1 **Influence of root components of celery on pyrene bioaccessibility, soil enzymes**
2 **and microbial communities in pyrene and pyrene-diesel spiked soils**

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Abstract:

Though phytoremediation is deemed as a promising approach to restore polycyclic aromatic hydrocarbon (PAHs) contaminated sites, studies about how the biodegradation of PAHs is enhanced still remains incomprehensive. Effects of root components on pyrene bioaccessibility, soil enzymes and microbial communities were explored in the paper, and their interactions in simulated pyrene and pyrene-diesel spiked microcosms were tried to give a reasonable explanation. Results indicated that root components enhanced the pyrene removal of bioaccessible and adsorbed fractions by 16.10 and 1.80 mg kg⁻¹, respectively, in pyrene-spiked soils at the end of the experiment. By contrast, root components increased the degradation of bioaccessible fraction by only 3.3 mg kg⁻¹ in pyrene-diesel spiked soils. Although the bound fractions of pyrene increased over time in treatments without root components, they remained relatively stable, ranging from 0.02 to 0.03 mg kg⁻¹, in root components amended treatments. Activities of soil enzymes (polyphenol oxidase, catalase, invertase, urease and alkaline phosphatase) varied differently in response to pollutants and root components. Analysis of phospholipid fatty acids revealed that root components increased the biomass of soil microorganisms and altered the microbial structure. Pearson correlation analysis proved positive correlations between all the microbial subgroups and pyrene removal in pyrene-spiked soils, but the degradation of bioaccessible pyrene was only positively related with microorganisms confirmed by monounsaturated fatty acids in pyrene-diesel spiked soils.

Keywords: Root components; Bioaccessibility; Soil enzyme; Soil microbial communities;

Pyrene-diesel spiked soils

1 Introduction

Due to extensive anthropogenic activities, such as coal burning, coking, automobile exhaust gas, refuse incineration and so on, polycyclic aromatic hydrocarbons (PAHs) are widely dispersed into the environment (2005). PAHs, resistant to be degraded, tend to accumulate in the soil and potentially threaten human health (Tejeda-Agredano et al., 2013). Phytoremediation emerges as an economically and environmentally feasible in situ technology in eliminating PAHs. In general, it is deemed that root components, an important interface process of the exchange between plants and the soil, can enhance the degradation of contaminants, which contributes greatly to the total removal of pollutants during phytoremediation (Meng and Zhu, 2011). Some reports suggested that root components boosted the growth of soil microorganisms (Meng and Zhu, 2011), while some thought root components affected the PAHs bioaccessibility through affecting the adsorption/desorption of PAHs in the soil (Bielská et al., 2012). However, little literature reported the comprehensive interaction among soil microbes, PAHs bioaccessibility and soil enzymes influenced by root components.

PAHs exist in the soil as freely desorbed fraction, weakly adsorbed fraction, strongly adsorbed fraction and combined fraction with soil organic matters (SOM). PAHs bioaccessibility represents all the fractions that are available to cross the cellular membrane of organisms, involving not only bioavailable but potentially bioavailable fractions in the soil (Crampon et al., 2016). It is thought that freely desorbed and weakly adsorbed fractions are bioaccessible to soil microorganisms, while strongly adsorbed fraction cannot be utilized until desorption. Though combined fraction plays only a small part in the total PAHs, it accounts for the main retention in the soil because of its resistance to degradation (Wei et al., 2014a). Mild chemicals, such as butanol, methanol, β -cyclodextrin, can be used to components bioaccessible PAHs to microbes in the soil and estimate their potential for biodegradation

(Meynet et al., 2014). The fractionation of PAHs in the soil can be detected by sequential extraction method (Duan et al., 2015). Someone thinks that organic acids and amino acids from root components can enhance the bioaccessibility of pyrene, probably by breaking the “bridges” of soil complexes formed through the linkage of metallic cations between soil minerals and SOM (Sun et al., 2013). Evidences are also observed that root exudates facilitate desorption of pyrene and phenanthrene to a great extent (Gao et al., 2010). However, the influences of root components on PAHs fractionation and their accessibility to various microbial groups are even unknown.

