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Microbiological study on bioremediation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) contaminated soil by agricultural waste composting

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Abstract This paper studied the degradation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in contaminated soil under composting and natural conditions, respectively. BDE-47 residue in agricultural waste-composting pile was determined during 45-day composting. The microbial communities were determined by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE), and the relationships between the DGGE results and physico-chemical parameters were evaluated by redundancy analysis (RDA) and heatmap-clustering analysis. The results showed that the degradation rate of BDE-47 was significantly higher in agricultural waste-composting pile compared with control group, which was enhanced up to almost 15 % at the end of composting. There were different environmental factors which affected the distribution of composting bacterial and fungal communities. The bacterial community composition was more significantly affected by the addition of BDE-47 compared with other physico-chemical parameters, and BDE-47 had stronger influences on bacterial community than fungal community during the composting. Meanwhile, the most variation in distribution of fungal community was explained by pile temperature.

Keywords BDE-47 · Degradation · Contaminated soil · Composting · PCR-DGGE · Community composition

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Introduction

Polybrominated diphenyl ethers (PBDEs) are a variety of persistent organic pollutants (POPs), which belong to the group of brominated flame retardants (BFRs) in response to the ban of previously used flame retardants, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) (Król et al. 2012). In consequence of being physically added into products (Chen et al. 2009), electronic, building material, textiles, polyurethane vehicles, and plastic (Kim et al. 2007; Nouira et al. 2013; Zou et al. 2007), plus no chemical bond between PBDEs and product materials (Zhang et al. 2013), PBDEs have been released and transported ubiquitously (Xie et al. 2014). Among them, a commercial penta-brominated diphenyl ether (PeBDE) flame-retardant mixture, whose main component is 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), has been extensively used for years (Liu et al. 2012; Wang et al. 2013; Yen et al. 2009).

BDE-47 usually can cause great concern due to its extensive occurrence and unfavorable effects. For its high hydrophobicity and high octanol/water partition coefficient (K_{ow}), BDE-47 tends to accumulate in soil, especially in e-waste disposal and recycling sites (Xie et al. 2014; Xin et al. 2012). Recent research in humans have suggested that exposure to BDE-47 is related to a higher risk of certain attention-deficit hyperactivity disorder symptoms and social competence in postnatal children

(Currier et al. 2015). Moreover, the mechanistic studies show that monohydroxylated metabolites of BDE-47 have greater influence than the parent BDE-47 in modulating γ-amino butyric acid (GABA) and α4β2 nicotinic acetylcholine (nACh) receptor function, changing spontaneous activity and cell viability in cultured cortical neurons, and competing with thyroxine (T4) for binding to human transthyretin (TTR) (Hamers et al. 2008; Hendriks et al. 2010; Kim et al. 2010), which indicate that BDE-47 increases the neurotoxic potential by oxidative metabolism (Feo et al. 2013). Therefore, the accumulation of BDE-47 in soil is regarded as a serious environmental problem.

Biodegradation is identified as a possible way of removing BDE-47 from the soil (Xin et al. 2014). Aerobic microorganisms (such as *Pseudomonas stutzier*) in soil can degrade BDE-47 by opening loop through oxidization (Sun et al. 2016). However, the BDE-47 which is contained in soil could hardly be biodegradated under natural condition due to some limiting factors, such as the lack of nitrogen (Zhang et al. 2014). In the meantime, composting is frequently used as an enhanced biotechnological process which mainly depends on the microorganisms to get better remediation and degradation of organic matters (Barrena et al. 2008; Raj and Antil 2011). Thus, it is considered to be feasible to degrade BDE-47 by composting. Additionally, inadequate research has examined the fate of BDE-47 in aerobic conditions in soil system (Xin et al. 2014; Zhang et al. 2013). Consequently, this experiment was designed to study the biodegradation of BDE-47 in aerobic composting process, in which could provide useful information for regarding composting as an efficiency method to remediate the soil contaminated by BDE-47 in engineering practice.

In the present paper, we conducted a study on the biodegradation of BDE-47 in soil through 45-day agricultural waste composting. Physico-chemical parameters were measured not only to ensure the compost quality (Benito et al. 2006) but also to determine whether they are associated with the changes of bacterial and fungal community compositions. The residue of BDE-47 was determined to examine the effect of composting on the degradation of BDE-47. Microbial community changes were also studied by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE), which have been successfully applied in detecting dynamic development of microbial communities (Cahyani et al. 2003; Ishii and Takii 2003). Quantifying those relationships between physico-chemical parameters and microbial community compositions was revealed by redundancy analysis (RDA) and heatmap clustering analyses, which would achieve a deeper understanding of the degradation of BDE-47 process during the composting.

