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CONTRASTIVE SOIL PROPERTIES, MICROBIAL STRUCTURE AND SOIL ENZYMES IN THE RHIZOSPHERE OF *Scirpus triqueter* AND BULK SOIL IN PETROLEUM-CONTAMINATED WETLAND

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Abstract

Though *Scirpus triqueter* plays an important role in protecting the ecological functions of Yangtze River estuary wetland which is frequently contaminated by petroleum, knowledge is limited about the mechanisms involved. In this study, comparative analysis of soil properties, microbial community structure and soil enzymes in the rhizosphere of *S. triqueter* and bulk soil was conducted throughout one year to provide an insight into the phytoremediation mechanism in the field. The total petroleum hydrocarbon content and soil pH in the rhizosphere were averagely 0.06 g kg^{-1} and 0.1 lower, respectively, than in the bulk soil. Analysis of phospholipid fatty acids shew the microbial community structure was quite different from that in the bulk soil with much higher activity and diversity. The relative abundance of Gram-positive to Gram-negative bacteria was 0.12 lower in the rhizosphere, while the relative abundance of aerobes to anaerobes was 0.74 higher, than in the bulk soil. Positively correlated with the relative abundance of fungi to bacteria and microbial diversity, the activities of dehydrogenase, catalase, polyphenol oxidase were significantly lower ($P < 0.05$) in the bulk soil than in the rhizosphere. Redundancy analysis and Pearson correlation shew that soil organic matter, total nitrogen, total phosphorus, as well as temperature were crucial factors influencing soil microbial communities and enzymes which were responsible for the degradation of petroleum hydrocarbons. These results would improve our understanding of the phytoremediation processes in fragile contaminated wetland and supply valuable information for its application in the restoration of damaged ecosystems.

Key words: microbial community structure, petroleum pollution, phytoremediation, *Scirpus triqueter*, soil enzymes

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1. Introduction

The Huangpu-Yangtze River estuary (HYRE) wetland is the most valuable natural estuarine wetland in Shanghai, China, which plays an important role in maintaining the ecological environment. It is also an ideal model to study the impact of human activities on estuarine wetland. However, HYRE wetland is suffering from frequent petroleum contamination in recent years. It is estimated that more than 760 tons of petroleum from oil leakage has intruded into the HYRE wetland in the last few years (Liu et al., 2010b). Fortunately, *Scirpus triqueter*, the dominant plant in HYRE wetland, helps greatly to protect, maintain and restore the wetland ecosystem through phytoremediation (Liu et al., 2011; Zhang et al., 2011). Though the petroleum-degrading ability of *S. triqueter* has been studied in greenhouse experiments, no field study is conducted till now. The situation is much more complicated in the field, the extremely intricate interactions among plants, environmental factors (such as weather, soil properties and tidewater) and pollution may lead to discriminating results. Therefore it is necessary to carry out field study in HYRE wetland to make up for the current inadequacy of scientific data.

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Soil microorganisms play an important role in the degradation of organic contaminants (Ciancio et al., 2016; Gerhardt et al., 2009). Liu et al. (2010a) found that 58.2% of the petroleum hydrocarbons were degraded by indigenous microbes after one year of bioremediation in a pot experiment. It is thought that plants can enhance the pollutant-removal ability of soil microbes through the so called rhizosphere effect (Liu et al., 2010a; Mihalache et al., 2016). Sun et al. (2010) proved that root-exudates enhanced the biodegradation of phenanthrene and pyrene by 15.5% and 21.3%, respectively, in a low organic matter soil. Meng and Zhu (2010) found that components from celery roots increased the removal of pyrene by around 22% in an industry soil, and lipophilic components enhanced the relative abundances of pyrene-degraders. In addition, the influences of plants on soil microorganisms are plant-dependent (Yao and Wu, 2010), which may lead to discrepant phytoremediation efficiencies (Wei et al., 2013). As a culture-independent approach to explore the fingerprints of soil microbes, phospholipid fatty acid (PLFA) model is a preferable method to monitor the complex soil microbial community structure (Cowie et al., 2010; Frostegård et al., 2011). Therefore, PLFA model was used to analyze the relative abundance of certain microflora, including bacteria (B), fungi (F), Gram-positive bacteria (GP) and Gram-negative bacteria (GN), aerobes (AE) and anaerobes (AN), in this study.

Soil enzymes, deriving primarily from soil microorganisms, plant roots, plant and animal residuals, are sensitive indicators for the soil function and environmental strain in affected ecosystems (Zhou et al., 2011). Their activities vary widely depending on the enzyme species, soil types, environmental factors, plants, as well as pollutants (Wang et al., 2009). Wyszkowska and Wyszkowski (2010) found that petroleum contamination inhibited the activities of soil dehydrogenases and urease to a large degree in spring rape and oat soils. Considering that soil enzymes can serve to measure the health and sustainability of disturbed ecosystems (Boerner et al., 2005), activities of important soil oxidoreductases and hydrolases, as well as the relationship between soil microbial communities and enzymes, were measured to probe into the influences of *S. triqueter* on the soil function in this study.

