Variation of microbial community structure in a simulated remediation process of BDE-47-contaminated soil

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Abstract. As a kind of persistent organic matter, it is important to study the effects of 2,2',4,4'-tetrabromobiphenyl ether (BDE-47) on microbial ecology in soil. The Illumina Miseq high-throughput sequencing technique was used to study the effects of pollutants and degrading bacteria on the microbial community structures in indoor simulated remediation of BDE-47-contaminated soil. The correlation between the microbial community structures and physiochemical properties of the soil was analyzed. The results showed that the addition of pollutant BDE-47 no significant effect on the number of microbial species, and the diversity of species reduced slightly. In contrast, the addition of degrading bacteria led to a dramatic decrease of both the species number and diversity. After the simulation, the degree of variation in microbial community structural composition was ranked as: simulation system E > C > A. The simulated systems included 27 microbial phyla, among which Acidobacteria, Proteobacteria, and Actinobacteria were the most dominant with a relative abundance of greater than 10%. Firmicutes was the common phylum in simulated systems A and C and was dominant in the system E. The distributions of Nitrospirae, Bacteroidetes, Saccharibacteria, Proteobacteria, and Gemmatimonadetes were similar. The distribution of Firmicutes was irrelevant to the physicochemical properties of soil.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), also known as persistent organic pollutants, are brominated flame retardants widely used in the fields of electronics, electrical appliances, household appliances, textiles, petroleum industry, and building materials [1,2]. Due to the lack of binding effect of chemical bonds, PBDEs added can readily diffuse into the atmosphere, water, soil, and other environmental components via volatilization, infiltration, etc. [3-5]. PBDEs are highly lipophilic, chemically stable, difficult to degrade, cause disruption of endocrine activity [6], developmental neurotoxicity [7] and immunotoxicity [8]. Among the 209 homologs of PBDEs, 2,2',4,4'-tetrabromobiphenyl ether (BDE-47) is the most common and highly concentrated throughout the globe [9]. BDE-47 is a great threat to human health and the environment because it is highly hydrophobic and recalcitrant. Consequently, BDE-47 can be enriched through the food chain [10] and can be transferred through breast milk, placenta and umbilical cord blood [11-13], and is thus a great threat to human health and the environment.

As a destination of BDE-47, soil plays an important role in its spatial and temporal distribution and geochemical cycling. To date, most of the studies on BDE-47 degradation using aerobic microorganisms were based on liquid medium environments [14,15], and the phytoremediation of BDE-47-contaminated soil was prevalent [16,17]. In this study, we used the un-polluted soil in
Northeast China to build indoor simulation systems of BDE-47-contaminated soil and to investigate the effects of BDE-47 and specific bacteria (derived from the selected PBDEs-contaminated soil in Taizhou City and could degrade BDE-47) on the structure of soil microbial communities. This work could be theoretically and technically beneficial for the subsequent bioremediation of contaminated soil.

2. Materials and methods

2.1. Simulation systems

The substrate was acquired from the surface soil (0–20 cm in depth) in a BDE-47-free region of Harbin Normal University, Heilongjiang Province, China. The plant residues and other impurities in the substrate were removed. The substrate soil was packed into aseptic bags and stored at below 0 ºC using ice. A flowerpot of height 18 cm and of diameter 21 cm was used to construct the simulation systems for the microbial remediation of BDE-47-contaminated soil.