Materials and methods

Composting materials and sampling setup

The raw soil was collected from Yuelu Mountain (Changsha, China), and then, it was air-dried and ground to pass through a 40-mesh screen (0.38 mm) to remove coarse plant debris. Bran was used to adjust the initial total nitrogen content in composting piles (Chen et al. 2014). Air-dried rice straw was cut into 1.0–1.5-cm lengths to be used as the recalcitrant organic composting material (Chen et al. 2015). Several kinds of vegetables (such as cabbage and celery) were chopped into 10–20-mm pieces as easy-metabolizable materials. BDE-47, 3,3′,4,4′-tetrabromodiphenyl ether (BDE-77), and decachlorobiphenyl (PCB-209) were all purchased from AccuStandard Inc. (New Havenand, USA). The stock solutions of BDE-47 (500 ppm), BDE-77 (1 ppm), and PCB-209 (10 ppm) were prepared in isooctane and stored under refrigeration at 4 °C. Approximately 100 g of the sieved soil was spiked with 20 mL of acetone and 12.5 mL of the BDE-47 stock solution as initial contaminated soil. Then, the initial uncontaminated soil was gradually added to a specimen container in 100-g aliquots and mixed with the contaminated soil, and this procedure was repeated until the entire amount of about 3.9-kg uncontaminated soil was added. Then, the acetone was left to evaporate under a flow hood. Two experimental composting systems (piles A and B) were both conducted with about 20-kg composting materials (moist weight). Soil, rice straw, vegetables, and bran were mixed at a ratio of 9:11:3:2 (moist weight). The pile A was free from BDE-47 contamination, while the composting material in pile B was added with 2-kg BDE-47 contaminated soil to replace the same amount of raw soil, and pile C only contained 9.5-kg raw soil and 2-kg BDE-47 contaminated soil without any other composting materials as control group. The initial concentration of BDE-47 in compost mixture was 0.366 mg/kg (dry weight). Simultaneously, the BDE-47 concentration in pile C was 0.369 mg/kg (dry weight). The experiment of piles A, B, and C were all under dark conditions to prevent the effects of light degradation of BDE-47 (Schenker et al. 2008). The mixture was adequate homogenization and good heat preservation for self heating. Initial organic matter (OM) content was 550 g/kg (dry weight), and TOC/TN ratio was 29.2:1. The water content was monitored every 3 days with the initial value of 50 %, and some water was added to maintain appropriate moisture. In order to provide enough aeration, the piles were turned twice a week during the first 2 weeks and once a week afterward.

The experiment was carried out for 45 days. Samples were collected on days 1, 3, 6, 10, 15, 20, 30, and 45, respectively. Three subsamples were taken at each sampling occasion from the different places of composting pile and then mixed for parameter analysis; parts of them

were freeze-dried, ground, and sieved through a 200-mesh sieve for Soxhlet extraction. Samples for total DNA extraction were stored at −20 °C before used.

Composting physical and chemical parameters

Temperature was monitored by inserting a thermometer into different points of the piles and calculating average values, while the pH was determined with a digital pH meter, after the fresh sample was mixed with deionized water at a ratio of 1:10 (*w*/*v*) and mechanically shaking at 200 rpm for 40 min. Organic matter was determined by subtraction of ash content (Lu et al. 2014). Moisture content was measured after drying the fresh samples for 24 h at 105 °C, and the dried samples were analyzed for TOC by dry combustion at 550 °C. TN content was determined by Kjeldahl digestion (Nelson and Sommers 1980). Water-soluble carbon (WSC) concentration was measured with a compost extract (water to wet compost ratio of 10:1) in a total organic carbon analyzer (TOC-5000 A, Shimadzu, Japan) after centrifuging at 12,000 rpm for 15 min and filtering through a 0.45-μm membrane filter (Serra-Wittling et al. 1995). Dehydrogenase activity (DA) was measured with the substrate of 3 % of 2,3,5-triphenyltetrazolium chloride (Brzezińska et al. 1998).

