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Responses of *Scirpus triqueter*, soil enzymes and microbial community during phytoremediation of pyrene contaminated soil in simulated wetland

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ABSTRACT

The aim of this study was to determine the enhancement of *Scirpus triqueter* in the dissipation of pyrene and the interaction of pyrene with plant, soil enzymes and microbial community. The results indicated that the dissipation ratios of pyrene in the rhizospheric and non-rhizospheric soil were 64.65 ± 3.86% and 54.49 ± 2.74%, respectively, and were higher than that in the unplanted soil (42.60 ± 0.71%) at 80d after planting *S. triqueter*. The pyrene was toxic to *S. triqueter*, as evidenced by growth inhibition in height, diameter, shoot number and biomass during the planting period. The activities of dehydrogenase decreased significantly at the presence of pyrene in soils, and increased remarkably with the introduction of *S. triqueter*. It was found that the pyrene addition increased the ratios of fungal/total fatty acids and gram-positive/gram-negative, but the presence of *S. triqueter* decreased the ratios of gram-positive/gram-negative. A larger stress level was found in the pyrene treated soils without *S. triqueter*. The ratio of aerobic/anaerobic bacteria decreased with increasing pyrene concentration, but increased when *S. triqueter* was planted. The principal analysis of phospholipid fatty acid signatures revealed that microbial community structures in the rhizospheric and non-rhizospheric soil were similar, but different from those in the unplanted and control soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) derived from natural sources and human inputs occur widely in coastal environments and accumulate in sediment [1]. The risk of being exposed to PAHs dramatically increases as the natural balance is being disturbed [2]. So it is very urgent to remove them from contaminated soil. Among remediation techniques for PAH contaminated sites, phytoremediation has been recognized as one of the most promising methods owing to its economic and ecological benefits [3]. During phytoremediation, organic contaminants could be removed by plant uptake, plant accumulation and plant enhanced dissipation; however, plant enhanced dissipation was the main way [4]. Moreover, the phytoremediation efficiency are influenced by many factors, including root exudates, microbial community, soil structure, root architecture, water and nutrient conditions, and pollutant stress [5].

Huesemann et al. [6] found that the presence of eelgrass stimulated the microbial biodegradation of PAHs and PCBs in the rhizosphere by releasing root exudates, plant enzymes, or even oxygen. During the 80-day experiment, Lee et al. [7] found that the dissipation of PAHs was more in planted soil than that in unplanted soil, and there were more obvious effects of plants on pyrene dissipation than on phenanthrene dissipation. The role of plants in bioremediation may be the support of diverse microbial communities that contain higher numbers, or more efficient strains of bacteria with the required physiological capabilities [2]. White clover could also dissipate pyrene by plant-promoted microbial degradation, direct uptake and accumulation [8]. The most probable number of PAH degraders was influenced by planting regime [9] and the composition of rhizospheric bacterial communities was influenced by the plant species [10]. The effect of roots was species specific, some roots might be beneficial to the degradation of contaminants; however, the others have no effect or even hinder the degradation [11]. Competition for nutrients between plants and microorganisms may have impeded the microbial degradation of PAHs in the rhizosphere [12,13].

PAHs cause positive or negative effect on plant growth, soil enzymes and microbial community, thereafter affect the plant-enhanced dissipation. Su and Yang found the soil PAHs had little toxicity to the growth of rice seedlings at 1.02–1.42, 1.32–4.77 and 2.98–18.5 mg kg^{-1} of naphthalene, phenanthrene and pyrene respectively [14]. Reynoso-Cuevas et al. also believed that the germination was not affected by any assayed concentration; however, the length of the stems and roots decreased when hydrocarbon mixture increased and the survival of the four species also

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diminished [15]. Increasing lindane concentrations significantly reduced ($p < 0.05$) the dry weight of *Withania somnifera*, and this was most pronounced in plants grown in 20 μg g^{-1} of lindane [16]. The enzyme activity is a sensitive indicator of fertility, metabolic processes and matter circulation, as well as the process and contamination of soil environment [17]. The enzymes involved in the degradation of PAHs are oxygenase, dehydrogenase and lignolytic enzymes. A significant positive correlation was observed between the soil enzyme activities (dehydrogenase, polyphenol oxidase) and the removal ratio of pyrene [18]. The potential for intrinsic bioremediation of contaminants can be assessed by investigating the interplay between the contaminant concentrations and the microbial community's populations and distributions [19]. The concentration of phospholipid fatty acids (PLFAs) indicative of gram-negative bacteria increased in the PAH hot spots, whereas PLFAs representing fungi and gram-positive bacteria (including actinomycetes) were negatively correlated to the PAH concentrations [20].