This study was carried out in HYRE wetland with the objectives: (1) to explore the differences of soil properties, microbial community structure and soil enzymes in the rhizosphere and bulk soil; (2) to monitor seasonal fluctuation of microbial communities and soil enzymes throughout one year; (3) to investigate the interactions between soil properties, soil microbial communities and soil enzymes during phytoremediation of petroleum polluted wetland.

2. Materials and methods

2.1. Field sampling

Sampling sites were set in the HYRE wetland, the north of Shanghai, China. Characterized by the north semi-tropical monsoon climate, the annual precipitation is 1022 mm and approximately 70 percent falls between May and September. The annual mean temperature is around 15.3ºC according to Shanghai Meteorological Center. Almost 80 percent of the mesolittoral zone in HYRE wetland is colonized by *S. triqueter*, a kind of perennial herb, whose growing period is from April to November.

In this study, rhizosphere and bulk soil samples were collected every month from March, 2011 to February, 2012. Rhizosphere soil samples (SS) were randomly collected from the root surface of *S. triqueter* at the depth of 0-20 cm and bulk soil samples (BS) were collected at the same depth, but at least 1 m away from the nearest plants (Toyama et al., 2011). All soil samples were processed within 6 h according to previous studies with some modifications (Nie et al., 2009). Plant roots and stones were hand-picked out. Soils were freeze-dried, then passed through 1 mm mesh to get homogenized. All the samples were divided into two parts: one was stored at 4ºC for the analysis of soil properties and soil enzymes as mentioned previously (Wei et al., 2013); the other part was stored at -80ºC to avoid the degradation of PLFAs and used for soil microorganism analysis.

2.2. Soil properties

Total petroleum hydrocarbon (TPH) was ultrasonically extracted from 5 g of treated soil samples with dichloromethane, then the content was determined through gravimetric analysis as described in previous studies (Liu et al., 2011; Mishra et al., 2001; Zhang et al., 2013). Values of pH and total dissolved solid (TDS) were determined by HI255 pH-TDS-conductivity-salinity meter after mixing the soil with deionized water (1:10, w/w); total nitrogen (TN) content was analyzed by Kjeldahl apparatus (Buchi Distillation Unit B-324); soil temperature-moisture meter (LZB-I) was used for the measurement of the soil temperature (T); total phosphorus (TP) and organic matter (OM) were determined by colorimetric and volumetry method, respectively (Rukun, 1999).

2.3. Soil microbial communities

The activity of fluorescein diacetate hydrolase (FDA), expressed in A_{490} g^{-1} dry soil 2 h^{-1}, was used to reflect the total activity of soil microorganisms (Green et al., 2006; Wei et al., 2013). To measure the FDA activity, 1 g of treated soil was mixed with 30 mL of phosphate buffer (pH 7.6) and 1 mL of fluorescein diacetate solution (2 g mL^{-1}, dissolved in acetone), then the mixture was incubated in the dark at 30ºC for 2 h. After centrifugal separation, the absorbance at 490 nm was detected.

Microbial community structure was analyzed using PLFA method. In general, crude fatty acids were extracted from 3 g of soil by a mixture of citrate buffer, methanol, and chloroform (Frostegård et al., 1993; Li

et al., 2010). Then the crude fatty acids were passed through silica gel columns with methanol as the eluent to obtain phospholipids. After that, phospholipids were methylated and quantified using GC-MS (Li et al., 2010). PLFAs were named according to the nomenclature described in previous study (Cao et al., 2012). Shannon index *H* was used to evaluate the diversity of microbial communities and calculated by Eq. (1):

$$H = -\sum_{i=1}^{R} pi \ln pi \quad (1)$$

where *H* represented the value of Shannon index, *pi* was the relative abundance of each detected PLFA, *R* was the total number of detected PLFAs (Yao and Wu, 2010; Zornoza et al., 2009)

2.4. Soil enzymes

Activities of three oxidoreductases [dehydrogenase (DHA, EC 1.1), catalase (CAT, EC 1.11.1.6) and polyphenoloxidase (PPO, EC 1.10.3.1)] and three hydrolases [sucrase (SUC, EC 3.2.1.26), urease (URE, EC 3.5.1.5) and alkaline phosphatase (PHOS, EC 3.1.3.1)] were measured.