Soil enzymes, secreted by plants, soil microorganisms and animals, act an important role in biological metabolism processes with high specificity and high catalytic efficiency (Shen et al., 2005). Oxidoreductases such as dehydrogenase, catalase, and polyphenol oxidase contribute directly to the degradation of PAHs by breaking the benzene rings (Štursová and Baldrian, 2011). Hydrolases such as invertase, phosphatase and urease can affect the degradation of organic pollutants indirectly by altering metabolic activities of degraders through nutrition limitation or supply. Soil properties like organic carbon content and pH, contamination and land use patterns affect the activity of soil enzyme greatly (Štursová and Baldrian, 2011). Though plants alter specifically the soil enzyme activities in the rhizosphere through root components, nothing is known till now about the relationship between this alteration and the degradation of different PAHs fractions (Zhou et al., 2011).

As the main degraders, soil microbes take an essential role in the phytoremediation of PAHs contaminated soil. Studies have proved that plants can reshape or redirect the microbial community structure in the rhizosphere by releasing compounds, such as sugars, amino acid, organic acid, fatty acid, sterols, growth hormone and so on, into the soil (Abhilash et al., 2012). In addition, the influence of root components varies according to the types of plants (Wang et al., 2011). Though it has been

proved that PAHs-degrading plants succeed to enhance the abundances of PAHs-degraders in their rhizosphere, the effect of root components on the whole microbial community structure, as well as the bioaccessibility of different PAHs fractions to various microbial subgroups remain unknown (Meng and Zhu, 2011).

The objective of this study is to investigate the root components effect on the fractionation of PAHs, soil enzymes and soil microorganisms, as well as their relationships. Hypotheses were proposed: (1) root components enhanced the bioaccessibility of PAHs in the soil; (2) root components facilitated the biodegradation of PAHs by reshaping the microflora; (3) root components promoted the biodegradation of PAHs by altering the activities of soil enzymes. To verify these hypotheses, pyrene was chosen as the model PAHs and celery (*ApiumGraveolens*) as the model PAHs-degrading plant. The degradation and fractionation of pyrene, activities of soil enzymes (invertase, urease, alkaline phosphatase, polyphenol oxidase, and catalase), as well as soil microbial community structure were detected in pyrene and pyrene-diesel spiked soils with or without root components.

2 Materials and methods

2.1 Chemicals and soils

Pyrene (98%purity) was purchased from Aladdin Reagent. The other chemicals, analytical grade or better, were bought from Sinopharm. Pollution free soil was collected from vacant land at Shanghai University, air-dried and sieved to pass through 2 mm mesh. The initial physicochemical properties of the used soil were determined: organic matter 19.60 gkg⁻¹, TN 700 mgkg⁻¹, TP 800 mgkg⁻¹, pH 7.30, and sand 32.20%, silt 60.40%, clay 7.40%. To reduce the disturbance to the soil microbes, the soil was spiked in the following steps: firstly, pyrene was dissolved in dichloromethane and added into the soil

to 500 mg kg⁻¹, the spiked soil was aged for 100 d in the dark at 4°C after volatilization in fume hood for a week; then the aged soil was mixed with fresh soil to a final pyrene concentration of 50 mg kg⁻¹ (the detected pyrene concentration was about 45 mg kg⁻¹); eventually, diesel was dissolved into dichloromethane and mixed into the pyrene spiked soil to get pyrene-diesel spiked soil (diesel content was about 5 000 mg kg⁻¹). Spiked soils were sieved to pass 2 mm mesh again to get homogeneous.

2.2 Root components

Celery was selected as the model plant because it was the most efficient one out of nine tested pyrene-degrading plants in the preliminary experiments, its great pyrene-degrading ability was also demonstrated in the previous study (Yi and Crowley, 2007). After germination, the seedlings in soil were incubated in a climate chamber for 40 d, the roots were collected as sources of root components and stored at -80°C (Štursová and Baldrian, 2011).

