R. Iannelli, Phytoremediation and bio-physical conditioning of dredged marine sediments for their re-use in the environment, Water, Air, Soil Pollut. 210 (2010) 187–195.
[13] J. Gurska, W. Wang, K.E. Gerhardt, A.M. Khalid, D.M. Isherwood, X.D. Huang, B.R. Glick, B.M. Greenberg, Three year field test of a plant growth promoting rhizobacteria enhanced phytoremediation system at a land farm for treatment of hydrocarbon waste, Environ. Sci. Technol. 43 (2009) 4472–4479.
[14] Y. Liang, X. Zhang, D. Dai, G. Li, Porous biocarrier-enhanced biodegradation of crude oil contaminated soil, Int. Biodeterior. Biodegrad. 63 (2009) 80–87.
[15] D.H.W. Nelson, L.E. Sommers, Total carbon, organic carbon and organic matter, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, American Society of Agronomy, Madison, WI, 1982, pp. 539–580.
[16] D.R. Keeney, D.W. Nelson, Nitrogen inorganic forms, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, American Society of Agronomy, Madison, WI, USA, 1982, pp. 643–698.
[17] V.S. Green, D.E. Stott, M. Diack, Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples, Soil Biol. Biochem. 38 (2006) 693–701.
[18] J. Dunbar, L.O. Ticknor, C.R. Kuske, Assessment of microbial diversity in four Southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis, Appl. Environ. Microbiol. 66 (2000) 2943–2950.
[19] E.R. Travis, N.C. Bruce, S.J. Rosser, Short term exposure to elevated trinitrotoluene concentrations induced structural and functional changes in the soil bacterial community, Environ. Pollut. 153 (2008) 432–439.
[20] P.I. Ravikovitch, B.W. Bogan, A.V. Neimark, Nitrogen and carbon dioxide adsorption by soils, Environ. Sci. Technol. 39 (2005) 4990–4995.
[21] W. Liu, Y. Luo, Y. Teng, Z. Li, P. Christie, Prepared bed bioremediation of oily sludge in an oilfield in northern China, J. Hazard. Mater. 161 (2009) 479–484.
[22] S. Lee, B. Oh, J. Kim, Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity, Bioresour. Technol. 99 (2008) 2578–2587.
[23] I. Gandolfi, M. Sicolo, A. Franzetti, E. Fontanarosa, A. Santagostino, G. Bestetti, Influence of compost amendment on microbial community and ecotoxicity of hydrocarbon-contaminated soils, Bioresour. Technol. 101 (2010) 568–575.
[24] G. Adam, H. Duncan, Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils, Soil Biol. Biochem. 33 (2001) 943–951.
[25] A. Godoy-Faúndez, B. Antizar-Ladislao, L. Reyes-Bozo, A. Camaño, C. Saez-Navarrete, Bioremediation of contaminated mixtures of desert mining soil and sawdust with fuel oil by aerated in-vessel composting in the Atacama Region (Chile), J. Hazard. Mater. 151 (2008) 649–657.

[26] C.R. Li, W.K. Wang, Y.Q. Cao, Bioremediation of petroleum-contaminated soil, J. Veg. Sci. (in Chinese) 13 (2002) 234–238.
[27] V. Bianchi, B. Ceccanti, A three components system (TRIAS) in the phytoremediation of polluted environmental matrices, Toxicol. Environ. Chem. 92 (2010) 477–493.
[28] M.B. Leigh, J.S. Fletcher, X. Fu, F.J. Schmitz, Root turnover: an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants, Environ. Sci. Technol. 36 (2002) 1579–1583.
[29] L.A. Phillips, C.W. Greer, J.J. Germida, Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil, Soil Biol. Biochem. 38 (2006) 2823–2833.
[30] Z.Y. Wang, Y.Z. Xin, D.M. Gao, F.M. Li, J. Morgan, B.S. Xing, Microbial community characteristics in a degraded wetland of the Yellow River Delta, Pedosphere 20 (2010) 466–478.
[31] X.Z. Lin, J.H. Shen, K.Z. Liu, X.H. Huang, Study on remediation effects of *Suaeda salsa* L. planting on coastal saline soil, Adv. Mar. Sci. (in Chinese) 23 (2005) 65–69.
[32] E.R. Travis, N.K. Hannink, C.J. van der Gast, I.P. Thompson, S.J. Rosser, N.C. Bruce, Impact of transgenic tobacco on trinitrotoluene (TNT) contaminated soil community, Environ. Sci. Technol. 41 (2007) 5854–5861.
[33] C.W. Yang, D.C. Shi, D.L. Wang, Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge.), Plant Growth Regul. 56 (2008) 179–190.
[34] Y. Gao, L. Ren, W. Ling, S. Gong, B. Sun, Y. Zhang, Desorption of phenanthrene and pyrene in soils by root exudates, Bioresour. Technol. 101 (2010) 1159–1165.
[35] J.C. White, Z.D. Parrish, M. Isleyen, M. Gent, W. Iannucci-Berger, B.D. Eitzer, J.W. Kelsey, M.I. Mattina, Influence of citric acid amendments on the availability of weathered PCBs to plant and earthworm species, Int. J. Phytoremediation 8 (2006) 63–79.