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Different responses to soil petroleum contamination in monocultured and mixed plant systems

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ABSTRACT

The role of plant composition should be considered during ecological risk assessment of soil petroleum contamination. To evaluate the influences of plant composition on phytotoxicity, petroleum degraders, and petroleum degradation, four treatments were arranged in the present study: unplanted, bristle grass only, alfalfa only, and bristle grass and alfalfa mixed planted in uncontaminated soil or petroleum contaminated soil (w/w, 1.0%). Petroleum contamination inhibited the growth of bristle grass and alfalfa significantly, and alfalfa growth inhibition was significantly alleviated when mixed planted with bristle grass ($p < 0.05$). MPN analysis indicated that the mixed plant treatment can gather the benefits of two species, and facilitate the development of alkane, total hydrocarbon and PAH degraders in contaminated soil, but not occur in uncontaminated soil. Compared with alfalfa only treatment, the degradation rates for total petroleum hydrocarbons (TPH) and aliphatic fraction were significantly increased in the mixed plant treatment ($p < 0.05$). However, the degradation of aromatic petroleum fraction was not received substantial improvement in the mixed plant treatment, despite containing an abundant PAH degraders. Overall, mixed plant cultivation had the significant influences on plant growth, microbial community and petroleum degradation in contaminated soils. The study provides valuable insights for vegetation restoration and remediation systems in petroleum contaminated sites of study area.

1. Introduction

With increasing levels of global industrialization, petroleum contamination continues to be of serious environmental concern, with ongoing growth in crude oil extraction, production and utilization, world-wide (Varjani and Upasani, 2017). Petroleum mainly composes of saturated hydrocarbons, aromatic hydrocarbons, asphaltenes and non- hydrocarbon compounds. Most petroleum compounds are toxic and have been intensively investigated in previous decades for their effects on living organisms and environmental safe (Kriksunov, 2011; Osse et al., 2018). Meanwhile, significant efforts are being made to develop sustainable remediation technologies for the recovery of petroleum contaminated environments.

Soil contaminants usually have the negative effects on plant growing, and the reduced plant germination, growth rate or yield caused by petroleum contamination were often reported (Shahsavari et al., 2013; Xie et al., 2017). The decrease was influenced by oil composition and concentration, and varied depending on the plant species present (Shahsavari et al., 2013). Notably, plant growing can boost the degradation of organic contaminants in soil, via stimulating the rhizosphere microbial community, as well as enzymatic activities (Hall et al., 2011). Similarly as petroleum tolerance, the effectiveness of phytodegradation differed significantly among plant species, with variation even observed among cultivars (Ionescu et al., 2009; Schwitzguébel, 2017). In previous reports, phytodegradation or plant tolerance was mostly assessed with single plant species, showing notable discrepancy with contaminated field sites, where usually occurred with multiple plant species simultaneously (Banks et al., 2003; Siciliano et al., 2003). In the mixed plant species system, the interaction of roots can modify the overall root distribution, architecture, exudation and deposition processes in the rhizosphere, and soil microbial communities would change accordingly (Duchene et al., 2017). Mijangosa et al. (2009) found that microbial activity in soils from the mixed plant species treatments, was significantly higher than in the single plant treatments, which led to the enhanced degradation of organic pollutants, such as pyrene and PCB-5 (Cheema et al., 2010; Li et al., 2011). Obviously, the accelerated dissipation of organic pollutants in soils can alleviate the relevant inhibition of plant growth. However, till now, the effects of plant species composition on phytotoxicity and transformation of soil contaminants were rarely reported. Given these, we

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hypothesized that the response to soil petroleum contamination would be different in the monocultured and mixed plant species systems, primarily reflected by the variation in plant growth and petroleum degradation.

Two plant species native to the Yellow River Delta, bristle grass and alfalfa, were thus employed singly or combinedly in the present study. The occurrence of oil contamination is commonly observed in this area, due to oil production, transportation and refining (Gao et al., 2015). These two native plant species were the important vegetation in petroleum contaminated region, and possessed different root morphology, high petroleum tolerance, and remediation potential (Xie et al., 2017). Here, the effects of petroleum contamination on i) plant growth; ii) the microbial petroleum degrading community; and iii) petroleum degradation were compared between the mixed and single plant species treatments. The objective was to explore whether plant species composition influenced the response of soil-plant system to petroleum exposure.

2. Materials and methods

2.1. Soil description and preparation

The used soil in this study was from a cotton-cultured field in the Yellow River Delta. After 2-mm sieving and air-drying, the used soil was added with petroleum to form a final concentration of 1.0% (w/w), then agitated and re-filtered through a 2-mm sieve to guarantee homogeneous distribution of petroleum. In our previous study, petroleum influenced plant growth and soil microbial community significantly at 1.0% contamination level. Prior to pot experiments, the contaminated soil was stored in laboratory for 7 days. The main properties of the soil utilized and the petroleum composition are presented in Table 1.