2.3 Experimental design

Four groups were set up: pyrene spiked soils without root components (PY), pyrene spiked soils with root components (PYR), pyrene-diesel spiked soils without root components (PYD), and pyrene-diesel spiked soils with root components (PYDR). Root tissues were ground under liquid nitrogen, and then 2.8 g of root tissues was mixed with 35 g of spiked soils. All treatments were incubated in the dark at 28°C for 40 d, the soil moisture was kept at about 30% by adding sterilized distilled water every three days. During the experiment, the microcosms were repositioned every three days to avoid edge effect. Each of the setups was triplicate and sampled at 10 d intervals. Soil samples were freeze-dried and sieved through a 0.125 mm mesh, then stored at -80°C till analysis.

2.4 Analytical methods

Pyrene fractionation. Sequential extraction was used to determine the bioaccessibility and fractionation of pyrene in the soil according to previous studies (Wei et al., 2014a). Briefly, 1 g of treated soil was mixed with 5 mL of *n*-butanol for thirty seconds in a vortex mixer, the mixture was centrifuged at 2600 g for 20 min, then filtered. The supernatant evaporated and redissolved with *n*-hexane for analysis of bioaccessible. Then associated pyrene (potentially bioaccessible fraction) was extracted ultrasonically by dichloromethane (DCM) from the remaining soil. Finally, bound pyrene (non-bioaccessible fraction) was extracted after heat-treating the DCM-free soil in the solution of sodium hydroxide at 100°C for 2 h under nitrogen flow. GC-MS (Agilent 6890N/5975B) equipped with a DB-5 column was used to determine the content of bound pyrene (Wei et al., 2014a).

Soil enzymes. Urea was used as the substrate to detect the activity of urease (URE, EC 3.5.1.5) which was expressed by mg NH₄-N g⁻¹ soil 24 h⁻¹ (Hoffmann and Teicher, 1961). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was measured according to the amount of purpurogallin produced by the oxidation of 1,2,3-trihydroxy benzene, and expressed as mg purpurogallin g⁻¹ soil 2 h⁻¹ (Tang et al., 2010). For the determination of invertase (INV, EC 3.2.1.26), five grams of soil was added into sucrose solution, incubated for 24 h at 37°C, and its activity was mg glucose equivalents g⁻¹ soil 24 h⁻¹ (He et al., 2015). To analyze the activity of catalase (CAT, EC 1.11.1.6) five grams of soil was mixed with 5 mL of hydrogen peroxide (0.3%, w/w) for 30 min at 30°C. After filtration, the filtrate was titrated with 0.1 M potassium permanganate (KMnO₄), and the unit was mL 0.1 M KMnO₄ g⁻¹ soil 30 min⁻¹ (Johnson and Temple, 1964). The activity of alkaline phosphatase (PHOS, EC 3.1.3.1) was determined according to the method described, and expressed by mg phenol g⁻¹ 3 h⁻¹ (Wyszkowska and Wyszkowski, 2010).

Microbial community structure. Soil microbial community structure was analyzed by phospholipid fatty acid (PLFA) pattern following previous studies (Zhang et al., 2011). In general, crude lipids were extracted from 3 g of freeze-dried soil samples with a mixture of citrate buffer, methanol, and chloroform, then separated by column chromatography to obtain PLFAs. Finally, PLFAs were methylated by methanol, dissolved in 1 mL of hexane, and quantified using GC-MS.

2.5 Statistical analysis

The two-way analysis of variance (ANOVA) and Pearson correlation matrix were conducted by SPSS 17.0. Paired comparison with the Duncan's multiple range tests was applied for testing significant differences among treatments. Canoco for Windows 4.5 was used to conduct principle component analysis (PCA).