Scirpus triqueter is a dominant species in wetland of Huangpu-Yangtze estuary. The use of native species for phytoremediation may be more ecologically beneficial and cost effective [21]. The wetlands also have specific macrophytes, microflora and microfauna which lead to a high rate of the autotrophic and heterotrophic processes [22]. Little is known about the effect of PAHs on the soil microbial activity and whether the effect is due to changes in the dominant species and/or the physiological activity of the microbial community [14]. The objectives of this study were: (1) to estimate the dissipation ratios of pyrene in simulated wetland in the presence of *S. triqueter*; (2) to ascertain the growth parameters of *S. triqueter* and the changes of the soil enzymes in different parts of the soils after pyrene treatment; (3) to investigate the response of the microbial communities to pyrene.

2. Materials and methods

2.1. Soil preparation

The soil (air-dried, 1 mm sieved) was collected from the east campus of Shanghai University and had no previous exposure to PAHs. The characteristics were: pH 8.3; organic matter, 38.6 g kg^{-1}; total nitrogen, 2.4 g kg^{-1}. Pyrene used in this experiment was purchased from Aladdin Reagent Incorporation with a purity of 98%. The pyrene was dissolved in ether to make the pyrene-ether stock solution (6400 mg kg^{-1}), which was added to the potted soil to produce a final concentration of 80 mg kg^{-1}. After the solvent was evaporated, the spiked soils were used to the pot experiment.

2.2. Experimental design

The treatments in this trial were as follows: uncontaminated soil without plant (C0); uncontaminated soil with plant; pyrene contaminated soil with the concentration of 80 mg kg^{-1} (C80); planted soil with the pyrene concentration of 80 mg kg^{-1} (rhizospheric soil, RS); pyrene contaminated soil without plants but receiving root exudates (non-rhizospheric soil, NS). The rhizo-bags (width 6 cm, and height 8 cm) made from nylon mesh (48 μm pore size) were used to distinguish the rhizospheric soils and non-rhizospheric soils. The rhizo-bags could prevent the translocation of *S. triqueter* roots while the exchange of water and root exudates was allowed. Each treatment was repeated for eight times, and the pots were randomized in experiment place. All treatments were irrigated with the running water every day and the depth of water was kept at 2–3 cm high at the surface of the soil. Soils and plants were destructively sampled after measuring the heights and diameters of *S. triqueter* every ten days. The plants were weighed to determine their fresh biomass. All the plants were stored at −20 °C and all soil samples were kept at −20 °C after freeze drying.

2.3. Dehydrogenase activity assays

The dehydrogenase (DHA) activity was determined by the transformation of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,2,5-triphenylformazan (TPF) [23]. A 2 ml aliquot of TTC-Tris buffer solution (1%) and 2 ml of 1% glucose solution were added to 4 g of soil in 50 ml glass flasks. For blank samples, no TTC solution was added. After 24 h incubation at 37 °C, the reaction product was extracted with methanol, and the absorbance was measured at 485 nm. DHA is reported as micrograms TPF per gram dry soil per day.

2.4. Analysis of soil pyrene

The method was reference to Sun et al. [13], and slightly modified. The soil sample (2 g) was ultrasonically extracted (45 kHz, 300 W) in 10 ml of 1:1 (v/v) mixture of dichloromethane and acetone for 30 min followed by 4000 × *g* centrifugation. This process was repeated two times. The solvent fractions were then evaporated and exchanged to 2 ml hexane, followed by filtration through silica gel with 5 ml of 1:1 (v/v) elution of hexane and dichloromethane. The samples were then evaporated and dissolved by hexane with a final volume of 1.0 ml.

The extract was stored in glass vials at 4 °C until analysis. The extracts were quantified using Agilent 7890 gas chromatography-5975mass spectrometry (7890GC-5975MS) equipped with a DB-5 capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium, delivered at 1 ml/min. The detector and injection port temperatures were 280 °C and 250 °C, respectively. The splitless injection volume of 1 μl was delivered by a 10 μl syringe. The initial oven temperature was maintained at 100 °C for 2 min, then increased at 12 °C/min to 300 °C, and maintained at 300 °C for 5 min. Full scan (m/z 50–550) mode was selected. The pyrene concentration was obtained by the external standard method. The dissipation ratios were defined according to the following formula:

$$\text{The dissipation ratios}\,(\%) = \frac{(\text{pyrene concentration})_{\text{initial soils}} - (\text{pyrene concentration})_{\text{experimental soils}}}{(\text{pyrene concentration})_{\text{initial soils}}} \times 100$$

2.5. Microbial assays

The extraction and analysis of the phospholipid fatty acid (PLFA) were performed according to previous studies [24,25], with slight modifications. Briefly, 3.0 g soil (freeze-dried) was placed in 15.8 ml mixture of citrate buffer solution, methanol, and chloroform (0.8:2.0:1.0, v/v). The sample was extracted ultrasonically and separated by centrifugation. The supernatant was transferred to a tube. The remainder was blended with 6.8 ml of the mixture, and then repeated the former process. Subsequently, 4.8 ml of citrate buffer solution and 6 ml of chloroform were then added to the combined supernatant and the mixture was left to separate overnight. The chloroform phase was reduced by rotary evaporation. Phospholipids were separated from the crude lipids by silica gel columns with methanol eluting. After mild, alkaline methanolysis of the phospholipids, the resulting fatty acid methyl esters (FAMEs), dissolved in hexane, were analyzed by GC–MS.