Three types of simulation systems (A, C and E) were built and the test of each type was repeated twice. The system A contained only the compacted substrate, which had been sieved with a 10-mm-pore sieve. The system C contained the substrate soil and BDE-47. In this system, the BDE-47 was added following this procedure: a solid standard sample was dissolved in organic solvent n-hexane and the solution was evenly added into the air-dried substrate that had been sieved with a 60-mm-pore sieve. This mixture was placed in an aseptic ventilator until the n-hexane was completely vaporized. From this treated soil, 100 g was used as the contaminated parent soil in the subsequent experiments. This parent soil was evenly added into the BDE-47-free substrate soil (sieved with a 10-mm-pore sieve) in a certain ratio until the BDE-47 content in a simulation system reached about 1500 ng/g. The third simulation system, E contained the substrate, 1500 ng/g BDE-47, and a Bacillus strain for degradation BDE-47. Before the simulation, the strain was incubated in a liquid medium containing inorganic salts and 500 μg/L BDE-47. The incubation period consisted of four cycles and each cycle lasted for 10 days. These incubated degrading bacteria were transferred into a liquid culture medium for enrichment and bacteria acquisition. The bacteria were rinsed with sterile water three times and then were mixed with sterile water to prepare a suspension, which was subsequently added to the relative simulation systems. The initial concentration of the degrading strain was $1.05 \times 10^5$ CFU/g.

The evaporated water was determined on the basis of weight loss. A certain amount of aseptic water was regularly added to maintain a constant moisture content in the soil. In the remediation days 0 (denoted by 1) and 90 (denoted by 5), each system was sampled at different sites and the sampling was repeated twice. The as-derived soil samples were homogeneously mixed. A proportion of the mixture was air-dried and sieved at room temperature for the determination of soil physiochemical properties. The other was separately stored at -80 and -20º C for the determination of soil microbial community structure and soil BDE-47 content, respectively.

2.2. Determination of microbial community genome structure before and after the simulation

The variation in soil microbial community structure during the simulation was probed by the high-throughput sequencing technology. The total genomic DNA of the microorganisms in soil was extracted for purity detection. The PCR reaction system consisted of 4 μL of 5× FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of Forward Primer (5 μM), 0.8 μL of Reverse Primer (5 μM), 0.4 μL of FastPfu Polymerase, 10 ng of template DNA, and 0.2 μL of BSA. The volume of PCR system was adjusted to 20 μL with dd H2O. The PCR reaction conditions were the following: 95ºC for 3 min; 27 cycles at 95ºC for 30 s, 55ºC for 30 s, and 72ºC for 45 s; a final extension at 72ºC for 10 min, and finally stored at 4º C until use. The PCR reaction of each sample was repeated three times and the products were recovered with an AxyPrep DNA gel recovery kit. The products were eluted using Tris-HCl and were detected by electrophoresis on a 2% agarose gel. For the preliminary electrophoresis, the PCR products were evaluated by a QuantiFluo™-ST blue fluorescence
quantification system. Then, the samples were mixed at a certain ratio based on the sequencing duty of each sample. After the library establishment and sequencing in the Illumina platform, OTU cluster analysis was carried out. Based on the results of cluster analysis, various diversity indices and sequencing depths were analyzed, and the statistical analysis of community structure at each classification level was conducted. This sequencing experiment was performed by the BMK Cloud Technology Co., Ltd., Beijing.

2.3. Data processing and analyses

The Mothur software was used to calculate \( \alpha \)-diversity indices, including Ace, Chao, Shannon and Simpson indices. The weighted Unifrac principal component analysis algorithm was applied to analyze \( \beta \)-diversity of bacteria in the soil community. The Origin 8.0 software was used to analyze the bacterial community composition and relative abundance at phylum and genus levels.

3. Results and analysis

3.1. \( \alpha \)-diversity of the microbial community structure

A total of 206954 sequences of 16S rRNA fragments were obtained from six soil samples through the high-throughput sequencing technique. The number of sequences with a fragment length of 421–460 bp accounted for 99.82% of the total number. The number of sequences in the soil of each sampling site is shown in table 1. The bioinformatics statistical analysis of OTU was carried out at a similarity level of 97%. A coverage index reflects whether the sequencing results truly represent all the microorganisms in a sample. In the present work, the coverage indices of all samples surpassed 0.99, indicating that the microorganisms in the samples were truly represented through the sequencing results.