3 Results

3.1 Pyrene fractionation and bioaccessibility

The degradation and fractionation of pyrene over time in pyrene and pyrene-diesel spiked soils are illustrated in Figs. 1a and 1b. Root components improved pyrene removal greatly from 20.7 to 35.5 mg kg⁻¹ and from 10.7 to 15.3 mg kg⁻¹ at 40 d in pyrene and pyrene-diesel spiked soils, respectively. Bioaccessible fraction contributed to the most degradation of pyrene decreasing from 35.1 to 16.6 mg kg⁻¹ in pyrene spiked soils (Fig. 1a) and from 36.8 to 24.4 mg kg⁻¹ in pyrene-diesel polluted soils (Fig. 1b) without root components. Root components decreased the remaining bioaccessible pyrene further with 16.1 mg kg⁻¹ in pyrene spiked soils and 3.3 mg kg⁻¹ in pyrene-diesel contaminated soils (Figs. 1a and 1b).

In pyrene spiked soils, the remaining adsorbed pyrene decreased over time from 9.5 to 7.4 and 5.6 mg kg⁻¹ when unamended and amended, respectively (Fig. 1a). While in pyrene-diesel spiked soils, the adsorbed fraction increased slightly from 9.6 to 11.2 and 10.7 mg kg⁻¹ in root components untreated and treated treatments, respectively (Fig. 1b).

3.2 Soil enzymes

Activities of three hydrolases (URE, INV, PHOS) and two oxidases (PPO, CAT) in pyrene and pyrene-diesel spiked soils were shown in Table 1. The activity varied with enzyme variety, incubation time, contamination and root components amendment. In both types of pollution, PPO activity, ranging from 0.12 to 0.23 mg g⁻¹ h⁻¹, was weakly affected by root components. For CAT, root components tended to enhance its activity in pyrene-diesel contaminated soils in the early 30 d, while only at 40 d in pyrene spiked soils ($P < 0.05$). In addition, root components increased the activities of URE and INV in both pyrene and pyrene-diesel contaminated soils. Though root components decreased the PHOS activity during the first 20 days especially in pyrene spiked soil, it acted a slight role during the last 20 days.

Pearson correlation coefficients were used to analyze the relationship between soil enzymes and the removal of bioaccessible and adsorbed pyrene (Table 2). In pyrene contaminated soil, the removal of bioaccessible fraction was negatively related with activities of URE, INV and PHOS, but positively correlated with CAT activity ($P < 0.01$). In pyrene-diesel contaminated soils, the activities of URE and INV were negatively correlated with the removal of bioaccessible fraction, while CAT activity was negatively correlated with the removal of adsorbed fraction ($P < 0.05$).

3.3 Microbial community structure

A total of 29 PLFAs were detected in all the treated soils, among which 16:0, 16:1 ω 5c and 18:2 ω 6,9 were the most abundant (Table 3). Based on their chemical structure, they were divided into five subgroups, namely straight-chain saturated fatty acids (SSFA), branched-chain fatty acids (BSFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and cyclopropyl fatty acid (CFA), and their abundances over time in both pyrene and pyrene-diesel contaminated soils were illustrated in Fig. 2. SSFA, ranging from 8.26-18.16 nmol g⁻¹, took the highest proportion of the total PLFAs, followed by BSFA and MUFA. While the proportions of PUFA and CFA with the abundance of 0.32-3.32 and 0.76-2.63 nmol g⁻¹ respectively, were much lower.

Principal component analysis was conducted to explore the differences in the microbial structure among soil samples in pyrene contaminated soils (Fig. 3) and pyrene-diesel spiked soils (Fig. 4). In pyrene spiked soils, the first principal component (PC1) and the second principal component (PC2) accounted for 61.3% and 28.7% of the total variance, respectively (Fig. 3). Soil microbial community structure was simultaneously affected by incubation time and root components. In particular, the sample dots of PY and PYR were separated distinctly from each other, indicating that their soil microbial structures were significantly different. However, the differences gradually diminished with shortened distance between PY and PYR samples over time, which was consistent with the results illustrated in the Fig. 2. In addition, the dominant PLFAs in root components amended and unamended soils were 18:2 ω 6,9, 18:2 ω 5c, 16:1 ω 9t, cy17:0 and 10Me16:0, 10Me18:0, 18:1 ω 9c, 17:1 ω 8c, respectively (Fig. 3a).