BDE-47 extraction, purification, and determination

Composting samples were freeze-dried, ground, and then sieved through a 200-mesh stainless steel sieve. The prepared samples were spiked with 50 μL PCB-209 as surrogate standard before extraction, then were Soxhlet extracted with 100 mL acetone:hexane mixture (1:1) for 48 h. One gram of activated copper powder was also added to remove elemental sulfur which was contained in soil. One milliliter of isooctane was added to the extract liquor and then dried by a rotary evaporator (R205B, SENCO, Shanghai, China). At last, the eluent was concentrated under nitrogen flow to about 1 mL of final volume.

Cleanup was firstly performed with Cleanert Florisil solid-phase extraction column (1000 mg/6 mL; Agela Technologies). The column was washed with 5 mL *n*-hexane/acetone (9:1 *v*/*v*) and 5 mL *n*-hexane to activate. Elution was first carried out by collecting 10 mL of *n*-hexane and then collected 10 mL of 1:1 *n*-hexane/dichloromethane (*v*/*v*) (Binelli et al. 2007). The two portions of eluent were mixed and then concentrated under gentle nitrogen stream with a final volume of ~1 mL. A known amount of BDE-77 was added to all the samples as the internal standard prior to analysis (Wang et al. 2011).

Sample analysis was performed with Shimadzu model 2010 gas chromatograph (GC) coupled with a model QP2010 mass spectrometer (MS; Shimadzu, Japan). The GC-MS was applied with electronic ionization (EI) in selective ion monitoring mode with helium as reagent gas. A DB-5MS silica capillary column (30 m × 0.25 mm i.d. and 0.25-μm film thickness) was used to separate the BDE-47. The inlet was set to a temperature of 280 °C. The oven temperature program was held at 70 °C for 2 min, followed by a ramp of 20 °C/min to 280 °C, and kept for 10 min. Temperatures of the interface and ion source were set at 280 and 230 °C, respectively. A splitless injection of 1 μL sample was injected, and the helium carrier gas flow was 1 mL/min.

DNA extraction and PCR-DGGE

Total genomic DNA was extracted by TIANamp Soil DNA Kit and then was purified by Multifunctional Recovered DNA Purification Kit (BioTeke, USA/CHN) according to the manufacturer's instructions. The fragments of 16S ribosomal DNA (rDNA) and 18S rDNA genes were amplified with bacterial and fungal universal primers, respectively. The primers used for bacteria were 338F (5′-CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) (LaPara et al. 2000; Muyzer et al. 1993), and the primers used for fungi were NS1 (5′-GTAGTCATATGCTTGTCTC-3′) and Fung (5′-ATTCCCCGTTACCCGTTG-3′) (May et al. 2001). The forward primer of each set was used with a GC clamp (May et al. 2001) for 338F and Fung. The PCR reaction mixture was prepared with 1 μL of template DNA, 25 μL ×2 Power Taq PCR Master Mix (Tiangen), and 1 μL of each primer (20 μmol) and adjusted to a final volume of 50 μL with sterile deionized water. The PCR thermal cycling scheme of 16S rDNA was consisted of 5 min at 94 °C for initial denaturation, 35 amplification cycles of denaturation (94 °C for 45 s), annealing (56 °C for 40 s) and extension (72 °C for 40 s), and a final extension of 10 min at 72 °C before holding at 4 °C (LaPara et al. 2000). Amplifications of 18 s rDNA were performed with an initial denaturation for 3 min at 94 °C, 40 amplification cycles of denaturation (94 °C for 1 min), annealing (50 °C for 1 min) and extension (72 °C for 3 min), and a final extension of 10 min at 72 °C before holding at 4 °C (May et al. 2001).

DGGE was carried out using a DCode universal mutation detection system (Bio-Rad, USA). The PCR samples (40 μL) were loaded onto the 1-mm-thick 8 % (*w*/*v*) polyacrylamide gels in ×1 TAE buffer using a denaturing gradient ranging from 35 to 65 % and 20 to 50 % for bacterial and fungal PCR products, respectively. Electrophoresis was operated at 60 °C and 80 V for 14 h. After stained with SYBR Green I nucleic acid gel stain (Molecular Probes, Carlsbad, CA, USA) for 30 min, the gels were visualized with the Gel Doc XR system (Bio-Rad, USA).

Statistical analysis

Parameters were presented as the mean values of triplicates. All physico-chemical parameters of different samplings were tested for normality of variance by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Correlations between physico-chemical parameters and degradation of BDE-47 were also calculated by SPSS 16.0. DGGE banding profiles were detected and digitized using Quantity One software (version 4.5, Bio-Rad Laboratories, USA). CANOCO software v4.5 was used to determinate multivariate relationships between microorganism community compositions and physico-chemical parameters. The similarity among the samples in piles A and B was performed by cluster 3.0 and TreeView-1.16r4.