URE activity, expressed as mg NH4-N g^{-1} soil 24 h^{-1}, was measured according to the hydrolysis rate of carbamide. SUC activity, expressed as mg glucose g^{-1} soil 24 h^{-1}, was determined by incubating treated soil with sucrose solution (Schinner and Mersi, 1990). PHOS (mg phenol g^{-1} soil 3 h^{-1}), DHA (mg TF g^{-1} soil 48 h^{-1}) and PPO (mg purpurogallin g^{-1} soil 2 h^{-1}) were measured following the methods previously described (Guan, 1986; Tang et al., 2010; Wyszkowska and Wyszkowski, 2010). Hydrogen peroxide was used as the substrate for CAT determination, and the unit of its activity was mL 0.1 M $KMnO_4$ g^{-1} soil 30 min^{-1} (Johnson and Temple, 1964).

2.5. Statistical analysis

Each data was the mean value (±SD) of three replicates. SPSS 17.0 was used for paired *t* test to examine the differences of rhizosphere and bulk soils in data sets of soil properties, soil microorganisms and soil enzymes. Principal component analysis (PCA) was used to compare microbial community structure in the rhizosphere and bulk soil. Redundancy analysis (RDA) performed by Canoco for windows 4.5 was used to analyze the microbial characteristics across soil property gradients, as well as soil enzymes across microbial population gradients. Pearson correlation matrix was used to assess the covariance between soil properties, microbial communities and enzyme activities.

3. Results and discussion

3.1. Soil properties

Paired *t*-test was conducted to compare the soil characteristics in the rhizosphere and bulk soil (Table 1). TPH content in the rhizosphere was significantly lower ($P < 0.05$) than in the bulk soil, indicating that *S. triqueter* can greatly enhance the degradation of petroleum hydrocarbons. In addition, the pH value (7.3 ± 0.1) in the bulk soil was significantly higher than that (7.2 ± 0.0) in the rhizosphere ($P < 0.001$), while the OM content of 75.03 ± 18.21 g kg^{-1} in the rhizosphere of *S. triqueter* was slightly higher than that of 61.28 ± 17.07 g kg^{-1} in unplanted soil. By contrast, differences of soil temperature, TN, TP and TDS contents, between the two types of soil were less remarkable.

Organic acids released by plants, including citric acid, malic acid, oxalic acid and so on, may contribute to the decrease of soil pH. It is thought that petroleum hydrocarbons can affect increase soil pH, deteriorate soil function and decrease soil permeability.

On the other hand, plants can improve soil properties through releasing ample carbon sources and mineral nutrients into the soil in the form of root exudate, mucilage, colloidal and so on (Bertin et al., 2003; Hinsinger, 2001; Richardson et al., 2009). Therefore different soil properties between the rhizosphere and bulk soil were the combined results by petroleum hydrocarbons and plants.

3.2. Soil microbial communities

A total of 23 PLFAs were detected, most of which had 15-18 carbon in their molecules. PLFAs i12:0, a13:0, 13:0, i14:0, i15:0, 15:0, a15:0, i16:0, 16:1ω7c, 16:1ω7t, i17:0, a17:0, 17:0, 18:0, cy17:0, 18:1ω7c and cy19:0 were used to indicate bacterial biomass; 16:1ω5t, 18:1ω9t, 18:1ω9c, 18:2ω6,9c were used as fungal biomarkers; 16:1ω7c, 16:1ω7t, 18:1ω7c, cy17:0, cy19:0 were chosen to represent GN; branched saturated fatty acids i14:0, i15:0, i16:0, i17:0, a15:0 and a17:0 indicated GP; 14:0 and 16:0 were common PLFAs in most microorganisms (Frostegård et al., 1993; Velasco et al., 2010; Zhang et al., 2010). The ratio of monounsaturated fatty acids to branched saturated fatty acids was used to indicate the relative abundance of aerobic to anaerobic microbes (AE/AN) (Li et al., 2010; Su and Yang, 2009).

PCA plot was used to compare the microbial community structure in the rhizosphere and bulk soil (Fig.1). The first principal component accounted for 75.4% of the total variance and the second explained 17.3%. Samples in the rhizosphere were completely separated with those in the bulk soil as illustrated in the PCA plot, therefore their microbial structures were quite different from each other. Microbial communities in the rhizosphere were characterized by monounsaturated fatty acids, such as 18:1ω7c, 16:1ω5t, 16:1ω7t and 16:1ω7c, with higher diversity and activity. By contrast, the relative abundances of saturated fatty acids were much higher in the bulk soil, resulting in the higher GP/GN.

Paired *t*-test analysis was used to compare the microbial community structure in the bulk and rhizosphere soil (Table 2).