PCA results of soil microbial community structure were shown in Fig. 4. PC1 and PC2 accounted for 59.8% and 17.1% of the total variance, respectively in pyrene-diesel spiked soils (Fig. 4). Similar with that in pyrene pollution, root components altered greatly the structure of soil microbes in pyrene-diesel

pollution. Samples from PYDR treatment were located at the lower position of PC1 and PC2, while those from PYD treatment were located at higher position of PC1 and PC2 (Fig. 4b). The dominant microbial communities in PYDR and PYD treatments were those indicated by i15:0, i19:0, 18:2 ω 6,9, 16:1 ω 9t, 18:2 ω 7c and 10Me16:0, cy17:0, 16:1 ω 5c, 18:1 ω 5c, respectively (Fig. 4a).

4 Discussion

It was proved previously that root exudates, one of the main components from root components, could improve the degradation of PAHs (Xie et al., 2012). In particular, butyric acid, a low molecular weight organic acid of root components, was found to be cometabolized with PAHs in the water utilizing dual-wavelength fluorimetric method (Wei et al., 2009). Recent reports have shown that petroleum hydrocarbons could be uptaken as carbon sources of microbial communities, but PAHs were considered as minor microbial carbon source (Ahad and Pakdel, 2013). Moreover, alkyl hydrocarbons were more readily to be biodegraded than PAHs. To this point, the competition for cometabolism between pyrene and diesel might account for the less degradation of pyrene in pyrene-diesel polluted soils.

The significant decrease of adsorbed fraction in pyrene contaminated soils treated by root components indicated that pyrene bioaccessibility was enhanced to a great extent by the root components. Similarly, it was also showed that root exudates could improve the availability of PAHs in previous studies (Sun et al., 2013). High level of soil organic substances could inhibit desorption of PAHs in the soil and decline their accessibility to microorganisms. And the oil hydrocarbons at high concentration could enhance the sorption of PAHs in the soil and decrease their bioaccessibility by co-adsorption (Du et al., 2012). Therefore, the presence of diesel contributed to the slight rise of adsorbed pyrene in pyrene-diesel contaminated soils.

Regarding the bound fraction, it is appealing that distinct differences between amendment and unamendment treatments were observed. Though bound pyrene increased greatly from 0.02 to 0.05 and 0.09 mg kg⁻¹ in pyrene and pyrene-diesel spiked soils without root components, respectively, it remained relatively stable ranging from 0.02 to 0.03 mg kg⁻¹ in both pyrene and pyrene-diesel contaminated soils amended with root components (Figs. 1a and 1b). Despite of its relative low concentration, bound fraction played an important role in assessing the ultimate extent of PAHs removal by phytoremediation. Ma et al. (2012) also found that the proportion of bound residues of phenanthrene and pyrene increased significantly with the cultivating time, which might be caused by the transfer of PAHs molecules into SOM holes or the combination of PAHs metabolites with SOM. Root components may influence the formation of bound pyrene by changing the structure of SOM or competing for combining position. However, the detailed mechanisms still need to be explored further.

Although the degradation rate of pyrene in pyrene-diesel polluted soils was significantly lower than that in pyrene spiked soils, the total amount of bioaccessible and potentially bioaccessible fractions were similar in both types of the soil (Fig. 1a and 1b). This phenomenon indicated that the coexistence of diesel had an effect on the efficiency of PAHs degradation in the short term, rather than the end point of phytoremediation.