The fatty acids were quantified with methyl nonadecanoate and expressed as nmol/g^{-1}. Individual fatty acids were designated in

terms of the total number of C atoms: number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The suffixes *c-* and *t-* indicate *cis-* and *trans*-configurations. The suffixes *i-* and *a-* indicate *iso-* and *anteiso*-branching, respectively, and *cy-* indicates cyclopropane fatty acid. Hydroxy groups are indicated by OH. *Me-* refers to the position of the methyl group from the carboxyl end of the chain [26].

Analysis of fatty acid methyl esters was performed on Agilent 7890 gas chromatography-5975mass spectrometry (7890GC-5975MS), which was fitted with a manual injector. The compounds were separated on DB-5 (30 m × 0.25 mm × 0.25 μm) silica capillary column. Samples (1 μl) were injected in the splitless mode. The injector temperature was 280 °C, the ion-source temperature was 200 °C, and the detector temperature was 250 °C. Helium was used as the carrier gas at a velocity of 1 ml/min. The temperature program used was 80 °C for 2 min then increasing to 150 °C at 50 °C/min, held for 2 min and to 195 °C at 2.5 °C/min, held for 3 min, finally to 240 °C at 2.5 °C/min. The concentrations (mole percentage) of the individual fatty acids in water samples were determined from the peak areas of each fatty acid relative to the peak area of the internal standard. The scan mode was full at m/z 50–550.

2.6. Statistical analysis

Statistical procedures were carried out using the software packages SPSS version 13.0 for windows. One-way analysis of variance followed by Duncan's multiple range test is used to compare the mean values. A principal component analysis (PCA) of PLFA data was conducted using Canoco for windows 4.5.

3. Results and discussion

3.1. The growth parameters of S. triqueter

The growth parameters were measured every ten days to explore the growth ability of *S. triqueter* in pyrene contaminated soil. The height, diameter, shoot number and fresh weight of *S. triqueter* grown in the soil contaminated with pyrene are shown in Table 1. The results have shown that all the growth parameters displayed same responses to the presence of pyrene in the soil. The height, diameter, shoot number and fresh weight were significantly lower in the pyrene-treated soils than those in control soils throughout the experimental period. The decrease of growth parameters can be attributed to the negative effect of pyrene on the *S. triqueter*. The results have also revealed that the presence of pyrene might delay the growth process (see from the height, the height of control plant reached the highest value at 50d, while the height of plant in pyrene soil reached the highest value at 60d).

The reduction in plant biomass grown in PAHs polluted soil might result from the inherent toxicity of PAHs [4]. The possible toxic effect of the contaminant could alter root morphology, simultaneously, the alterations in the root morphology directly influence the water and nutrient uptake and thus affect plant growth [15]. In previous studies, the toxic effect of PAH on the growth of different species was found [5,27]. The study of Ma et al. [5] indicated that PAHs had a moderate negative effect size on the growth of roots ($d+ = -0.75$) and shoots ($d+ = -0.57$) in soils contaminated with the mixed PAHs, whereas small negative effects were found in the soils contaminated with single PAHs ($d+ = -0.27$ and −0.31 for roots and shoots, respectively). In another study, the *J. subsecundus* shoot numbers significantly decreased due to the PAH treatment after 35 days of growth [28].

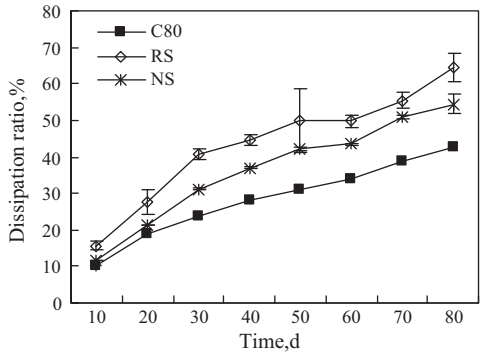

Fig. 1. Dissipation ratios of pyrene in different parts of soils during 80d. Error bars represent ±S.D ($n = 3$).