| System | Number of sequences | Coverage index | Chao index | Ace index | Shannon index | Simpson index |
|--------|---------------------|----------------|------------|-----------|---------------|---------------|
| A1     | 32200               | 0.994          | 1442       | 1432      | 6.34          | 0.0045        |
| A5     | 33944               | 0.994          | 1399       | 1402      | 6.37          | 0.0033        |
| C1     | 41558               | 0.996          | 1451       | 1444      | 6.27          | 0.0050        |
| C5     | 30463               | 0.992          | 1436       | 1425      | 6.09          | 0.0057        |
| E1     | 35670               | 0.993          | 1343       | 1329      | 5.98          | 0.0164        |
| E5     | 35819               | 0.993          | 1405       | 1399      | 4.41          | 0.1609        |

The Chao and Ace indices evaluate the total number of species in a sample and both their values are directly proportional to the number of species. In this work, the addition of BDE-47 had an insignificant effect on the total number of species in the simulated systems, but the addition of degrading bacteria significantly decreased the total number of species, though a slight increase was observed in the simulation process.

The Shannon and Simpson indices can estimate the microbial diversity in a sample. While the Shannon indicator is directly proportional, the Simpson indicator is inversely proportional to the diversity of communities. The addition of degrading bacteria reduced the species diversity in the simulation systems E, and along with the simulation, an even more obvious reduction in diversity was observed, as shown in table 1.

3.2. \( \beta \)-diversity of the microbial community structure

The \( \beta \)-diversity is an inter-habitat diversity, often analyzed by the weighted Unifrac algorithm. Here, the differences between samples are expressed by the distances of the phylogenetic relationships
between OTUs of different communities. According to the evaluation and information on the abundance of microorganisms in different samples, the different’ distance matrices between these samples would be obtained for PCoA analysis. In this study, as shown in figure 1, the first principal component (PC1) was most weighted with a contribution rate of 67.22%, and the second principal component (PC2) had a contribution rate of 25.11%. Taken together, their total contribution rate was 92.33%. The distribution of different samples on the PC axis was obviously different. On the PC1 axis, the simulation system C was distributed in the positive direction, and the others were distributed in the negative direction. On the PC2 axis, A5 was distributed in the negative direction, and the others were distributed in the positive direction. Considering the situations before and after the simulation, the sample distance of the system E was greater than that of system C, and that of system A was the smallest. These results show that the microbial community structure of system E changed most obviously, followed by that of the system C, and only a minimal change - to the smallest extent - was observed in the system A.

![Figure 1. Weighted Unifrac analysis of the samples.](image)

3.3. Structural analysis of microbial community at phyla level

![Figure 2. Analysis of heatmap of microbial community structure in each sample.](image)
Based on the high-throughput sequencing results, that the microorganisms in all the systems could be divided into 27 phyla (figure 2). The dominant phyla with a relative abundance of > 10% included Acidobacteria, Proteobacteria, and Actinobacteria. Firmicutes was common in the simulated systems A and C and was the most dominant in the system E. The separate relative abundance of Chloroflexi, Bacteroidetes, Gemmatimonadetes, Verrucomicrobi, Nitrospirae, and Saccharibacteria was observed in the range of 1%–10% and these phyla were common in all the simulation systems. On the other hand, the remaining phyla were rare in all the simulation systems.

The phyla with a relative abundance of 1% in each system were further studied (phyla with a relative abundance of < 1% were integrated and denoted as “Others”, as shown in figure 3). At the beginning of the simulation, the structural compositions of the microbial community in the systems A1 and C1 were similar, indicating that the addition of BDE-47 scarcely affected the compositions of microbial community structures. However, in the system E1, the addition of degrading strain led to significant changes in relative abundance of the microbial community structure at the phylum level.