The activities of soil enzymes are the combined results of pollutants, soil components, plants and environmental factors (Hendriksen et al., 2016). Lu et al. (2010) found that the catalase and polyphenoloxidase activities in pyrene polluted soil were both higher in *Bidens maximowicziana* planted soil than those of unplanted soil. In this paper, we found the influences of root components on soil enzymes changed with time and types of pollution. Tang et al. (2010) found that PPO took an important part in the degradation of petroleum hydrocarbons, however, no significant correlation

between PPO and pyrene degradation was observed in this paper. This inconsistent may be caused by different pollutants and soil properties. Released mainly by aerobes in the soil, CAT activity is closely linked to the activity of soil enzymes (He et al., 2015), therefore, higher CAT activity represents higher degradation of bioaccessible pyrene in pyrene spiked soils. While in pyrene-diesel contaminated soil, diesel hydrocarbons can compete with pyrene as the substrates of catalytic oxidation, consequently breaking the correlation of CAT and pyrene degradation. URE, INV and PHOS were responsible for the hydrolysis of urea, organic carbohydrate and organically bound phosphate, respectively (Wei et al., 2014b). They can affect the biodegradation of pyrene mainly in two ways: (1) pyrene bioaccessibility can be influenced by hydrolases through catalyzing the hydrolysis of soil organic matters, moreover, products in these processes such as phosphate can also impact pyrene adsorption/desorption (Wei et al., 2015); (2) hydrolases play an important role in the growth and function of soil microorganisms and thus affect the microbial degradation of pyrene.

In general, root components enhanced significantly the abundance of every subgroup in both types of pollution, except that of CFA in pyrene-diesel spiked soils. These results showed that root components promoted the biomass of all the microbial subgroups, mostly by providing carbon sources, nitrogen sources and other nutrients for the growth of soil microorganisms (Kamath et al., 2004). However, the enhancing effect of root components weakened over time, for example, TPLFA was 11.42 nmol g⁻¹ higher in PYR treatment than that in PY treatment at 10 d, but only 2.51 nmol g⁻¹ higher at 40 d. This may be reduced by the depletion of root components by soil microbes. Moreover, the significant promotion of various soil microorganisms induced by root components must account for the enhancement of pyrene removal to some extent.

The abundance of total PLFAs is positively correlated with the whole biomass of soil microorganisms (Plaza et al., 2015), while SSFAs and BSFAs are usually used as indicators for soil bacteria, PUFAs for soil fungi, MUFAs and CFAs for Gram-negative and aerobic bacteria respectively (Zhang et al., 2015). Pearson correlation analysis was used to reveal the function of different microbial subgroups in pyrene degradation, and the coefficients were listed in Table 4. In pyrene spiked soils, the abundances of all the microbial subgroups were positively correlated with the removal of bioaccessible and adsorbed fractions, however, the positive correlation was only observed between the degradation of bioaccessible fraction and MUFA abundance ($P < 0.01$). In pyrene contaminated soils, all the microbial subgroups can get access to the bioaccessible fraction, degrade it through direct metabolism or cometabolism. Absolutely, the degradation of bioaccessible fraction can enhance desorption of adsorbed fraction, therefore promote its removal, which must contribute to the positive correlation between adsorbed pyrene and microbial subgroups. In contrast, though microbes can still get access to the bioaccessible fraction directly, diesel hydrocarbons prevented pyrene biodegradation through competing as carbon sources. Common PAHs degraders are Gram-negative bacteria, aerobes and fungi (Zhang et al., 2011), among which Gram-negative bacteria, indicated by MUFAs are more tolerant to PAHs contaminants with higher PAHs-degrading ability (Meng et al., 2015). The significant positive correlation between MUFA abundance and the removal of bioaccessible pyrene also demonstrated the great pyrene-degrading ability of Gram-negative bacteria.

Plants can alter the function and structure of soil microbial communities in the rhizosphere specifically through changing the properties of rhizospheric microenvironment, for example pH and redox potential, offering habitat point and supplying nutrients. Cao et al. (2012) found that the most abundant PLFA subgroup was SSFA in the rhizosphere of *Acorus calamus*, *Cyperus rotundus* and

Zizaniaaquatica, but BSFA in the rhizosphere of *Phragmitesaustrali*, under the stress of diesel hydrocarbons. Wei et al. (2014b) compared the microbial characteristics in the rhizosphere of various diesel-degrading flowering plants through greenhouse experiment, and found that PLFAs 15:0, i15:0, cy17:0, 16:1 ω 5c and 18:1 ω 5c dominated in the rhizosphere of efficient diesel-degrading *Viola tricolor*, while 18:1 ω 7c, 18:0, 10Me18:0, i13:0 and a14:0 were dominant in the rhizosphere of *Viola tricolor*. Therefore, the shifts of soil microbial communities when amended by root components must account for the enhanced pyrene degradation of both bioaccessible and adsorbed fractions.

