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Pyrene dissipation potential varies with soil type and associated bacterial community changes

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ABSTRACT

Understanding the relationship between polycyclic aromatic hydrocarbon (PAH) dissipation potential in different soils and the bacterial community changes is of great importance for bioremediation and ecological evaluation. Soil microcosms were constructed with four different types of soils, including Anthrosol (Ant), Black soil (Black), Fluvo-aquic soil (Flu), and Red soil (Red), by spiking with pyrene at three concentrations (5, 30, and 70 mg kg^{-1}). Real-time PCR and Illumina sequencing were employed to examine the bacterial community changes. The results showed that the pyrene dissipation rate differed with soil type. The fastest pyrene dissipation was observed in Ant soil: greater than 90% reduction was achieved by day 14 at higher pyrene dosages (30 and 70 mg kg^{-1}) and no lag time existed before onset of pyrene dissipation at all spiked dosages. A 7-day and 14-day lag time was needed in Black and Flu soils, respectively before pyrene dissipation at pyrene concentration of 70 mg kg^{-1}, more than 90% reduction of pyrene could still be achieved by day 21 for the Black soil and by day 42 for the Flu soil at all pyrene dosages. The evident reduction of pyrene in Ant, Black, and Flu soils was significantly correlated ($r = 0.847–0.914$; $P < 0.05$) with the increase of the pyrene dioxygenase gene (*nidA*) abundance. Moreover, the enrichment of the specific bacterial phylotypes at the phylum/class and genus level was strongest in the Ant soil but was relatively lower in the Black and Flu soils. However, pyrene reduction didn't occur in the Red soil during the entire incubation (42 days). This poor degradation ability was associated with the stability or decrease of the *nidA* gene quantity and very different types of the enriched genera from that in Ant, Black, and Flu soils. This work is helpful for understanding of the mechanisms that led to the contrasting pyrene fates in different types of soils from the view point of microbiology.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are aromatic hydrocarbons composed of two or more fused benzene rings. They are ubiquitous hazardous environmental pollutants and have continuously accumulated in the environment, primarily resulting from human activities, such as the combustion of coal, wood, and hydrocarbon fuels. Owing to their high recalcitrance, (geno)toxicity, mutagenicity, and carcinogenicity, PAHs pose a serious risk for both human health and the function of soil ecosystems. With the increase in anthropogenic activities, unpolluted soils are under the threat of PAH pollution. Although PAHs may undergo adsorption, volatilization, photolysis, and chemical degradation, microbe-mediated degradation is the main process of natural decontamination (Haritash and Kaushik, 2009; Viñas et al., 2005). Previous studies (Muangchinda et al., 2013; Niepceron et al., 2014) have demonstrated that PAHs can be degraded by native microbiota. Furthermore, PAHs-degradative populations have been isolated and their ability to utilize PAHs as the sole carbon or energy source has been examined (Heitkamp et al., 1988; Ping et al., 2014; Song et al., 2011; Widada et al., 2002). These strains often belong to a limited number of genera such as *Mycobacterium*, *Rhodococcus*, *Sphingomonas*, *Burkholderia*, *Pseudomonas, Haemophilus,* and *Paenibacillus* (Haritash and Kaushik, 2009; Uyttebroek et al., 2006). Molecular ecological tools have also been used to study PAHs-degrading microbiota. For example, DeBruyn et al. (2009)

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examined the PAH-biodegradation potential of indigenous microbial communities through quantification of the key PAH-degradative genes (pyrene dioxygenase gene: *nidA*) and the 16SrDNA gene of *Mycobacterium*, which are often found to be capable of degrading environmentally recalcitrant high molecular weight PAHs (DeBruyn et al., 2007; Heitkamp et al., 1988; Miller et al., 2004; Schneider et al., 1996), using quantitative real time PCR technique. The authors revealed that the *Mycobacterium* had a broad geographical distribution and may play an important but overlooked role in natural attenuation and cycling of high molecular weight PAHs in Lake Erie sediments.

PAH degradation depends on various biotic and abiotic factors (Haritash and Kaushik, 2009). For instance, the nature and chemical structure of the PAHs (Haritash and Kaushik, 2009), plant exudate (Sipilä et al., 2008), and soil size fraction (Uyttebroek et al., 2006) have been found to selectively enrich PAH degradation-associated bacterial groups. Additionally, the type of microorganism (Haritash and Kaushik, 2009), the composition and structure of native microbial communities (Haritash and Kaushik, 2009), and the microbial density and biomass (Romero et al., 1998) have also been associated with PAH degradation. Most studies that examine biodegradation potential and associated microbial populations have focused on the degradation potential of historically PAHs-polluted soil. Few studies have examined the potential change in the microbial community, and the biodegradation potential of previously unpolluted soils. Anthrosol, Black soil, Fluvo-aquic soil, and Red soil differ greatly in their properties and represent four typical soil types in China. They are widely distributed and constitute important soils used for food production in China. Understanding the relationship between the PAH dissipation potential of soils and their associated microbial community changes is of importance for the ecological evaluation of the effect of PAHs on soil ecosystems from the viewpoint of microbial ecology. Furthermore, an increased understanding is a prerequisite for directing the management of PAH-polluted soils.