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Table 1. Comparison of soil properties in the bulk and rhizosphere soil through paired *t*-test

	Bulk	***Rhizosphere***	***Difference***	***t***	***P***
T（°C）	8.3 ± 7.0	8.2 ± 7.0	0.1 ± 0.2	1.129	0.283
TN（g kg^{-1}）	1.01 ± 0.18	1.07 ± 0.19	0.06 ± 0.13	-1.642	0.129
TP（g kg^{-1}）	0.11 ± 0.02	0.11 ± 0.01	0.00 ± 0.01	1.687	0.120
OM（g kg^{-1}）	61.3 ± 17.1	75.0 ± 18.2	13.8 ± 24.7	-1.931	0.080
pH	7.3 ± 0.1	7.2 ± 0.0	0.1 ± 0.1	6.934	0.000
TPH（mg kg^{-1}）	712 ± 178	470 ± 244	241 ± 221	3.781	0.003
TDS（mg L^{-1}）	113 ± 55	91 ± 33	21 ± 45	1.601	0.138

T, soil temperature; TN, total nitrogen; TP, total phosphorus; OM, organic matter; TPH, total petroleum hydrocarbon; TDS, total dissolved solid

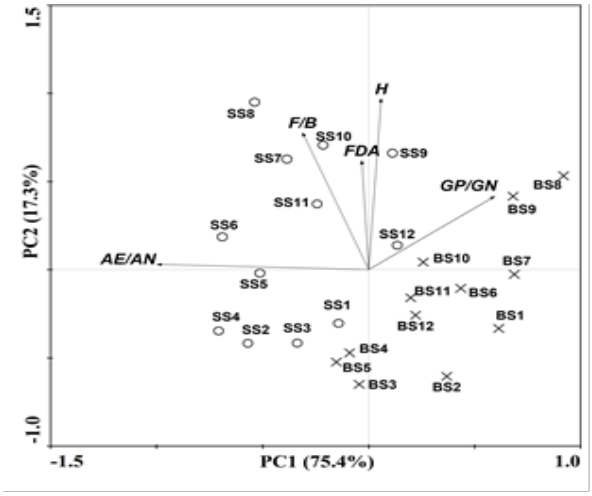

Fig. 1. PCA plot of microbial communities in the rhizosphere and bulk soil (FDA, fluorescein diacetate hydrolase; AE/AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index. BS, bulk soil; SS, soil in the rhizosphere of S. triqueter, digitals reflect the sampling month)

Table 2. Comparison of soil microbial structure in the bulk and rhizosphere soil by paired *t*-test

	Bulk	***Rhizosphere***	***Difference***	***t***	***P***
FDA (abs g^{-1})	0.22 ± 0.10	0.29 ± 0.14	0.45 ± 0.32	-2.352	0.038
AE/AN	1.89 ± 0.43	2.63 ± 0.33	0.07 ± 0.10	-4.996	0.000
GP/GN	0.62 ± 0.18	0.50 ± 0.14	0.74 ± 0.51	4.017	0.002
F/B	0.03 ± 0.03	0.12 ± 0.09	0.12 ± 0.10	-4.267	0.001
H	1.94 ± 0.20	2.10 ± 0.25	0.16 ± 0.13	-4.203	0.001

FDA, fluorescein diacetate hydrolase; AE/ AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index

FDA activity was significantly higher ($P < 0.05$) in the rhizosphere (0.29 ± 0.14 abs g^{-1}) than that in bulk soil (0.22 ± 0.10 abs g^{-1}), which indicated the soil microbes were more active in the rhizosphere than in the bulk soil. The activity of soil microorganisms was thought to be largely related with petroleum biodegradation (Kirk et al., 2005; Liu et al., 2011).

The relative abundance of AE to AN was distinctly higher ($P < 0.001$) in the rhizosphere than in bulk soil (Table 2), and this disparity reached to the summit in August (Fig.2A). Similarly, the relative abundance of fungi to bacteria in the rhizosphere was 3-4 times higher than in the bulk soil (Table 2), and the biggest difference appeared in October (Fig.2B). However, the relative abundance of GP to GN decreased by about 20% in the rhizosphere compared with that in bulk soil (Table 2) during the whole vegetative period of *S. Triqueter* (Fig.2C). In addition, the average value of *H* was 2.10 ± 0.25 in the rhizosphere, while 1.94 ± 0.20 in the bulk soil, reflecting that soil microbes in the rhizosphere were more diverse during the whole year (Table 2, Fig.2D).

The lower TPH content and moderate pH (Table 1) in the rhizosphere, as well as various organic carbons released by plant roots, provided a better habitat for soil microbes and accounted for the higher activity and diversity of microbial communities in the rhizosphere (Table 2). It was believed that the microbial community structure was plant-dependent in the rhizosphere (Smalla et al., 2001). For example, soil microbial populations were dominated by cy17:0, 16:1ω5c and 18:1ω5c in the rhizosphere of *V. tricolor*, while by 18:1ω7c, 18:0 and 10Me18:0 in the rhizosphere of *C. comosum* (Wei et al., 2013).