5 Conclusions

Root components of celery significantly enhanced the degradation of pyrene, through influencing pyrene fractionation and bioaccessibility, activities of soil enzymes and microbial community structure, in pyrene and pyrene-diesel spiked soils. The removal of pyrene resulted from the degradation of bioaccessible and adsorbed fractions in pyrene spiked soils, but only bioaccessible fraction in pyrene-diesel spiked soils during the experiment. Root components increased the biodegradation of bioaccessible fraction with 16.1 mg kg⁻¹ and 3.3 mg kg⁻¹ in pyrene and pyrene-diesel spiked soils, respectively. In addition, the formation of bound residual pyrene was inhibited by root components in both types of soils. Activities of soil enzymes varied according to enzyme variety, time of incubation, types of pollutants and root components. The degradation of bioaccessible pyrene was positively correlated with CAT activity and negatively with activities of URE, INV and PHOS, while significant negative correlation between PHOS and adsorbed pyrene was observed. In both types of contaminated soils, root components shifted the structure of soil microflora greatly by promoting their biomass and altering their superiority communities. Interestingly, the differences in microflora structure between

two treatments faded gradually over time, suggesting a time-limited effect of root components. Though the removal of bioaccessible and adsorbed pyrene was positively correlated with abundances of all the microbial subgroups in pyrene contaminated soils, there was only a positive correlation between bioaccessible pyrene and Gram-negative bacteria indicated by MUFAs in pyrene-diesel spiked soils.

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403

Tables

Table 1 Activities of soil enzymes in pyrene and pyrene-diesel spiked soils

		PPO	CAT	URE	INV	PHOS
10d	PY	0.20±0.01	7.59±0.53	0.26±0.02	1.43±0.16	1.43±0.21
	PYR	0.21±0.03	7.47±0.38	0.28±0.03*	1.32±0.08	1.17±0.28*
	PYD	0.20±0.02	6.58±0.35	0.27±0.01	1.45±0.18	1.26±0.28
	PYDR	0.18±0.02	7.02±0.14*	0.32±0.03*	1.27±0.34	1.57±0.16
20d	PY	0.19±0.01	7.32±0.52	0.34±0.06	4.39±0.34	1.30±0.08
	PYR	0.16±0.01	7.67±0.06	0.51±0.05*	5.35±0.22*	1.26±0.08*
	PYD	0.14±0.02	7.44±0.35	0.39±0.06	5.14±0.77	1.17±0.08
	PYDR	0.12±0.00*	7.32±0.51	0.30±0.08	5.05±0.57	0.94±0.00*
30d	PY	0.23±0.02	7.18±0.24	0.33±0.01	2.51±0.16	1.35±0.14
	PYR	0.22±0.00	7.22±0.18	0.36±0.02	2.60±0.17*	1.39±0.16
	PYD	0.15±0.01	6.56±0.27	0.34±0.05	2.06±0.36	1.44±0.08
	PYDR	0.15±0.02	7.28±0.20*	0.40±0.04	2.38±0.01*	1.53±0.08
40d	PY	0.15±0.01	6.61±0.23	0.55±0.03	4.44±0.11	1.44±0.28
	PYR	0.19±0.01*	6.90±0.17*	0.60±0.06*	5.19±0.43*	1.57±0.16
	PYD	0.12±0.02	7.26±0.28	0.52±0.01	4.10±0.40	1.57±0.08
	PYDR	0.15±0.01*	6.94±0.48*	0.56±0.06*	4.61±0.08*	1.75±0.49