Results

Physico-chemical parameters and residue of BDE-47

The physico-chemical parameters were shown in Table 1. The WSC of piles A and B showed almost the same change trends on the whole. It increased significantly in the first 6 days for both piles A and B. After then, the content of WSC decreased sharply, and it was lower in pile A at the end of the process.

The DA levels in pile A significantly increased and peaked on day 10 (2.284 mg TPF/g). Similarly, in pile B, DA levels increased from 0.484 mg TPF/g at day 1 to 2.065 mg TPF/g at day 10. Then, the DA levels in piles A and B decreased with final values of 0.786 and 0.824 mg TPF/g, respectively (Table 1). The DA in pile A was higher than B until the 15th day, and afterward, the DA in pile A was lower than B.

The pile temperatures in pile A and pile B were similar. It rose fast in the initial stage of composting. The thermophilic stage was maintained above 50 °C for nearly 10 days, which is long enough to ensure the organic matter stabilization and the pathogenic microorganism suppression (Awasthi et al. 2014), after that the pile temperature decreased gradually.

The pH value rose rapidly in piles A (from 6.79 to 8.67) and B (from 6.83 to 8.61) after a brief drop. The moisture content was adjusted to about 58 % in the initial stage and then to about 48–50 % during the second phase to ensure a suitable condition for bacterial and fungal activities (Liang et al. 2003).

The organic matter has been declining in both piles and reached 288 and 283 mg/kg in the final for piles A and B, respectively (Table 1). The continuing decreasing trend was also demonstrated by the TOC. In pile A, the value of TOC decreased from 330 to 228 mg/kg, and that in pile B decreased from 322 to 227 mg/kg (Table 1). Meanwhile, the TN contents were similar in the two piles during the composting process, and the TOC/TN ratio showed a significant decreasing trend from initial values of around 29 to final values of 17.5 and 18.3 for piles A and B, respectively.

The residues of BDE-47 (representing tetrabrominated diphenyl ether) in piles B and C for 45 days were shown in Fig. 1. Residues (%) indicated the ratio between BDE-47 measured value and initial value. As seen in Fig. 1, it was clear that the removal efficiency of BDE-47 in pile B was superior to that of pile C, while initial concentrations were almost

Table 1 Physico-chemical parameters during composting

Pile	Days	WSC (mg/g)	DA (mg TPF/g)	Temperature (°C)	pH	Moisture (%)	OM (%)	TOC (g/kg)	TN (g/kg)	TOC/TN
A	1	20.51 d	0.578 a	19.6 a	6.79 a	58.41 cd	53.93 f	330.8 e	11.42 a	28.86 g
	3	23.37 de	1.614 e	38.5 c	6.51 a	54.56 bc	48.42 e	327.2 e	12.02 b	27.24 f
	6	24.92 e	2.072 f	55.3 f	8.67 e	59.78 d	39.95 d	301.4 d	11.96 b	25.06 e
	10	21.63 d	2.284 g	51.6 ef	8.39 de	58.13 cd	35.81 c	267.7 c	11.60 a	23.14 d
	15	16.49 c	1.386 d	48.5 de	8.14 cd	56.32 cd	33.42 bc	258.2 bc	12.14 bc	21.32 c
	20	14.04 b	1.144 cd	44.4 d	8.04 cd	53.36 bc	31.49 ab	251.4 bc	12.22 bc	20.52 bc
	30	11.57 ab	0.994 c	32.7 b	7.71 bc	51.29 ab	30.11 ab	239.2 ab	12.38 c	19.23 b
	45	10.30 a	0.786 b	22.6 a	7.55 c	48.47 a	28.84 a	227.6 a	13.14 d	17.36 a
B	1	20.11 cd	0.484 a	19.6 a	6.83 a	58.12 cd	54.07 f	321.7 e	11.09 a	29.29 e
	3	21.68 de	1.502 e	37.6 c	6.61 a	55.43 bc	48.54 e	313.2 de	11.27 ab	27.72 de
	6	24.04 d	1.936 f	53.1 e	8.61 e	59.91 d	41.62 d	296.6 d	11.34 ab	26.19 d
	10	21.96 de	2.065 f	50.5 e	8.48 de	57.96 cd	36.50 c	264.5 c	11.39 ab	23.22 c
	15	17.81 c	1.337 d	50.1 e	8.25 cd	55.81 bc	34.55 bc	253.1 bc	11.61 b	21.81 bc
	20	14.62 b	1.249 cd	44.4 d	8.16 cd	55.14 bc	32.82 ab	246.5 abc	11.75 b	21.02 b
	30	10.22 a	1.121 c	33.5 b	7.85 bc	52.63 b	31.24 a	238.3 ab	11.67 b	20.35 b
	45	8.83 a	0.824 b	22.6 a	7.7 b	49.04 a	29.75 a	226.9 a	12.35 c	18.26 a