2.2. Pot experiments

Alfalfa and bristle grass seeds were collected from a deserted land surrounding an oil well in the Yellow River Delta. Two groups were established, petroleum contaminated and uncontaminated. Each group contained four treatments: alfalfa only, bristle grass only, bristle grass and alfalfa mixed and unplanted, with the unplanted treatment used as the control (CK). The plastic pots (16 cm diameter and 14 cm in height) were used in the present study, and each pot was filled with 2.0 kg of contaminated or un-contaminated soils. Root bag (6 cm diameter and 12 cm in height) was placed along the central geometric axis in each planted pot. Fifteen alfalfa seeds and fifteen bristle grass seeds were sown in root bags of the mixed plant treatment, while thirty seeds of each species were sown in root bags of the monoculture treatments. Soil moisture content was maintained at about 65% of soil water-holding capacity through addition of water.

All treatments were performed in triplicate and grown in a greenhouse, with temperatures ranging from 20° to 30°C during the day and 15–20 °C at night. Rhizosphere soils were collected from the root bags, and bulk soils were from CK.

Table 1
The properties of soil utilized and the petroleum composition applied.

Soil properties	Contents	Oil composition	Contents
pH	8.47	Saturated hydrocarbons (%)	55.4
Salt (g/kg)	2.97	Aromatics (%)	24.5
Organic C (g/kg)	7.82	Asphaltenes (%)	12.1
Total N (g/kg)	0.63	Non-hydrocarbon compounds (%)	7.0
Available N (mg/kg)	47.3		
Available P (mg/kg)	24.1		
Available K (mg/kg)	227.4		

2.3. Plant growth analysis

Following plant emergence, 6 robust seedlings (3 each of alfalfa and bristle grass seedlings for the mixed treatment) were chosen from each planted treatment. After 70 days of sowing, the rate of photosynthesis (*Pn*) for alfalfa or bristle grass was determined using a Li-6400 XT portable photosynthesis system (Li-COR Inc., Lincoln, USA) under an external CO_2 concentration of 360 μmol/mol, and at a light intensity of 1700 μmol/m^2s. Leaf chlorophyll (Chl) was monitored simultaneously with a SPAD-502 apparatus (Minolta CO., Ltd., Osaka, Japan). To account for leaf heterogeneity, SPAD measurements were taken at 5 different locations of leaves. At day 100 post-sowing, 6 plants of each treatment were harvested, and the dry biomass was recorded after drying. Both bulk and rhizosphere soils were collected and stored at 4 °C until analysis.

The inhibition rate (IR) of plant growth or *Pn* due to petroleum contamination was calculated according to Eq. (1):

$$IR(\%) = \frac{Duc - Dpc}{Duc} * 100 \quad (1)$$

Where *Duc* represents plant dry biomass or *Pn* in un-contaminated treatments; and *Dpc* represents plant dry biomass or *Pn* in the corresponding petroleum contaminated treatments.

2.4. Soil and microbial analysis

Organic C and total N contents of soil samples were measured using a vario EL III analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) after inorganic C being removed by 1N HCl. Soil dissolved organic C (DOC) extracted with ultrapure water and measured by a liquiTOC II analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) after filtration through a 0.45 μm millipore filter. Soil pH was measured in soil-water suspension (v/v, 1:2.5). Available N was extracted with 2 M KCl and analyzed with a segmented flow AA3 analyzer (Seal Analytical Gmbh, Norderstedt, Germany). Available P and available K were extracted by sodium bicarbonate and ammonium acetate, respectively (Lu, 2000).

The fumigation extraction method was used to determine soil microbial biomass carbon concentration (MBC; Lu, 2000). Based on the method reported by Roberts et al. (2002) and Horel et al. (2012), MPN technique was employed to estimate the number of hydrocarbon-degrading bacteria present within soils. Alkane, total hydrocarbon and polycyclic aromatic hydrocarbon (PAH) degraders were determined in this study, which were elaborated in our previous study (Xie et al., 2017).

2.5. Petroleum degradation analysis

Residues of total petroleum hydrocarbons (TPH) in soils were extracted with dichloromethane by an E-916 Speed Extractor (BUCHI Labortechnik AG, Switzerland). TPH concentrations were determined gravimetrically (Moubasher et al., 2015). Residues of aliphatic and aromatic hydrocarbons in soils were measured according to the methods by Hou et al. (2015) and Zhang et al. (2016). Briefly, TPH extract residues were suspended in 10 mL of hexane, then centrifuged and 3 mL of supernatant was chromatographically separated using a 0.5 cm (i.d.) × 22 cm glass column containing 0.5 g anhydrous sodium sulfate, 2 g aluminum oxide and 3 g activated silica gel. The fraction of aliphatic hydrocarbons was eluted using 30 mL of hexane. The Following aromatic hydrocarbon fraction was eluted using 15 mL of a 2/1 (v/v) mixture of dichloromethane/hexane from the column. Eluents were concentrated to 2 mL for 7890 GC-5975MSD analysis (Agilent Technologies Inc., Santa Clara, USA). A DB-5 capillary column was used with helium as the carrier gas. For fractions of aliphatic hydrocarbons, the injection temperature was 300 °C; the oven temperature was initially set at 40 °C holding for 5 min, then increased to 45 °C at 2 °C