PY, pyrene spiked treatment without root components; PYR, pyrene spiked soil with root components; PYD, pyrene-diesel spiked treatment without root components; PYDR, pyrene-diesel spiked treatment with root components; PPO, polyphenol oxidase; CAT, catalase; URE, urease; INV, invertase; PHOS, alkaline phosphatase. * Significantly different from the same type of polluted soils without root components at 0.05 level

9 **Table 2** Correlation between activities of soil enzymes and the removal extents of bioaccessible and adsorbed

10 pyrene in pyrene and pyrene-diesel contaminated soils

Pollution	Pyrene fraction	PPO	CAT	URE	INV	PHOS
Pyrene	Bioaccessible	0.183	0.520**	-0.463*	-0.499*	-0.559**
	Adsorbed	0.033	0.305	-0.261	-0.327	-0.484*
Pyrene-diesel	Bioaccessible	0.177	0.266	-0.595**	-0.452*	-0.258
	Adsorbed	-0.355	-0.435*	0.244	0.342	0.159

11 PPO, polyphenol oxidase; CAT, catalase; URE, urease; INV, invertase; PHOS, alkaline phosphatase.

12 Significance of correlation is marked with asterisk (* $P < 0.05$, ** $P < 0.01$)

13

14

Table 3 All the PLFAs detected in treated soils

Number	Rt ^a (min)	PLFA	Number	Rt (min)	PLFA	Number	Rt(min)	PLFA
1	8.998	12:0	11	20.305	16:1ω5c	21	26.656	18:2ω6,9
2	12.944	i13:0	12	20.503	16:0	22	26.906	18:1ω9t
3	13.995	14:0	13	21.641	10Me16:0	23	27.356	18:1ω5c
4	15.914	i15:0	14	21.838	7Me17:0	24	27.866	18:1ω7c
5	16.152	a15:0	15	22.087	i17:0	25	29.458	18:0
6	17.085	15:0	16	22.540	a17:0	26	30.710	10Me18:0
7	18.909	i16:0	17	23.599	17:1ω7c	27	31.280	cy19:0
8	19.285	16:1ω9t	18	23.917	17:1ω8c	28	31.950	19:0 ^b
9	19.541	16:1ω7c	19	24.343	cy17:0	29	34.290	i20:0
10	19.886	16:1ω7t	20	26.321	17:0	30	35.751	20:0

15 ^aRt, Retention time; ^b19:0, Internal standard.

16

17 **Table 4** Correlation between abundances of PLFA subgroups and the removal extents of bioaccessible and

18 adsorbed pyrene in pyrene and pyrene-diesel polluted soils

Pollution	Pyrene fraction	TPLFAs	SSFAs	BSFAs	MUFAs	PUFAs	CFAs
Pyrene	Bioaccessible	0.818**	0.767**	0.695**	0.848**	0.855**	0.771**
	Adsorbed	0.605**	0.587**	0.469*	0.642**	0.666**	0.571**
Pyrene-	Bioaccessible	0.317	0.058	0.267	0.525**	0.368	-0.309
	Adsorbed	-0.083	0.026	-0.191	-0.149	-0.005	0.142

19 Significance of correlation is marked with asterisk (* $P < 0.05$, ** $P < 0.01$)

20

1 **Figures**

Fig. 1 Pyrene degradation and fractionation in pyrene (a) and pyrene-diesel (b) contaminated soils.

Fig. 2 Abundances of PLFA subgroups in pyrene and pyrene-diesel spiked soils (all the subgroups are expressed as nmol g⁻¹). TPLFA, total phospholipid fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BSFA, branched-chain saturated fatty acid; SSFA, straight-chain saturated fatty acid; CFA, cyclopropyl fatty acid. PY, pyrene spiked soils without root components; PYR, pyrene spiked soils with root components; PYD, pyrene-diesel spiked soils without root components; PYDR, pyrene-diesel spiked soils with root components.

Fig. 3 PCA figures of soil microbial community structure in pyrene spiked soils. (a) ecological niche of individual PLFA; (b) the whole microbial communities in soil samples. Symbols: ○ unamended soils (PY); ● amended soils (PYR)

Graphical Abstract