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Interactive effects of hypoxia and polybrominated diphenyl ethers (PBDEs) on microbial community assembly in surface marine sediments

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ABSTRACT

Hypoxia alters the oxidation–reduction balance and the biogeochemical processes in sediments, but little is known about its impacts on the microbial community that is responsible for such processes. In this study, we investigated the effects of hypoxia and the ubiquitously dispersed flame-retardant BDE47 on the bacterial communities in marine surface sediments during a 28-days microcosm experiment. Both hypoxia and BDE47 alone significantly altered the bacterial community and reduced the species and genetic diversity. UniFrac analysis revealed that BDE47 selected certain bacterial species and resulted in major community shifts, whereas hypoxia changed the relative abundances of taxa, suggesting slower but nonetheless significant community shifts. These two stressors targeted mostly different taxa, but they both favored Bacteroidetes and suppressed Gammaproteobacteria. Importantly, the impacts of BDE47 on bacterial communities were different under hypoxic and normoxic conditions, highlighting the need to consider risk assessments for BDE47 in a broader context of interaction with hypoxia.

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1. Introduction

Hypoxia, defined as dissolved oxygen (DO) level that falls below 2.8 mg O_2 L^{-1}, affects several 100,000 km^2 of marine water worldwide (UNEP, 2011). Previous studies have shown that hypoxia results in aberrant behaviors among benthic fauna, collapse of fisheries, and major changes in the structure and trophodynamics of marine ecosystems (Wu, 2002; Díaz and Rosenberg, 2008; Liu et al., 2011a, b). Although hypoxia may be a natural phenomenon, increasing eutrophication due to anthropogenic activities, especially sewage discharge and the wide-use of agricultural fertilizers, have exacerbated both the occurrence and severity of hypoxia in coastal waters worldwide (Gilbert et al., 2010; Rabalais et al., 2010). It was shown that the number of hypoxic areas has doubled each decade since the 1960s (Díaz and Rosenberg, 2008, 2011; Díaz and Rabalais, 2009; Levin et al., 2009; Rabalais et al., 2010). Recently, the United Nations Environment Programme (UNEP) predicted that the largest increase in the number of hypoxic areas in the coming decade would occur in Asia (UNEP, 2011).

Microorganisms play a pivotal role in regulating major geochemical, ecological and environmental processes in marine ecosystems, particularly trophodynamics and biogeochemical cycles (Nogales et al., 2011). Limited evidence has suggested that hypoxia can alter the oxidation–reduction balance in coastal sediments and the associated biogeochemical processes (Brüchert et al., 2003; Baird et al., 2004; Montagna and Ritter, 2006; Díaz and Rosenberg, 2008; Levin et al., 2009; Middelburg and Levin, 2009; Doney, 2010; Rabalais et al., 2010). For instance, under hypoxic conditions, marine microbes use nitrate instead of oxygen as a terminal electron acceptor for respiration, resulting in reactive nitrogen loss and net production of nitrous oxide, a potent greenhouse gas (Kemp et al., 2009; Codispoti, 2010; Doney, 2010). The biogeochemical processes are largely regulated by microbial activities in marine sediments, yet the effects of hypoxia on the sediment microbial communities remain virtually unknown.

Most studies examining the effects of hypoxia on the microbial community structure within marine sediments thus far have only focused on a single functional group of bacteria (Schulz and De

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Beer, 2002; Brüchert et al., 2003). Under severe hypoxia, sediment surfaces are often covered with signature microbial mats formed by sulfur-oxidizing bacteria, particularly *Beggiatoa*, *Thioploca*, *Thiovulum* and *Thiomargarita*, which are capable of removing sulfide using nitrate as an electron receptor (Divya et al., 2011; Schulz and De Beer, 2002; Brüchert et al., 2003; Levin et al., 2009). It is not known whether, and if so how, the microbial community structure in marine surface sediments may be altered under hypoxic conditions.

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants in plastics, textiles, resins, building materials and electronic equipment since the 1970s, and now become ubiquitous in the global environment (WHO, 1994; EPA, 2008). The harmful effects of penta- and octa-BDEs on humans have been well documented (Darnerud, 2003) and correspondingly their production has been banned by the European Union and United States in 2004 (EU, 2004; EPA, 2008). In contrast, there is no regulation over the production or usage of PBDEs of all kinds in Asia thus far, including China. PBDEs levels in the environment, wildlife and humans saw an alarming increase in the last 30 years (Darnerud, 2003; Wang et al., 2007; Frederiksen et al., 2009; Lam et al., 2010) South China has now become a major global dumping site of electronic wastes, and concentrations of PBDEs in marine sediments in the region (>7340 ng g^{-1} dry wt) and its proximity, Hong Kong (up to 53.6 ng g^{-1} dry wt) are amongst the highest in the world (Liu et al., 2005; Mai et al., 2005) and it is clear that PBDEs contamination of marine sediments is very serious in South China and Hong Kong.