However, some reports suggested that PAHs had no significant effect on plant growth, which might be dependent on the concentrations used as well as the plant species. Zhang et al. [18] observed a slight but non-significant ($p < 0.05$) decrease in the roots and shoots dry weights in the pyrene spiked soils compared with that for the un-spiked soils. Su and Yang [14] suggested that the soil pyrene seemed to have little toxicity to the growth of rice seedlings at the concentrations of 6.6, 13.4, 20.0 and 26.6 mg kg^{-1}. In the present study, *S. triqueter* tends to decrease the growth (including height, diameter, shoot number and biomass) in the relatively high concentration of 80 mg kg^{-1}. Furthermore, in wetland pyrene dissolved in water under the action of the exudates of root and microbe, it was easy to be absorbed by *S. triqueter*. So the toxicity of pyrene increased in wetland.

3.2. Dissipation ratio of pyrene in soil

Pyrene in both the planted (including rhizospheric and non-rhizospheric soil) and unplanted soil was greatly decreased at the end of the 80-day experiment, accounting for 57.4 ± 0.71% of the initial extractable concentration in the unplanted (control) soil 45.5 ± 2.74% in the non-rhizospheric soil and 35.4 ± 3.86% in the rhizospheric soil. As shown in Fig. 1, the dissipation ratio of pyrene in the rhizospheric soil was the highest among the three parts of

Table 1
Growth response of *Scirpus triqueter* in pyrene spiked soils (means ± S.E).

Treatment time (d)	Height (cm)		Diameter (mm)		Shoot number		Fresh weight	
	Control	80 mg kg^{-1}	Control	80 mg kg^{-1}	Control	80 mg kg^{-1}	Control	80 mg kg^{-1}
10	10.6 ± 1.3	9.3 ± 1.1	1.22 ± 0.07	1.21 ± 0.11	4.7 ± 0.6	3.0 ± 1.0*	–	4.3 ± 0.2
20	15.3 ± 3.2	9.9 ± 2.0	1.80 ± 0.09	1.22 ± 0.08**	9.7 ± 1.5	5.3 ± 0.6**	–	6.2 ± 1.3
30	30.8 ± 0.7	20.9 ± 3.6*	1.67 ± 0.21	1.58 ± 0.12	15.7 ± 1.5	7.3 ± 0.6**	18.3 ± 0.9	9.2 ± 1.3**
40	41.8 ± 1.3	29.2 ± 0.1**	2.21 ± 0.12	1.57 ± 0.04**	15.7 ± 0.6	9.3 ± 1.2**	–	17.7 ± 1.5
50	47.5 ± 2.4	35.4 ± 0.3**	2.23 ± 0.13	1.66 ± 0.11**	18.7 ± 1.2	10.7 ± 1.2**	–	18.0 ± 1.0
60	38.0 ± 2.8	38.2 ± 1.1	2.34 ± 0.07	1.87 ± 0.04**	19.3 ± 1.2	11.7 ± 0.6**	44.6 ± 0.6	31.1 ± 1.0**
70	37.2 ± 0.6	31.0 ± 1.8**	2.21 ± 0.09	1.86 ± 0.01**	24.3 ± 0.6	17.7 ± 1.5**	–	23.2 ± 2.3
80	36.9 ± 1.8	27.7 ± 1.6**	2.17 ± 0.05	1.87 ± 0.11*	30.0 ± 4.0	19.3 ± 0.6**	44.4 ± 1.5	25.2 ± 4.6**

* Means are significantly different at the 0.1 level comparing with control.
** Means are significantly different at the 0.05 level comparing with control.

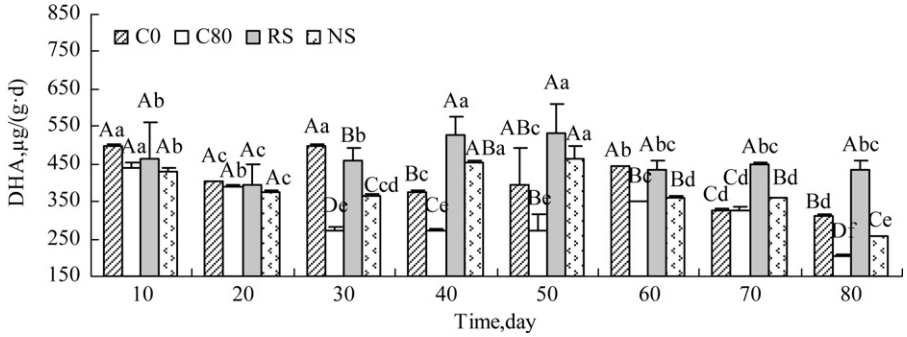

Fig. 2. Dehydrogenase activity in different parts of soils during 80d. Error bars represent ±S.D. ($n = 3$). Different small letters indicate significant differences between groups ($P < 0.05$). Different capital letters indicate significant differences within groups ($P < 0.05$).

soils. In addition, the non-rhizospheric soil had a higher dissipation ratio comparing with the control soil.