In the simulation system A, the relative abundance of the dominant phylum Acidobacteria and Actinobacteria, and the relative abundance of the common phylum Verrucomicrobi changed evidently during the simulation process. In detail, starting from the early stage to the later stage, the relative abundance of Acidobacteria decreased from 34.21% to 27.80%, the relative abundance of Actinobacteria increased from 16.14% to 21.26%, and the relative abundance of Verrucomicrobi decreased by 1.37%. These results indicated that the simulation process had a significant influence on the community structural compositions of these three phyla. While at the initial stage of simulation, the microbial community structure of system B was similar to that of the system A, in the late stage of simulation the microbial community structure of system C was affected by BDE-47, resulting in significant changes in the microbial community structure. The variation in relative abundance of dominant phyla (Acidobacteria and Proteobacteria) and common phyla (Gemmatimonadetes, Verrucomicrobi, Nitrospirae, and Chloroflexi) were largely different from that of the system A, indicating that BDE-47 had a significant effect on the community structural compositions of these phyla. In detail, from the early to the later stage, the relative abundance of Proteobacteria decreased from 22.52% to 16.28%; the relative abundance of Chloroflexi was increased by 3.60%; the relative abundance of Verrucomicrobi was increased by 2.96%. On the other hand, in the system C, the addition of BDE-47 and free degrading strain led to more obvious changes in the composition of microbial community structure. Here, irrespective of the simulation stage, the relative abundance of Proteobacteria and Firmicutes varied drastically - the former was decreased from 33.16% to 14.03%, and the latter increased from 12.02% to 40.04%.

Figure 3. Composition of microbial community structure with relative abundance of more than 1% in the sample.
4. Discussion
In community ecology, the analysis of diversity in a sample can reflect the abundance and diversity of microbial communities, which are estimated by a series of statistical analysis indices. The stability and complexity of a bacterial community structure are often expressed by $\alpha$- and $\beta$-diversities. The $\alpha$-diversity reflects the species diversity within a single sample and is quantified by many indicators such as Chao, Ace, Shannon, Simpson and other indices [18]. The $\beta$-diversity is an indicator of inter-habitat diversity and is often presented through the weighted Unifrac analysis, in which the differences between samples are expressed by comparing the phylogenetic relationship distances between OTUs of different communities. In the present study, the results of $\alpha$- and $\beta$-diversities indicate that the addition of pollutant BDE-47 had an inappreciable effect on the total number of species, but slightly reduced the diversity of species. In this study, the addition of degrading bacteria led to a significant decrease in the total number and diversity of species. In the three simulation systems, the degree of variation in microbial community structural composition was ranked as follows: system E > C > A. According to the literature, the BDE-47 homologs including BDE-15, BDE-153, BDE-154, and BDE-209 could also change the microbial community structures of soil [19,20], and concentrated BDE-209 could significantly reduce the diversity in the microbial community and inhibit potential nitrification in soil [21].

In our study, free BDE-47-degrading bacteria were added to solid degradation simulation systems E. Although the relative abundance of these bacteria increased significantly during the simulation process, the degradation rate was only as low as 6.46% over a period of 90 days. This may be because, during the degradation process, the pollutants first get enriched through extracellular adsorption, and then are transported into the microbial cells through their own systems, finally, degrading the pollutants through intracellular redox reactions. The low degradation efficiency of the simulated systems in our study may be attributed to abundant nutrients in the soil, which are readily utilized by the microorganisms for growth and reproduction. Another possible reason may be that the simulated systems were almost motionless, unlike in the liquid medium environment, resulting in the inadequate contact between the strain and BDE-47 for a good degradation efficiency.