For each pile, values in a column followed by different letters are statistically different according to S-N-K test ($p < 0.05$)

[a] *WSC* water soluble carbon, *DA* dehydrogenase activity

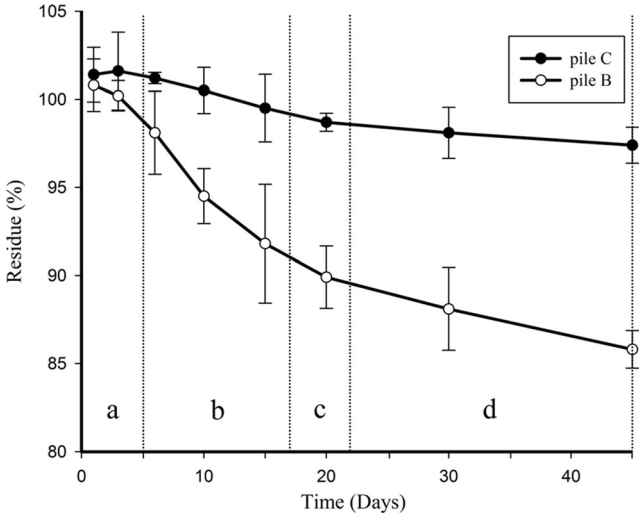

Fig. 1 Degradation of BDE-47 in piles B and C for 45 days. The *bars* represent the standard deviations of mean values (n = 3). **a–d** Heating, thermophilic, cooling, and maturity period during the composting, respectively

same. The residue rate of BDE-47 in pile C was found to be more than 97 % on day 45. On the other hand, BDE-47 removal presented a significant increasing trend in pile B. It was around 15 % of BDE-47 that was degraded by the end of the composting. And, decomposition rate was faster in the thermophilic phase of composting.

The Pearson's correlation analysis of BDE-47 residues and physico-chemical properties was shown in Table 2. Positive correlation was found among WSC, moisture, OM, TOC, and TOC/TN. No significant correlation was found among DA, pH, and temperature, and only TN was significantly negative correlated with BDE-47 residues.

Microbial community variation

The DNA band profiles of 16S rDNA and 18S rDNA were shown in Figs. 2 and Fig. 3, which implied the information of bacterial and fungal community compositions in piles A and B, respectively. It was obvious that the number of DGGE bands for bacteria was significantly larger than that for fungi. In pile A, the band number of bacterial DGGE profiles started with low value, and peaked on day 3, then decreased in the thermophilic phase, at last showed a stabilization trend. Similar changes were also observed in pile B. However, the band number of fungal DGGE profiles presented different trends. In general, it presented an upward trend after fall during the whole process, with the lowest value in the thermophilic phase.

The bacterial and fungal DGGE profiles were both analyzed by redundancy analysis to test the relationship between physico-chemical parameters and microbial community compositions. These results were shown in Figs. 4 and 5. The first two axes for bacterial DGGE fingerprints explained 18.0 and 14.8 % of the variation between the environment and species data, while 48.6 and 16.0 % of the variation were explained by the first two axes for fungal species and environment data. It was found that the samples from pile A were all in the second and the third quadrants of RDA ordination diagram, while the samples from pile B were all in the first and the fourth quadrants. Besides, the connecting arrowed vector lines representing the BDE-47 (Fig. 4) and temperature (Fig. 5) were both the longest.

For fingerprinting analysis by heatmap (Figs. 6 and Fig. 7), the bacterial band intensity did not show significant differences for all the samples, but the samples for fungi showed clearly differences. The tree view revealed similarity coefficient between the banding patterns from those samples. Similarity of bacteria was lower than that of fungi with final values of 0.34 and 0.45, respectively. As shown in Fig. 6, for the tree view of bacteria, the samples of A1, B1, and B3 had far clustering distance from the rest of samples. Meanwhile, the heatmap for fungi (Fig. 7) showed that the band intensity has followed basically a drop before rising trend in piles A and B.