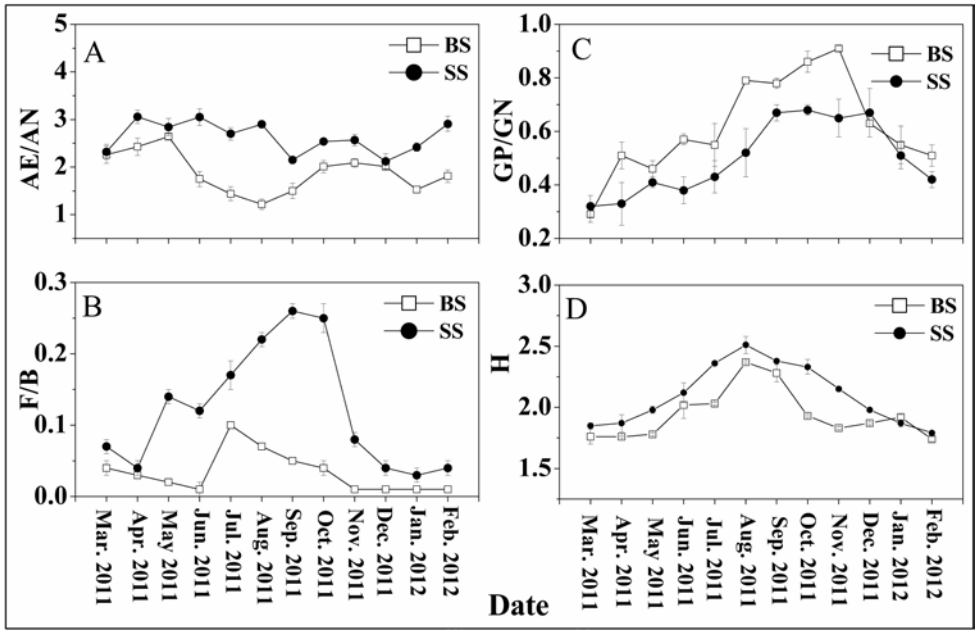

Fig. 2. Soil microbial community structure in the rhizosphere and bulk soil throughout one year
AE/AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index. BS, bulk soil; SS, soil in the rhizosphere of S. triqueter

The relative abundances of effective petroleum-degrading microorganisms (AE and GN) were much higher in the rhizosphere of *S. triqueter*, which can explain the reason why *S. triqueter* had a good ability to enhance the degradation of petroleum hydrocarbons in the HYRE wetland (Wei et al., 2014; Zhang et al., 2011). RDA was used to explore the effect of soil properties on microbial communities. The first two axes explained 69.4% and 25.4% of the total variance respectively (Fig.3).

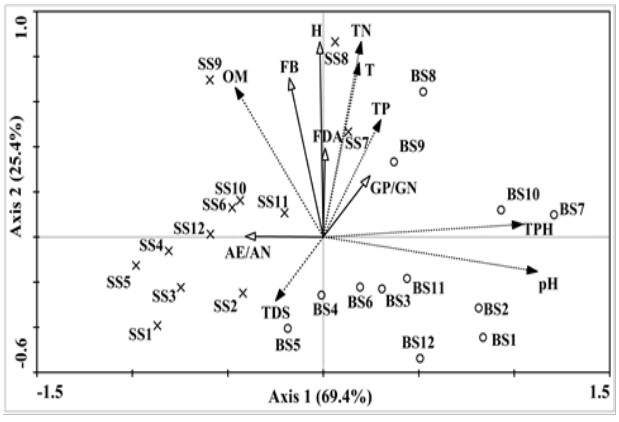

Fig. 3. RDA plot based on microbial communities and soil properties in the rhizosphere and bulk soils throughout one year (FDA, fluorescein diacetate hydrolase; AE/AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index. T, soil temperature; TN, total nitrogen; TP, total phosphorus; OM, organic matter; TPH, total petroleum hydrocarbon; TDS, total dissolved solid. BS, bulk soil; SS, soil in the rhizosphere of S. triqueter, digitals reflect the sampling month)

Rhizosphere samples were located on the left of Axis1, while bulk samples on the right. With the greatest arrow lengths among all the environmental factors, pH and TPH concentration were two main determinants of the soil microbial community structure. By contrast, TDS affected the soil microbial communities slightly. The completely contrary tendencies of TPH and AE/AN revealed that TPH concentration made a negative influence on the relative abundance of AE/AN. However, OM, TP and TN influenced soil microbial structure by increasing microbial diversity and activity. In addition, soil microbial structure appeared to be similar in the same season, for example, rhizosphere samples in March, April and May located adjacently in the ordination diagram of RDA.