The fates of PAHs in the spiked soil include volatilization, leaching, photo-degradation (contaminated at the surface), plant uptake, biodegradation, and other abiotic losses [29]. The phytoremediation of organics is primarily based on the beneficial effects of roots on biodegradation [18]. The plant species vary widely in their efficiency of PAH dissipation [5]. The presence of *S. triqueter* increased significantly the pyrene degradations in the soil, which confirmed the beneficial effect of vegetation. A positive effect of the root exudates on the dissipation of pyrene ($p < 0.05$) was also found in this study (Fig. 1). However, Sun et al. [13] demonstrated the dissipation of pyrene was higher in the non-rhizospheric soil than that in the rhizospheric soil. The rhizospheric effects are influenced by many factors, including root exudates, microbial communities, soil structure, root architecture, water and nutrient conditions, and pollutant stress [5]. Sun et al. [13] believed the competition between the plant roots and the soil microbes for soil nutrients and the suberization should be responsible for the lower dissipation ratio of the planted soil. In this study, the contents of organic matter, total nitrogen, phosphorus, and potassium were 2667 ± 236, 255 ± 30, 11.1 ± 0.2, $107 \pm 18\ mg\ kg^{-1}$, respectively. The soil nutrients were sufficient for the growth of microbes and plant during the whole experimental period. So no competition occurred between the plant and microbes for nutrients.

3.3. The response of dehydrogenase activity

Fig. 2 demonstrated the activities of the dehydrogenase in different parts of the soils. There was no significant difference in the dehydrogenase activity among the treatments during the first 20 days of plant growth. After 30-day treatment, the dehydrogenase activities in the rhizospheric soil were significantly higher than that in C80 and the non-rhizospheric soil. The highest dehydrogenase activities in the rhizospheric soils were observed at 40 and 50-day treatment. The similar trend was seen in the non-rhizospheric soils. And the dehydrogenase activities in C80 soils were significantly lower than those in the other treatments during the whole treatment time except for 10 and 20-day.

The dehydrogenase is an important oxidoreductase in soils, which is the catalyst for important metabolic processes, including the decomposition of organic inputs and the detoxification of xenobiotics [30]. Soil dehydrogenase activities are quite sensitive to the pollution [18]. Cheema et al. [4] indicated that the presence of PAHs could stimulate the dehydrogenase activity in the rhizosphere of plant treatments. In this study, the dehydrogenase activity in the rhizospheric and non-rhizospheric soil was higher than that in the C80 soil, which should be attributed to the active and effective carbon source supplemented by the root exudates. The results could not confirm the views of Cheema et al., on the contrary, they exhibited a negative effect of pyrene on soil dehydrogenase (see C80 in Fig. 2). The increment of the dehydrogenase activity in the rhizospheric soils was the role of *S. triqueter*. So the acceptable explanation might be planting stimulated dehydrogenase activity [31]. The dehydrogenase activity assays in soil have often been used to obtain correlative information on the biological activity of microbial populations in soil, i.e., as an index of total microbial activity [32]. The higher dehydrogenase activity in the PAHs contaminated soil might be attributed to the increased microbial activity as a result of enhanced root exudation of plants [4].

3.4. Signature microbial community changes during cultivation

Fig. 3 shows the chromatogram obtained from the pyrene contaminated soil sample in a full scan mode. At least twenty different

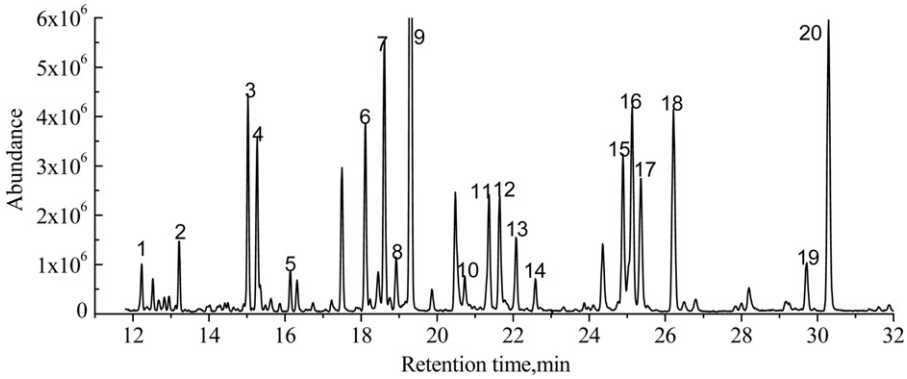

Fig. 3. Chromatogram obtained in full-scan mode from soil samples. 1, 2Me14:0; 2,14:0; 3, 2Me15:0; 4, 6Me15:0; 5, 15:0; 6, 2Me16:0; 7, 16:1ω7c; 8, 16:1ω7c; 9, 16:0; 10, 7Me17:0; 11, 2Me17:0; 12, 3Me17:0; 13, 7,8cy17:0; 14, 17:0; 15, 18:2ω6c,9c; 16, 18:1ω9t; 17, 18:1ω7t; 18, 18:0; 19, 9,10cy19:0; 20, 19:0 (internal standard).