Discussion

Assessment of compost maturity

Composting had advantages compared to other types of technologies, including relatively low costs, simplicity of operation, and design (Namkoong et al. 2002). During composting, the physico-chemical parameters should be determined because inappropriate parameter may retard or inhibit microbial activity (Benito et al. 2006). According to some relevant researches, those parameters we chose could not only reflect the whole changing process of composting well but also affect the

Table 2 Correlation coefficients of BDE-47 residues and physico-chemical properties

	WSC	DA	Temperature	pH	Moisture	OM	TOC	TN	TOC/TN
Residue	0.881*	0.182	0.084	−0.147	0.788*	0.945*	0.986*	−0.905*	0.987**

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

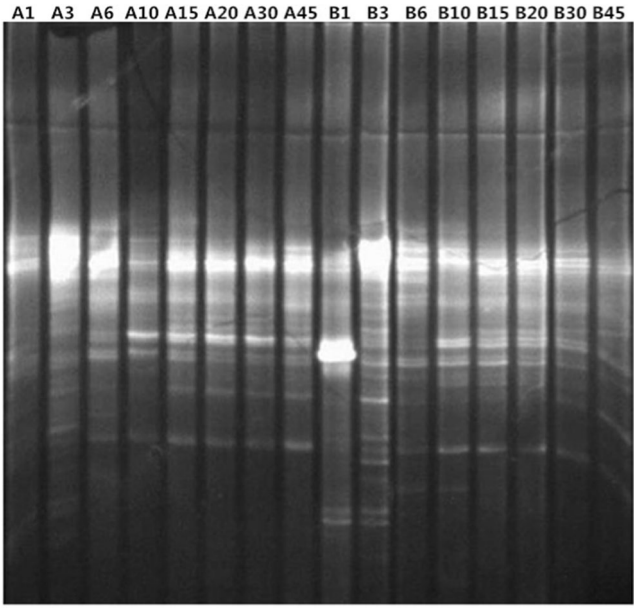

Fig. 2 Denaturing gradient gel electrophoresis (DGGE) profiles of amplified 16S rDNA fragments from piles A and B. The *letter* denoted the pile type, and the *number* referred to the age of the sample

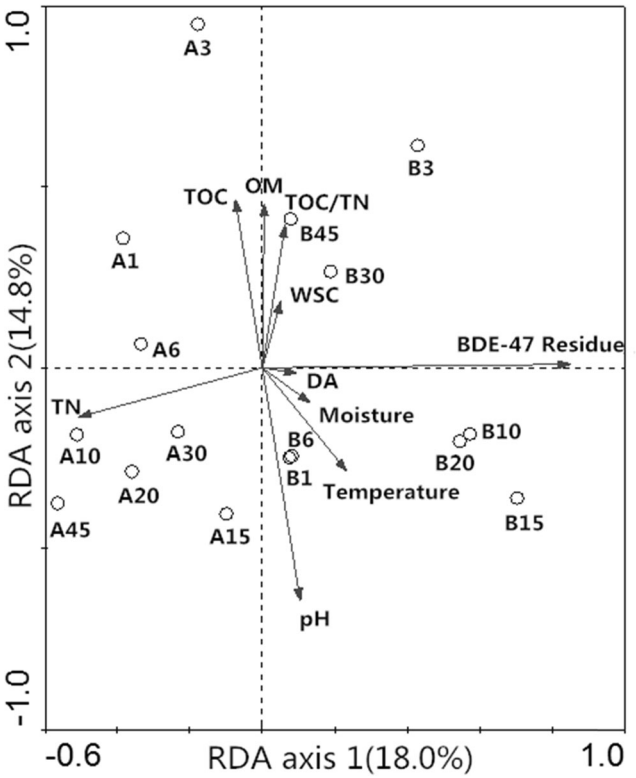

Fig. 4 Redundancy analysis (RDA) of bacterial community. Environmental variables and bacterial species were represented as *arrows* and *empty circles*, respectively

microbial activities greatly (Liang et al. 2003). The conventional parameters for the evaluation of compost quality such as WSC and TOC/TN ratio have showed good criterion of the end products for composting. It was commented that the WSC content decreased to below 17 mg/g when the composts were mature (Bernai et al. 1998). Meanwhile, TOC/TN ratio value lower than 20 was thought to be the threshold value for compost maturity (Garcia et al. 1992). The final values of WSC and TOC/TN ratio for piles A and B were 10.30, 8.83, 17.36, and 18.26 mg/g, respectively. So, the two piles got matured eventually, although the soil of composting for pile B was contaminated.