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References
[1] Wang Y, Jiang G, Lam P K et al 2007 Polybrominated diphenyl ether in the east Asian environment: a critical review Environ Int. 33 963-73
[2] Oliver W, Paul K S L and Jeffrey P O 2006 Occurrence and distribution of polybrominated diphenyl ethers (PBDEs) in the dissolved and suspended phases of the sea-surface microlayer and seawater in Hong Kong, China Chemosphere 65 1600-6
[3] Dong Y, Li L, Bie P J et al 2014 Polybrominated diphenyl ethers in farmland soils: source characterization, deposition contribution and apportionment SCI Total Environ 466 524-32
[4] Lohmann R, Klanova J, Kukucka P et al 2013 Concentrations, fluxes, and residence time of PBDEs across the tropical atlantic ocean Environ SCI Technol 47 13967-75
[5] Ma J, Qiu X H, Zhang J L et al 2012 State of polybrominated diphenyl ethers in China: An overview Chemosphere 88 769-78
[6] Kyla M W, Yan P L, Philip H K et al 2017 Association of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) with hyperthyroidism in domestic felines, sentinels for thyroid hormone disruption BMC Vet Res 13 120-31
[7] Aimin C, Kimberly Y, Stephen A R et al 2014 Prenatal Polybrominated Diphenyl Ether Exposures and Neurodevelopment in U.S. Children through 5 Years of Age: The HOME study Environ Health Persp 122 856-62
[8] Chris E T 2008 Overview of toxicological aspects of polybrominated diphenyl ethers: A
flame-retardant additive in several consumer products *Environ Res* **108** 158-67

[9] Costa L G, Giordano G, Tagliaferri S et al 2008 Polybrominated diphenyl ether (PBDE) flame retardants: Environmental contamination, human body burden and potential adverse health effects *Acta Biomed* **79** 172-83

[10] Zhou Y, Chen Q, Du X et al 2016 Occurrence and trophic magnification of polybrominated diphenyl ethers (PBDEs) and their methoxylated derivatives in freshwater fish from Dianshan Lake, Shanghai, China *Environ Pollut* **219** 932-8

[11] Doucet J, Tague B, Arnold D L et al 2009 Persistent organic pollutant residues in human fetal liver and placenta from Greater Montreal Quebec: A longitudinal study from 1998 through 2006 *Environ Health Persp* **117** 605-10

[12] Chen A, Yolton K, Rauch S A et al 2014 Prenatal polybrominated diphenyl ether exposures and neurodevelopment in U.S. children through 5 years of age: The HOME study *Environ Health Persp* **122** 856-62

[13] Bentuo X, Minghong W, Mingnan W et al 2017 Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs in human serum from Shanghai, China: A study on their presence and correlations *Environ SCI Pollut Res* **25** 3518-26

[14] Zhang S W, Xia X H, Xia N et al 2013 Identification and biodegradation efficiency of a newly isolated 2,20,4,40-tetrabromodiphenyl ether (BDE-47) aerobic degrading bacterial strain *Int Biodeter Biodegr* **76** 24-31

[15] Tang S Y, Yin H, Zhou S et al 2016 Simultaneous Cr(VI) removal and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) biodegradation by Pseudomonas aeruginosa in liquid medium *Chemosphere* **150** 24-32

[16] Chen J, Wang C, Shen Z J et al 2017 Insight into the long-term effect of mangrove species on removal of polybrominated diphenyl ethers (PBDEs) from BDE-47 contaminated sediments *SCI Total Environ* **575** 390-9

[17] Huang H L, Wang S, Lv J T et al 2016 Influences of artificial root exudate components on the behaviors of BDE-28 and BDE-47 in soils: Desorption, availability, and biodegradation *Environ SCI Pollut Res* **23** 7702-11

[18] Lozupone C and Knight R 2005 A new phylogenetic method for comparing microbial communities *Appl Environ Microb* **71** 8228-35

[19] Lu L, Wei Z, Lin X et al 2011 Effect of decabromodiphenyl ether (BDE 209) and dibromodiphenyl ether (BDE-15) on soil microbial activity and bacterial community composition *J Hazard Mater* **186** 883-90

[20] Chong C W, Silvaraj S, Supramaniam Y et al 2018 Efect of temperature on bacterial community in petroleum hydrocarbon-contaminated and uncontaminated Antarctic soil *Polar Biology* **41** 1763-75

[21] Yen J H, Liao W C, Chen W C et al 2009 Interaction of polybrominated diphenyl ethers (PBDEs) with anaerobic mixed bacterial cultures isolated from river sediment *J Hazard Mater* **165** 518-24