Effect of enhanced reactive nitrogen availability on plant-sediment mediated degradation of polycyclic aromatic hydrocarbons in contaminated mangrove sediment

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ABSTRACT

As land–ocean interaction zones, mangrove systems receive substantial polycyclic aromatic hydrocarbons (PAHs) from sewage and combustion of fossil fuel. In this study, we investigated the relationship between dissolved inorganic nitrogen (DIN) availability and degradation rate of phenanthrene, a typical PAH compound, in mangrove plant-sediment systems, using *Avicennia marina* as a model plant. After 50 day incubation, phenanthrene removal ratios in sediments ranged from 53.8% to 97.2%. In non-rhizosphere sediment, increasing DIN accessibility increased microbial biomass and total microbial activity, while enhancements in population size of phenanthrene degradation bacteria (PDB) and phenanthrene degradation rates were insignificant. In contrast, the presence of excessive DIN in rhizosphere sediment resulted in a significantly large number of PDB, leading to a rapid dissipation rate of phenanthrene. The differences in degradation rates and abundances of degrader in sediment may be explained by the enhanced root activity due to the elevation in DIN accessibility.

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1. Introduction

Mangrove wetland is located in estuaries or along the coastlines in tropical and sub-tropical regions, usually between 35°N and 35°S latitude (Wu et al., 2008). It receives a spectrum of anthropogenic pollutants derived from both fresh and saline waters (Lu et al., 2011). Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds, consisting of two or more fused benzene rings. PAHs are contaminants of great concerns due to carcinogenic and mutagenic (Kim et al., 2013). The majority of PAHs in natural environment is derived from human activities, such as incomplete combustion of coal, petroleum, fuel oil and garbage, pyrolysis of organic materials and exhaust emission of vehicles (Wilcke, 2000; Thiele and Brümmer, 2002). A substantial portion of anthropogenic PAHs is injected into surface runoff and transferred from inland to coastal regions (Wang et al., 2013). As compounds with a high lipophilic potential (Kim et al., 2013), PAHs are prone to be adsorbed in the organic matter-enriched sediment. Mangrove forest is one of the most productive wetlands. The debris from mangrove plant is rapidly buried in the sediment, creating an environment with large organic matter inventory (Sanders et al., 2012). As a result, accumulation of PAHs in mangrove sediment

has been widely reported (Tian et al., 2008; Vane et al., 2009; Cao et al., 2011; Zhao et al., 2012; Raza et al., 2013; Li et al., 2014; Sukhdhane et al., 2015). In heavily polluted coasts, the concentration of total PAHs (16 PAH species on U.S. EPA monitoring list, including naphthalene, phenanthrene and pyrene) in mangrove sediment can reach more than 10 mg kg^{-1} (Yu et al., 2005).

Mangrove swamp is an important habitat for numerous molluscs, tardigrades, and fishes (Chaves and Boucherau, 2000; Bodin et al., 2011). The PAHs, accumulated in the mangrove sediment, may be assimilated into benthos and then transferred along food chains to these high level consumers. In particular, because of bio-magnification effect, mariculture products harvested from adjacent coastal regions may contain a high level of PAH residue, which poses a potential risk to human beings. As a consequence, biogeochemical factors involved in degradation of PAHs in mangrove wetland have drawn increasing concerns and research interests. Previous research mainly focused on influences of different electron acceptors in modulation of PAH removal, such as Fe (III), Mn (IV), and CO_3^{2-} (e.g. Li et al., 2010, 2011, 2014); as well as factors that influence adsorption of PAHs in mangrove sediment, like humic acid (Ke et al., 2003). In comparison, the importance of nutrient accessibility, especially for dissolved inorganic nitrogen (DIN) in biodegradation of PHAs in mangrove wetland, received less attention. Nitrogen (N) is an essential element in the metabolism of microorganism. Prior studies revealed the important role reactive nitrogen played in removal of PAHs in coastal waters and terrestrial soils (Zaidi and Imam, 1999; Straube et al., 2003; Yang et al., 2011; Vauramo et al.,

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2011). As a land–ocean interaction zone, mangrove wetland also receives a substantial amount of allochthonous N (Sanders et al., 2014), mainly in the dissolved inorganic forms (NH_4^+ and NO_3^-), from agriculture fertilization, leakage of septic tanks, industrial spillages and the use of manure. In addition, mangrove wetland is often adjacent to aquaculture ponds that discharge waste water with a high level of DIN (Wu et al., 2014). Therefore, it is interesting to examine whether and to what extent DIN can influence PAH dissipation in mangrove plant-sediment systems.

To address this research question, phenanthrene (hereafter Phe), a typical PAH compound with a moderate degradation rate in mangrove sediment (Li et al., 2011), was selected to be a contamination source in the experiment. *Avicennia marina* (Forsk.) Vierh., one of the dominated mangrove species in China, was chosen as the model plant. The temporal change of Phe concentrations in a designed microcosm (rhizobox) under different levels of NH_4NO_3 (source of DIN) amendment was examined. We hypothesized that increasing accessibility of DIN in sediment could lead to a significant enhancement in degradation capability of Phe. The objectives in this study were to test this research hypothesis and elaborate the linkage between the availability of DIN and the degradation of Phe in the mangrove sediment environment.