As an important indicator for the soil microbial ecology, the ratio of F/B has been used to indicate the shift of soil microbial community structure under environmental stress (Kaur et al., 2005; Yao and Wu, 2010). It has been reported that F/B ratio was positively correlated with soil fertility quality, but negatively with pollution level (Liu and Herbert, 2002; Zhang et al., 2012). Therefore, the higher relative abundance of fungi in the rhizosphere might result from the lighter pollution level to some extent. pH deviation could impose great stress on soil microorganisms by changing the integrity and function of cellular membranes or altering the bioavailability of nutrients and contaminants (Baath and Anderson, 2003; Fierer and Jackson, 2006; Wick et al., 2010), therefore it was one of the main factors determining microbial community structure (Fig.3).

Pearson correlation coefficients between soil properties and microbial characteristics were listed in

Table 3. In the rhizosphere, microbial activity (indicated by FDA activity) and diversity (indicated by *H*) were both positively correlated with OM content ($P < 0.05$). In addition, OM was positively associated with the relative abundances of F/B and GP/GN. Plant residues are one of the main sources of OM in the soil (Rukun, 1999).

Therefore one of the pathways that plants act on soil microbial communities is by controlling the soil organic matters. Microbial diversity and the ratio of F/B were positively correlated with soil temperature both in the bulk soil and in the rhizosphere ($P < 0.001$), therefore, microbiota was more diverse in Summer with higher relative abundance of F/B than in Winter (Fig. 2). The positive correlation ($P < 0.001$) between microbial diversity and TN was also observed. Nitrogen is one of the most important nutrients that regulate the growth and metabolism of microbes. In most polluted sites, nitrogen is the limiting factor that controls the activity and function of soil microbiota (Nannipieri et al., 2003).

3.3. Soil enzymes

Paired *t*-test analysis revealed that activities of CAT, PPO, SUC, PHOS and DHA were significantly higher ($P < 0.001$) in the rhizosphere than in bulk soil (Table 4). Soil enzymes participate in various biogeochemical processes, such as the degradation of organic pollutants, the decomposition of humus and the redox of inorganic substances (Zhou et al., 2011).

The obvious higher activities of oxidoreductases, related with the degradation of organic pollutants, in the rhizosphere should be the results of the more active soil microorganisms, and made a good contribution to the degradation of petroleum hydrocarbons. The higher activities of SUC and PHOS, responsible for the transformation of organic nitrogen and organic phosphorus, respectively, indicated better soil fertility in the rhizosphere. Seasonal shifts of soil enzyme activities were observed in both types of soil (Fig. 4). The highest CAT activity (3.73-4.33 mL 0.1M $KMnO_4$ g^{-1} soil $30min^{-1}$) appeared in June, while the submit of PPO (0.39-0.47 mg purpurogallin g^{-1} soil 2 h^{-1}) was in August. Activities of URE, DHA and PHOS were 2-4 times higher in summer (June, July and August) than in winter (December, January and February). SUC was 0.78 ± 0.06 and 0.26 ± 0.05 mg glucose g^{-1} soil 24 h^{-1} in May and February, respectively.

RDA was also performed to explore the relationships between microbial communities and soil enzymes (Fig. 5). The first and second axes explained 86.7% and 12.0% of the total variance respectively. The similar trends of *H* and F/B in the RDA plot demonstrated that they affected soil enzymes alikely. The acute angles between F/B, CAT, DHA and PPO indicated that the relative abundance of F/B was positively correlated with activities of CAT, DHA and PPO. Similarly, GP/GN exerted a positive effect on DHA activity but a negative influence on PHOS activity.

Table 3. Coefficients of Pearson correlation between soil properties and microbial characteristics

	T	***TN***	***TP***	***OM***	***pH***	***TPH***	***TDS***
Bulk							
FDA	0.374	0.444	0.394	-0.358	-0.279	0.317	-0.280
AE/AN	-0.423	**-0.632***	-0.091	0.007	-0.154	0.204	**0.667***
GP/GN	0.284	**0.656***	**0.604***	-0.298	-0.568	0.223	-0.455
F/B	**0.711****	0.546	0.320	0.447	0.215	0.133	-0.178
H	**0.781****	**0.799****	0.495	0.278	-0.055	-0.182	-0.296
Rhizosphere							
FDA	0.080	0.341	**0.709****	**0.690***	0.501	**0.688***	**0.578***
AE/AN	0.271	0.117	0.112	-0.252	0.456	-0.465	-0.168
GP/GN	0.020	0.156	0.276	**0.617***	-0.082	**0.626***	0.078
F/B	**0.825****	**0.663***	0.225	**0.637***	0.391	0.137	-0.121
H	**0.823****	**0.801****	0.549	**0.812****	0.563	0.387	-0.108

*Asterisks mark different levels of significance: *P < 0.05, **P < 0.01. FDA, fluorescein diacetate hydrolase; AE/AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index, represents the microbial diversity. T, soil temperature; TN, total nitrogen; TP, total phosphorus; OM, organic matter; TPH, total petroleum hydrocarbon; TDS, total dissolved solid*