No study thus far has investigated the impact of PBDEs on microbial communities in marine sediments. Nevertheless, previous studies on the effects of PBDEs on natural microbial communities in soils and river sediments have primarily employed PCR–DGGE (denaturing gradient gel electrophoresis) fingerprinting of the 16S rRNA genes for community profiling and FISH (fluorescence in situ hybridization) cell counting. Most commercial PBDEs investigated, including the congeners 15, 47, 99, 100, 153, 154 and 209, altered the bacterial communities in soils and river sediments and reduced the bacterial diversity (Yen et al., 2009; Zhu et al., 2010; Liu et al., 2011c; Huang et al., 2012; Zhang et al., 2012). It has been further demonstrated that PBDEs and heavy metals, surfactants or carbon substrates interactively affected the bacterial communities (Liu et al., 2011c; Huang et al., 2012; Zhang et al., 2012; Qiu et al., 2012). More recently, it has been shown that significant shifts of PBDE-degrading bacterial communities were caused by electron donor amendments (Xu et al., 2012).

We hypothesized in this study that the interaction or co-occurrence of hypoxia and PBDEs in the marine environment would have exacerbated or synergistic impacts on the microbiota by further reducing the bacterial diversity and exaggerating the shifts in community composition (for instance, hypoxia would selectively favor the anaerobic PBDE-degrading microbiota over the aerobic counterparts enriched in the PBDEs treatments). Since PBDEs is ubiquitous while hypoxia affects very large areas in the marine environments, both individual and combined impacts of PBDEs and hypoxia must be considered in environmental risk assessment and bioremediation strategies.

In the present study, laboratory experiments were carried out to test the hypothesis that hypoxia and PBDEs would alter the bacterial community composition in marine surface sediments. Microcosms with different combinations of PBDEs and dissolved oxygen levels were set up, and the changes in the bacterial community composition on marine surface sediments over 28 days were characterized using terminal-restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene clone library construction. BDE47 was used as the model PBDE toxicant in this study because: (a) it is the predominant PBDE congener found in the marine environment and biota worldwide (Christensen and Platz, 2001; Christensen et al., 2002; Bayen et al., 2005; Liu et al., 2005; Guo et al., 2007; EPA, 2008) (b) it is one of the most abundant PBDE congeners reported in seawater and marine sediments in Hong Kong (Liu et al., 2005; Wurl et al., 2006), and (c) the possible adverse effects of BDE47 on aquatic wildlife have raised increasing concern (Darnerud, 2003; Muirhead et al., 2005; Lema et al., 2007; Chen et al., 2010; Chou et al., 2010; Lam et al., 2010).

2. Materials and methods

2.1. Microcosm setup and sample collection

Surface sediments used for this study was collected from Tung Lung Chau, a pristine site in Hong Kong (22°192′N/114°162′E), using a 0.1 m^2 van Veen grab. The homogenized sediments were sieved through a mesh (pore size: 250 μm) and frozen at −80 °C overnight to remove the metazoans. Microcosms were setup in a 2×4 factorial design experiment, with two levels of dissolved oxygen (DO) (hypoxia: 1.5 mg O_2 L^{-1}, and normoxia: 6.0 mg O_2 L^{-1}) and 4 levels of BDE47/control (i.e. control, solvent control, sediments spiked with 0.5 ng BDE47 g^{-1} sediment dry weight: 0.5 ppb, and sediments spiked with 3 ng BDE47 g^{-1} sediment dry weight: 3 ppb) treatments. The sediments were spiked with BDE47 following the procedures described in our previous study (Lam et al., 2010). Briefly, BDE47 (99% purity; Wellington Laboratories, USA) stock solution was prepared by dissolving 0.5 mg of BDE 47 in 10 mL of hexane and working stock solution at 1 ppm was prepared in turn using the stock solution. Sediments spiked with hexane were used in the solvent control. The testing BDE47 concentrations were within the reported range in the Pearl River Delta region (Liu et al., 2005).

Three replicate microcosms (diameter: 3 cm, depth: 1.5 cm) were set up for each combination of DO and BDE47, so there were a total of 8 treatments and 24 microcosms. Each microcosm contained 10 g of sediments. The microcosms were positioned in the experimental chambers (Fig. 1), following the design described in our previous study (Liu et al., 2011a). The chambers were filled with 2 L of filtered seawater. Surface sediments were sampled from each of the microcosms after 4 weeks for extraction of bacterial community DNA. The mean (±S.D.) wet weight of the sediment samples was 3.38 (±0.88) g (n = 24). These sediment samples were placed in extraction buffer and immediately frozen at −80 °C.

2.2. Extraction of bacterial community DNA and terminal-restriction fragment length polymorphism (T-RFLP)

Total genomic DNA from each sediment sample was extracted and purified using UltraClean soil DNA isolation kit (MoBio Laboratories Inc., CA, USA). The amount and quality of DNA was estimated using NanoDrop (Thermo-Scientific, DE, USA). The extracted DNA samples were stored at −20 °C. T-RFLP and cluster analyses were carried out following the procedures described in our previous studies (Chiu et al., 2007; Chiu et al., 2008). Bacterial 16S rRNA genes were amplified by PCR using the forward primer 341F (5′-CCTACGGGAGGCAGCAG) (Muyzer et al., 1993) and fluorescein tagged reverse primer 907R (5′FAM-CCGTCAATTCCTTTRAGTTT) (Rölleke et al., 1998) with the KapaTaq Hot Start Ready Mix System (Kapa Biosystems Inc., USA). The fluorescently-labeled PCR products were digested with 20 U *Msp*I (Boehringer Mannheim) at 37 °C for 6 h. The digested amplicons were purified with the Wizard® PCR prep DNA purification system (Promega). Aliquots of purified products were mixed with internal size standard ET-900 (Amersham) before capillary electrophoresis on a MegaBACE® genetic analyzer (Amersham) operating in Genotyping mode.

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