2. Materials and methods

2.1. Preparation of spiked sediment

Test sediment was collected by PVC corers from a depth of 30–10 cm in Jiulong Estuary mangrove wetland, Fujian Province, China. The pH of the sediment was 6.63 ± 0.08. The redox potential was -123.6 mV, measured with a FJA-4 ORP probe (Nanjing Zhuan-Di Instrument & Equipment CO., LTD, China). Water content [$58.4(\pm 2.1)\%$] of the sediment was calculated on the basis of weight difference between fresh and oven-dried sediment samples. Total organic matter content in the sediment was $4.2(\pm 0.2)\%$, which was analyzed by loss of ignition in a muffle furnace at 450 °C. Total organic carbon/nitrogen in the sediment was determined by acid fumigation method following Harris et al. (2001). They were $2.45(\pm 0.12)\%$ and $0.24(\pm 0.03)\%$, respectively. The collected sediment was sieved through a 0.5 cm pore size sieve to remove coarse particles. Subsequently, the sediment was spiked with Phe (Sigma-Aldrich Co. Ltd. UK, purity >97%) and NH_4NO_3 (purity >99%) as following: (1) 200 g freeze-dried sediment was accurately weighed and then spiked by Phe-acetone solution; (2) the contaminated sediment was placed in the dark environment overnight and allowed acetone to evaporate; (3) the spiked sediment was mixed with approximately 25% of the total sediment. Concurrently, NH_4NO_3 solution with two concentration levels was added in this sediment matrix; and (4) the well-mixed sediment from step (3) was stirred with all the remaining sediment and followed by intensive mechanical mixing. Furthermore, in order to determine the abiotic degradation rate of Phe in the sediment, we autoclaved the sieved sediment at 121 °C for 30 min and spiked the sterilized sediment with Phe. Subsequently, all the sediments were placed in a dark room. The concentrations of Phe and DIN in the spiked sediment samples were determined prior to incubation experiments, as presented in Table 1.

2.2. Design of rhizobox

A laboratory rhizobox was designed on the basis of previous studies in our research group (Lu et al., 2011). The rhizobox contains four compartments from the central section to the periphery (Fig. 1). Between each section, the sediment was separated by nylon mesh (500 mesh), viz. a central zone (rhizosphere) for plant growth (28 mm in width), near rhizosphere zones (1.5 mm in width), far rhizosphere zones (2 mm in width), and bulk sediment/non-rhizosphere zones (20 mm in width). For simplicity, these layers were numbered in an order, ranging from S1 to S4 (Fig. 1). This design successfully separates the sediment between different layers and permits transfers of sediment microfauna and root exudates between distinct compartments. It also prevents the extension of fibrous root from the inner section to the adjacent zones and facilitates the observation of the influences of root activity to the degradation of Phe.

One *A. marina* seedling that pre-cultured in sandy medium was planted in the central zone of the box. Subsequently, approximately 0.7 kg of the spiked sediment was injected into a rhizobox. Each treatment had three replicates. The plants in the rhizoboxes were grown in an illumination incubator with relative humidity of 85%. The temperature in the incubator was 25 °C in day time and 20 °C in the dark. The day length was set to be 12 h. The incubation experiment was carried out for 50 days. The sediment moisture content was adjusted to 100% of the water holding capacity every 3 days by Millipore water. Within the incubator, rhizoboxes were arranged in a randomized design and their positions were rotated daily to ensure the uniformity of physical conditions. Before harvesting, rhizoboxes were withheld from watering for 2 days. Harvesting involved the sequential dismantling of each rhizobox, separating the layers of each sediment zone of the rhizoboxes and removing the plants from the root compartment. Root was manually separated from the sediment and gently cleaned with deionized water, and then blotted dry with a piece of filter paper. The sediment samples gathered from different zones of each rhizobox were homogenized separately before analysis.

2.3. Determination of biomass of A. marina

After the cleaning process, root, stem and leaves from each plant were separated. The stem and leaves were directly freeze-dried until reaching constant weight. A portion of root was weighed and then kept for the analysis of root activity. The fresh biomass of the remaining root was documented again before freeze-dried. Subsequently, the weight of dry root was recorded and the moisture ratio of root was calculated on the basis of differences in weight between two measurements. Finally, the initial biomass of root can be calculated as a product of fresh weight of entire root and moisture ratio.

2.4. Measurement of Phe concentrations

Freeze-dried sediment and plant tissue were mashed and extracted with an accelerated microwave extraction system modified from Lu et al. (2011). The surrogate Phe-d10 (Sigma-Aldrich, UK) was added to the samples prior to extraction in order to determine the systematic recovery rate. The concentrations of the Phe in the extracts were determined by an Agilent 1100 High Performance Liquid Chromatography equipped with a RP-18 (250 mm × 4.6 mm) column. Detection was carried out at 254 nm wavelength. The detection limit derived from replicates and procedural blanks was 6.8 µg l^{-1}. The mean recoveries of deuterated surrogate were 94.2%.

Table 1
Detailed information of measured concentration for Phe and DIN (NH_4^+ and NO_3^-) in each treatment. The concentrations were determined prior to the incubation test. The analysis methods were described in Section 2. Nominal concentrations were 12.5 mg kg^{-1} for Phe, and 35 mg kg^{-1} (low level)/100 mg kg^{-1} (high level) for NH_4^+ as well as NO_3^- for the three contaminated groups. The unit for all the concentrations in the table is mg kg^{-1}.

Treatments	Concentration of Phe	Concentration of NH_4^+	Concentration of NO_3^-
Control	$3.8(\pm 0.4) \times 10^{-2}$	10.1 ± 0.3	1.4 ± 0.1
Sterilized	10.7 ± 0.3	17.6 ± 0.4	2.5 ± 0.3
Phe	10.3 ± 0.2	11.0 ± 0.2	1.2 ± 0.1
Phe + Low DIN	10.2 ± 0.1	43.7 ± 2.4	33.8 ± 1.2
Phe + High DIN	10.2 ± 0.2	104.5 ± 4.5	94.7 ± 3.4