Table 4. Comparison of soil enzymes in bulk and rhizosphere soil by paired *t*-test

	Bulk	***Rhizosphere***	***Difference***	***t***	***P***
CAT (mL 0.1M $KMnO_4$ g^{-1} soil $30min^{-1}$)	2.41 ± 0.76	2.93 ± 0.72	0.52 ± 0.40	-4.576	0.001
PPO (mg purpurogallin g^{-1} soil 2 h^{-1})	0.17 ± 0.10	0.23 ± 0.12	0.06 ± 0.04	-4.345	0.001
SUC (mg glucose g^{-1} soil 24 h^{-1})	0.43 ± 0.17	0.53 ± 0.18	0.01 ± 0.04	-8.285	0.000
URE (mg NH_4-N g^{-1} soil 24 h^{-1})	0.17 ± 0.08	0.14 ± 0.85	0.02 ± 0.10	-0.852	0.412
PHOS (mg phenol g^{-1} soil 3 h^{-1})	0.04 ± 0.01	0.08 ± 0.03	0.03 ± 0.02	-6.717	0.000
DHA (mg TF g^{-1} soil 48 h^{-1})	1.02 ± 0.43	1.47 ± 0.44	0.45 ± 0.32	-4.852	0.001

DHA, dehydrogenase; CAT, catalase; PPO, polyphenoloxidase; SUC, sucrase; URE, urease; PHOS, alkaline phosphatase

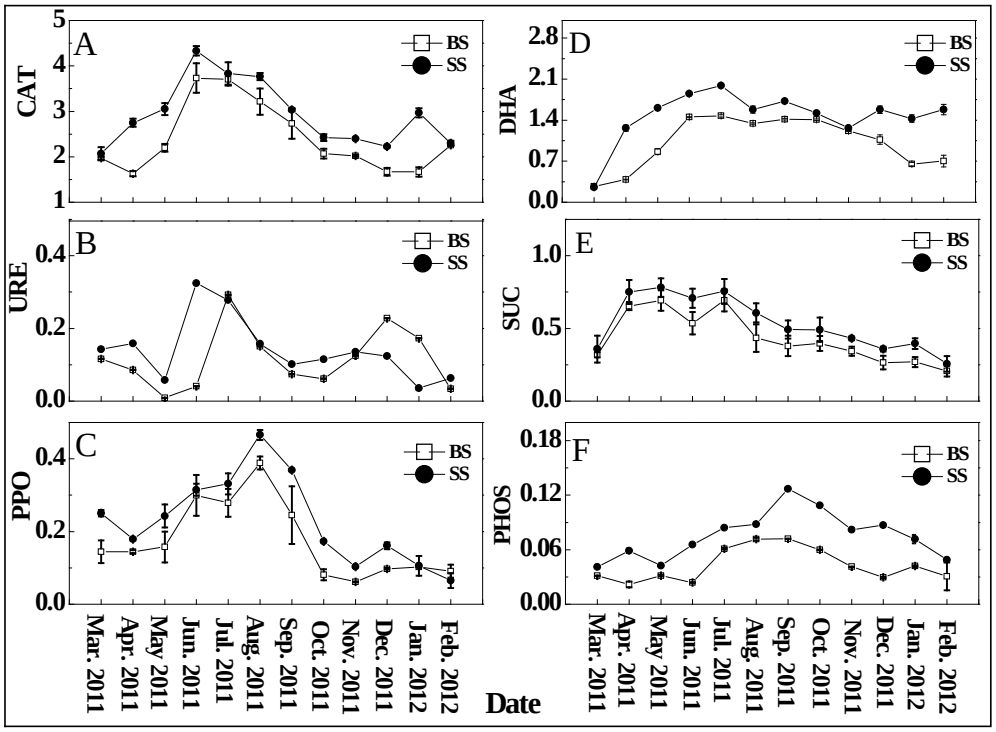

Fig. 4. Variations of soil enzyme activities in the rhizosphere and bulk soil throughout one year (DHA, dehydrogenase, mg TF g^{-1} soil 48 h^{-1}; CAT, catalase, mL 0.1M $KMnO_4$ g^{-1} soil $30min^{-1}$; PPO, polyphenoloxidase, mg purpurogallin g^{-1} soil 2 h^{-1}; SUC, sucrase, mg glucose g^{-1} soil 24 h^{-1}; URE, urease, mg NH_4-N g^{-1} soil 24 h^{-1}; PHOS, alkaline phosphatase, mg phenol g^{-1} soil 3 h^{-1}. BS, bulk soil; SS, soil in the rhizosphere of *S. triqueter*)