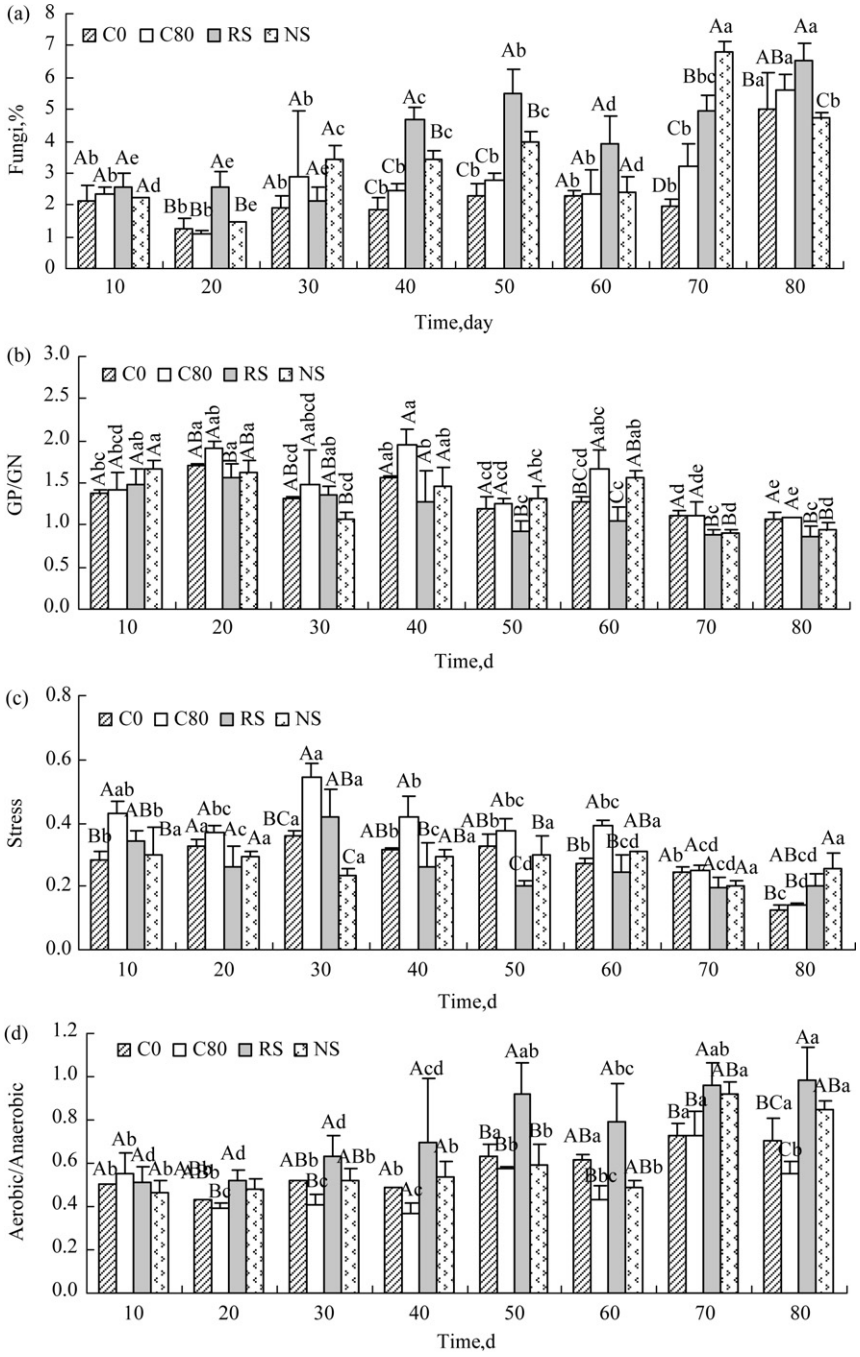

Fig. 4. Fungi, GP/GN, stress level and aerobic/anaerobic of different parts of soils at different incubation times. (A) Fungi; (B) GP/GN; (C) stress level; (D) aerobic/anaerobic. Different small letters indicate significant differences between groups ($P < 0.05$). Different capital letters indicate significant differences within groups ($P < 0.05$).

fatty acids were distinguished, including the internal standard peak. All the soils contained a variety of PLFAs of saturated, unsaturated, methyl-branched, cyclopropyl, and polyunsaturated fatty acids. The change of PLFAs is indicative of a stressful environment for the soil microorganisms. The pattern of twenty PLFAs with chain length from C14 to C20 varied in response to different treatments. The ubiquitous fatty acid C16:0 showed the highest abundance in all soil samples. The detailed characterization of the soil microbial community on the basis of the results from PLFA analysis was shown in Fig. 4.