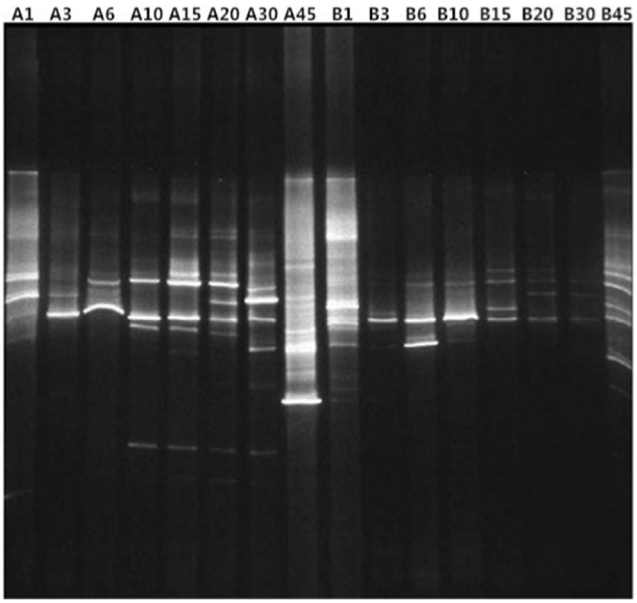

Fig. 3 Denaturing gradient gel electrophoresis (DGGE) profiles of amplified 18S rDNA fragments from piles A and B. The *letter* denoted the pile type, and the *number* referred to the age of the sample

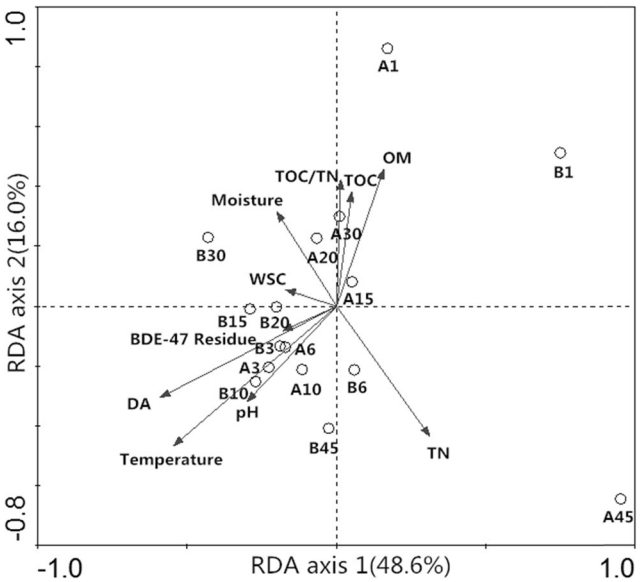

Fig. 5 Redundancy analysis (RDA) of fungal community. Environmental variables and fungal species were represented as *arrows* and *empty circles*, respectively

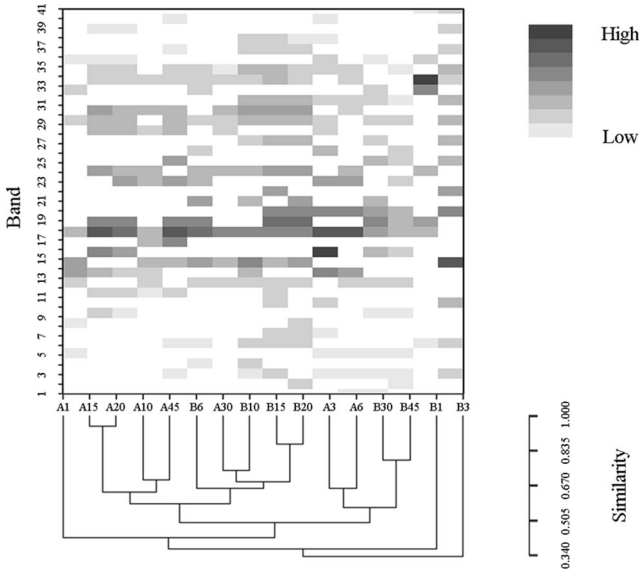

Fig. 6 Heatmap and multiple-sample similarity analyses of bacterial community. The *darker color* indicates the higher relative band intensity