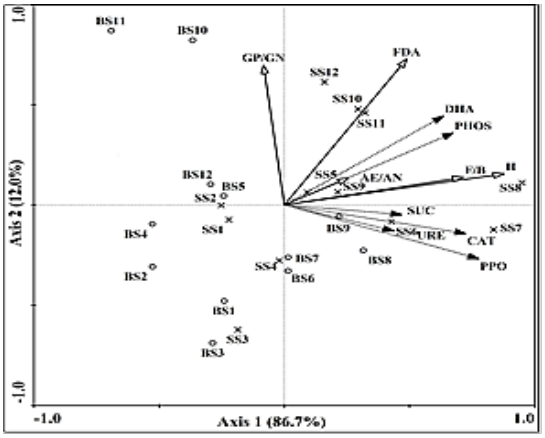

Fig. 5. RDA plot based on microbial communities and soil enzymes in rhizosphere and bulk soil (FDA, fluorescein diacetate hydrolase; AE/AN, the relative abundance of aerobes to anaerobes; GP/GN, the relative abundance of Gram-positive bacteria to Gram-negative bacteria; F/B, the relative abundance of fungi to bacteria; H, Shannon index. DHA, dehydrogenase; CAT, catalase; PPO, polyphenoloxidase; SUC, sucrase; URE, urease; PHOS, alkaline phosphatase. BS, bulk soil; SS, soil in the rhizosphere of S. triqueter, digitals reflect the sampling month)

Therefore, soil microbes can indirectly influence the degradation of petroleum by regulating activities of soil enzymes. Coefficients of Pearson's correlation between soil characteristics and enzymes were listed in Table 5. In both types of soil, CAT, PPO and SUC were positively related with soil temperature. PHOS was positively correlated with soil temperature and TN, but negatively with TDS in the bulk soil. However, PHOS was only positively correlated with OM content in the rhizosphere. Moreover, CAT, PPO and SUC were positively correlated with pH in the rhizosphere rather than in bulk soil.

DHA, PPO and CAT are important oxidoreductases and responsible for vital metabolic processes including the decomposition and detoxification of xenobiotics in the soil (Tang et al., 2010; Zhang et al., 2009). As an important participant in the natural recycle of phosphorus, phosphatase is responsible for the hydrolysis of organically bound phosphate into free ions; urease and sucrase are responsible for the hydrolysis of urea and organic carbohydrate, respectively (Wang et al., 2009). Previous studies proved that plants can alleviate the adverse effects of petroleum hydrocarbons on soil enzymes, however, the relationships of soil enzymes and microbial community structure were firstly explored in this paper (Wang et al., 2009; Wyszkowska and Wyszkowski, 2010; Zhang et al., 2011).

4. Conclusions

In this study, rhizosphere effects on soil properties, microbial community structure and soil enzymes, as well as their interactions, were explored at the HYRE wetland for the first time. Characterized with higher microbial activity and diversity, the microbial community structure in the rhizosphere was quite different from that in the bulk soil.

Table 5. Coefficients of Pearson's correlation between soil characteristics and enzymes

	T	*TN*	*TP*	*OM*	*pH*	*TPH*	*TDS*
Bulk							
CAT	**0.782****	0.369	0.249	0.366	0.378	-0.378	-0.093
PPO	**0.855****	0.486	0.407	**0.674***	0.382	-0.409	0.097
SUC	**0.589***	-0.193	0.166	**0.675***	0.531	-0.313	0.563
URE	-0.023	0.133	0.035	-0.074	0.270	0.247	-0.218
PHOS	**0.643***	**0.843****	0.404	0.031	-0.264	0.135	**-0.585***
DHA	**0.638***	**0.590***	0.480	-0.157	-0.137	-0.030	-0.293
Rhizosphere							
CAT	**0.743****	**0.636***	0.244	0.290	**0.772****	-0.240	0.033
PPO	**0.893****	**0.894****	0.446	**0.618***	**0.652***	-0.013	0.084
SUC	**0.662***	0.416	0.255	0.206	**0.677***	-0.425	0.174
URE	0.486	0.365	0.351	0.230	0.502	0.103	-0.041
PHOS	0.452	0.462	0.266	**0.696***	0.044	0.479	-0.180
DHA	0.499	0.435	0.205	0.419	0.551	0.142	0.199

Asterisks mark different levels of significance: $^{*}P<0.05$, $^{**}P<0.01$. DHA, dehydrogenase; CAT, catalase; PPO, polyphenoloxidase; SUC, sucrase; URE, urease; PHOS, alkaline phosphatase. T, soil temprature; TN, total nitrogen; TP, total phosphorus; OM, organic matter; TPH, total petroleum hydrocarbon; TDS, total dissolved solid

Positively correlated with *H* and the relative abundance of F/B, activities of DHA, CAT, PPO in the bulk soil were significantly lower ($P < 0.05$) than in the rhizosphere. The microbial structure and activities of soil enzymes were greatly affected by OM, TN, TP and pH.

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