Although molecular techniques such as terminal restriction fragment length polymorphism (T-RFLP), denaturing gel gradient electrophoresis (DGGE), and 16S rRNA-based clone library construction, which bypass the step of culturing microorganisms and thus can directly characterize the whole microbial community, have been used to analyze the microbial community in PAH polluted soil (Andreoni et al., 2004; Muckian et al., 2007; Sawulski et al., 2014; Viñas et al., 2005) or sediment (Bacosa and Inoue, 2015; Wu et al., 2014), the bacterial community in PAH polluted environment is far from being understood because of the overwhelming diversity of bacterial communities in the environment and relatively lower resolution of these molecular techniques. Next generation sequencing provides unprecedented levels of coverage and resolution of the microbial community (Hollister et al., 2010; Huse et al., 2008). It has been employed to identify bacteria correlated with PAH-degradation (Kawasaki et al., 2012; Singleton et al., 2011; Sun et al., 2014), to analyze the bacterial community changes during bioremediation of PAH contaminated soil (Kawasaki et al., 2012; Singleton et al., 2013), to determine the spatial pattern of microbial diversity and its relationship with pollutant distribution (including PAHs and total hydrocarbons (C10–C40)) (Mukherjee et al., 2014), as well as to determine the genome sequences of PAHs-degrading bacterial strains (Gai et al., 2012; Jin et al., 2011; Wang et al., 2014).

In this study, a microcosm incubation experiment was performed with four different types of unpolluted soils (Anthrosol, Black soil, Fluvo-aquic soil, and Red soil) supplemented with a high-molecular-weight PAH (pyrene). Illumina sequencing and real-time PCR techniques were used to investigate the bacterial community changes. Of particular interest were (1) the differences in the pyrene dissipation rate with soil type and (2) understanding the associations between the *nidA* gene abundance and bacterial community changes, and the pyrene fate in different soils.

2. Materials and methods

2.1. Site description and soil sampling

The Anthrosol, Black soil, Fluvo-aquic soil, and Red soil (classified according to Chinese Soil Taxonomy (Cooperative Research Group on Chinese Soil Taxonomy, 2001)) were collected from the topsoil at a depth of 0–15 cm from four long-term field experiment stations of the Chinese Academy of Sciences located in Changshu, Jiangsu province (31°33′N, 120°38′E); Changchun, Jilin province (43°59′N, 125°24′E); Fengqiu, Henan province (35°00′N, 114°24′E); and Yingtan, Jiangxi province (28° 15′N, 116° 55′E), respectively. These four soil types are called Ant, Black, Flu, and Red, respectively. All soils were passed through a 2 mm mesh to remove stones and plant debris. The soil properties are shown in Table 1. The initial total concentrations of the 16 USEPA priority PAHs (∑16PAHs) in the tested soils ranged from 38.4 to 196.3 μg kg^{-1} soil (dry weight soil, i.e., *d.w.s.*); the initial concentration of pyrene was 5.6–50.4 μg kg^{-1} *d.w.s.*. This indicated that these four soils were not polluted with PAHs as soils with a concentration of ∑16PAHs <200 μg kg^{-1} are classified as non-contaminated soils (Maliszewska-Kordybach, 1996).

2.2. Microcosm experiment

The soils were kept at a temperature of 28 °C for one week to acclimatize the soil microorganisms before the microcosm experiment. Pyrene-treated soils at concentrations of 5, 30, and 70 mg kg^{-1} *d.w.s.* were included in the experiment. Stock solutions (500, 3,000, and 7,000 mg L^{-1}) were prepared by dissolving the pyrene in acetone. The detail of contaminating the soils with pyrene was as follows: 25 g soil (*d.w.s.*) was contaminated by spiking 2.5 mL of pyrene stock solutions to give final concentrations of 50, 300, and 700 mg kg^{-1} *d.w.s.*. The control (CK) soil received only 2.5 mL of acetone. The contaminated soils were placed in a fume hood overnight, to allow the evaporation of acetone, and then mixed thoroughly with 225 g of unadulterated soil to achieve the targeted pyrene concentrations of 5, 30, and 70 mg kg^{-1} *d.w.s.*. After contaminating soil with pyrene, 10 g soil (*d.w.s.*) was placed into 120-mL serum bottles. Then, soil moisture was adjusted to 60% of the SWHC (soil water holding capacity) by adding sterilized water. The bottles were covered with black butyl stoppers, and the soil microcosms were incubated at 28 °C, in darkness, for 42 days. Triplicate samples for each treatment were taken at days 0, 4, 7, 14, 21, 28, and 42 for pyrene extraction or DNA extraction. Bottles were flushed with synthetic air (20% O_2 and 80% N_2) for 45 s after sampling at days 7, 14, 21, 28, and 42 to maintain oxic conditions.

2.3. Pyrene analysis

Pyrene in the soil was extracted and purified according to previously reported methods (Mao et al., 2012; Qian et al., 2007) with slight modifications. In brief, soils were freeze-dried with a vacuum freeze drier and passed through a 0.25 mm mesh. Then, 2 g of pyrene-treated soil was Soxhlet-extracted with 70 mL of dichloromethane for 24 h, and the extract was concentrated in a rotary evaporator to dryness. Then, 2 mL of cyclohexane was added to dissolve the residue and 0.5 mL of solution was then transferred and purified using a silica gel column (8 × 220 mm) and washed with a hexane-dichloromethane mixture (1:1, v/v). The first 1 mL of eluate was discarded because it contained nonpolar chemicals such