The major shifts in the microbial community during pyrene treatment could be ascertained using the percentage of fungi, gram-positive to gram-negative bacteria (GP/GN), stress level and the ratios of aerobic/anaerobic. Fig. 4a shows the temporal profile of fungi to total PLFAs ratios for all experimental conditions investigated. The ratios of fungi/total PLFAs in the rhizosphere and nonrhizosphere showed an ascending trend at first and then descended during the initial 60d cultivation period. However, there was no significant difference ($p < 0.05$) in the C0 soils during the initial 70d cultivation period. And the ratio of fungi was extremely high in different parts of the soils at 70 and 80-day cultivation. The ratios of fungi/total PLFAs in C80, rhizospheric and non-rhizospheric soils were higher than that in C0 soil in nearly the whole cultivation period. Simultaneously, the content of fungi

was significantly higher in the rhizospheric soil than that in C80 soil except for 30 and 70-day cultivation.

Fig. 4b shows the temporal profile of the Gram-positive to Gram-negative bacterial ratios at all experimental conditions under investigation. The ratio of GP/GN decreased in the mass during the whole cultivation period especially to the rhizospheric and non-rhizospheric soils. Meanwhile, the ratios of GP/GN were significantly higher in C80 soils than those in C0 soils. And the ratios in the rhizospheric soils were the lowest comparing with those in the other parts of soils.

As shown in Fig. 4c, the stress level of microbes in the unplanted soils tended to be higher in the pyrene treatments (C80) than that in the control samples (C0) during the initial 60d of cultivation. The rhizospheric and non-rhizospheric soils had lower stress level comparing with the C80 soils, while the stress level in the rhizospheric soils was lower than those in the non-rhizospheric soils. The descending trends of the stress level were seen in all parts of soils with cultivation time increasing.

The ratios of aerobic/anaerobic bacteria, characterized by the sum of the monounsaturated fatty acids to total branched, saturated fatty acids reduced markedly in the C80 soils comparing with those in C0 soils (Fig. 4d). However, it appeared that the ratios of aerobic/anaerobic bacteria showed an obvious increase in the rhizospheric and non-rhizospheric soils comparing with those in the C80 soils. Meanwhile, the ratios of aerobic/anaerobic bacteria in the rhizospheric soils were higher than those in the non-rhizospheric soils.

PLFA profiles can provide insight into the microbial community structure because of a relative abundance of certain PLFAs which differ considerably among specific groups of microorganisms [33]. Different bacterial strains in soil could be stimulated by both PAHs and vegetation [34]. The ratios of fungi/total PLFAs, GP/GN, cyc/precursor (stress level) and aerobic/anaerobic bacteria are considered to be important indicators for the soil microbial structure. The unsaturated PLFA 18:2ω6,9 was used as an indicator of fungal biomass [35]. The relatively low amounts of 18:2ω6,9 suggested that they were sparsely distributed in the different parts of soils treated in this experiment. Pyrene spiked soil was rich in fungi compared with the unspiked soil. The content of fungi was higher in the planted soil than that in the unplanted soil. Phytoremediation is a complex process in which the soil microbiota (fungi and bacteria) interacts with the plant favouring the degradation of pollutants. Fungi are known for their degradation activity of aromatic pollutants and they could have an important role in the bioremediation process, in particular in the case of soils polluted with heavy molecular weight PAHs as pyrene. *S. triqueter* could stimulate the growth of fungi. Zhang et al. [36] showed that fungi present in the agricultural soils were especially sensitive to the herbicide used in their study. The branched phospholipids i14:0, i15:0, a15:0, i16:0 and i17:0 were used as indicators of GP bacteria, which seemed to be sensitive to the PAH toxicity according to the study of Su and Yang [14]. GN bacteria, characterized by the branched phospholipids are known to increase when easily utilizable carbon source is available [37]. The results in the present study also indicated that GP bacteria were more sensitive to the pyrene toxicity and GN bacteria were more sensitive to the utilizable carbon source provided by the plant. The stress indicators were calculated based on the ratios of the relative abundance of cyclopropyl fatty acids to their monoenoic precursors [38]. The stress level of the microflora was affected by the soil properties, treatments and the incubation time [36]. A larger stress level was found in the pyrene contaminated but unplanted treatment groups. *S. triqueter* could alleviate this effect of stress, in other words, the plant was benefit to the monounsaturated fatty acids. Besides, the stress level decreased with increasing the treatment time. The ratios of monounsaturated fatty acids to branched fatty acids are

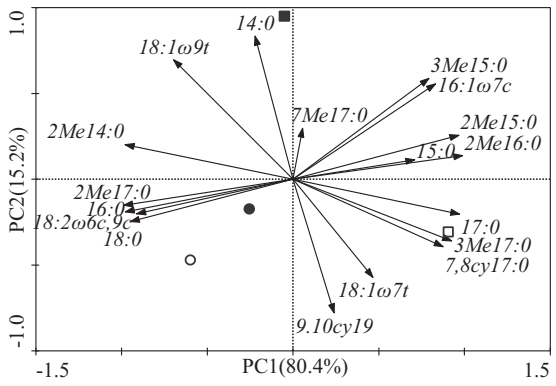

Fig. 5. Principle component analysis of soil microbial community composition based on relative concentrations of individual PLFA molecules. Symbols: □: C0; ■: C80; ◯: RS; ●: NS.

subjected to relative abundance of aerobic to anaerobic microbes [39]. The findings of Yang and Su [34] suggested that the anaerobic bacteria were more tolerant to the PAH toxicity than the aerobic bacteria, while the presence of plants could alleviate the PAH toxicity to aerobic bacteria by supplying oxygen to improve their activity. The results of this study suggested that pyrene reduced the relative abundance of aerobic to anaerobic bacteria. However, *S. triqueter* enhanced the relative abundance of aerobic to anaerobic bacteria. Wetlands have perfect anaerobic conditions, they provide ideal conditions for anaerobic bacteria. So the anaerobic bacteria dominate the soil's microbial community, as shown in Fig. 4d. The growth of *S. triqueter* could supply oxygen to soil, which could promote the growth of aerobic. Consequently, pyrene had more deleterious effects on aerobic bacteria than anaerobic bacteria, while *S. triqueter* benefited to the propagation of aerobic bacteria.

3.5. Microbial community structure reflected by PLFAs patterns

PCA was performed to analyze the changes of the soil microbial community treated with pyrene and plant. The first principal component (PC1) of the principal component analysis of the PLFA data could explain 80.4% of the total variation in the PLFA pattern and the second (PC2) for 15.2% (Fig. 5). Most of branched fatty acids (2Me15:0, 6Me15:0, 2Me16:0, 7Me17:0, and 2Me17:0), some saturated fatty acids (15:0, 16:0, and 18:0) and polyunsaturated fatty acid (18:2ω6c,9c) correlated more with PC1, while cyclopropyl fatty acid (cy17:0, cy19:0), some saturated fatty acids (14:0, 17:0) and monounsaturated fatty acids (18:1ω9t, 18:1ω7t) correlated more with PC2. The result indicated that gram-positive bacteria (represented by branched fatty acids) and fungi (18:2ω6c,9c) were easy to change when added pyrene to soil or planted *S. triqueter*. The microbial communities of the C0 soil without pyrene were clearly separated along the first axis from the C80 soil with pyrene. The abundances of gram-positive bacteria increased when pyrene were added to the soil. The presence of *S. triqueter* can enhance the abundances of bacteria (represented by 16:0), fungi, some gram-positive bacteria (2Me14:0, 2Me17:0) and some gram-negative bacteria (18:1ω9t) in rhizospheric and non-rhizospheric soils, as revealed by the PCA plots.

4. Conclusions

The results indicated that the dissipation of pyrene was enhanced by the presence of *S. triqueter*. It is conceivable that root exudates of *S. triqueter* had a beneficial effect on soil biota and enzyme activity, thereby improving the pyrene degradation. And the considerable diminution in plant growth subjected to the toxic

effect of pyrene, while *S. triqueter* could alleviate the toxic effect. This study provides insight into microbial biomass and community structure in rhizospheric and non-rhizospheric soils by means of phospholipid ester-linked fatty acid biomarkers. The introduction of *S. triqueter* was benefit to fungi, gram-negative bacteria and aerobic bacteria, furthermore, it could alleviate the stress of pyrene. It was evident that the wetland system was dominated by anaerobic bacteria. Additionally, there was discrimination in microbial community structure under the stress of pyrene or the benefit of *S. triqueter*. Nevertheless, the introduction of *S. triqueter* had alleviated the toxicity of pyrene and enhanced the dissipation ratio of pyrene. Soils at contaminated sited generally contain complex mixtures of chemicals and are often not readily bioavailable, so greenhouse experiments with soils collected from contaminated sites should be performed before implementing a field-level remediation [40]. In order to improve the bioavailability of contaminant in historically contaminated soil, surfactants or biosurfactants may be added to the soil.

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