The degradation of BDE-47

The experiment using the composting with contaminated soil found that BDE-47 could be degraded under aerobic condition. In fact, the aerobic PCB-degrading species *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400 (Robrock et al. 2009) were found to be capable of transforming PDBE, active toward some fraction of all of the mono-BDE through penta-BDE congeners. According to Fig. 1, the initial residue of BDE-47 was above 100 %, which was due to the deviation of BDE-47 measured by internal standard method. Considering that the BDE-47 residues in piles B and C were 85.8 and 97.4 %, respectively, composting could effectively promote the degradation of BDE-47. Therefore, it was feasible to restore the BDE-47-contaminated soil by composting. In Fig. 1, according to the temperature changes of the pile B, the composting process was roughly divided into four stages, which were heating, thermophilic, cooling, and maturity period (Fogarty and Tuovinen 1991). Residual curve slope in pile B changed obviously in different phases, which signified that the degradation rate of BDE-47 was variational during the composting. This may be due to the changing of preponderant microbe in different stages and thus affected the removal of BDE-47 (Yen et al. 2009). In the Pearson's correlation analysis, correlations between different physico-chemical parameters and the residue of BDE-47 were discrepant. It was because of the difference of physical and chemical parameter value variation trends in the composting process. The values of WSC, moisture, OM, TOC, and TOC/TN were all presented downward trends during the composting, which agree with the changing trend of BDE-47 residual rate. Meanwhile, the values of DA, pH, and temperature did not show similar downward trends to the residual rate of BDE-47. Moreover, the value of TN presented upward trend during the composting, which was opposite to the changing trend of BDE-47 residual rate. Therefore, there are significant different correlations between different physico-chemical parameters and the residue of BDE-47.

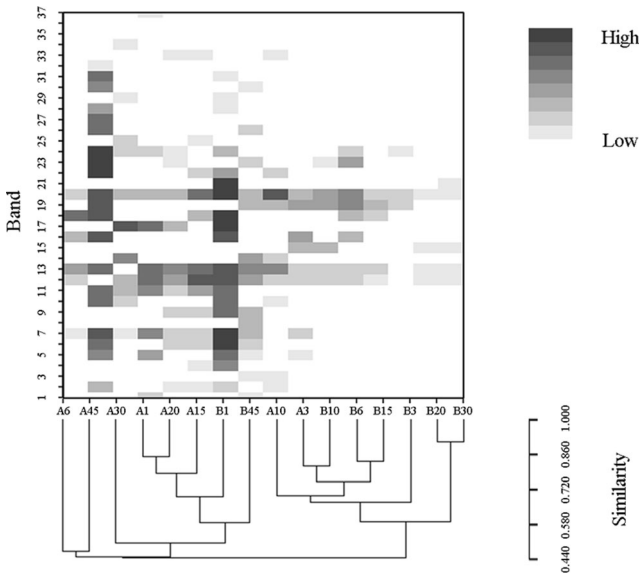

Fig. 7 Heatmap and multiple-sample similarity analyses of fungal community. The *darker color* indicates the higher relative band intensity

The community of microorganism

Samples taken at different time intervals could be simultaneously analyzed through DGGE, which was a powerful tool for monitoring community behavior (Muyzer and Smalla 1998). In this research, the difference of DGGE profiles among the samples of different ages indicated that microbial communities changed sharply throughout the whole composting process. For fungi, the numbers of bands were significantly less than bacteria; this was mainly because the species of bacteria were far more than fungi in the composting system (Deng et al. 2015). Different changing trends between bacteria and fungi could be the result of different responses to the physical and chemical parameters during the composting (Zhang et al. 2011).

The increases or decreases of a given band intensity in different samples were able to indicate a relative increase or decrease in abundance of this species (Marschner et al. 2001). Therefore, the relative band intensities which were calculated via Quantity One could manifest how the external environment parameters exerted influences on the changing of microbial community compositions in the agricultural waste compost system (Zhang et al. 2011).

In the two-dimensional sequence diagrams of composting samples and process factors, since the length of the connecting arrowed vector line represents the correlation coefficient size of the samples with process factors (Deng et al. 2015), it was evident that the distribution of bacterial community was mainly affected by BDE-47. Bacteria could grow using BDE-47 as the sole carbon source (Zhang et al. 2013), and they may have more advantageous of utilizing the BDE-47. Therefore, the addition of BDE-47 may change the structure of bacterial community and make the samples of piles A and B being in different quadrants of bacterial RDA ordination diagram. Moreover, for the distribution of fungal community, the main influential factor was temperature.

The angle between the arrowed connecting line and ordination axes represents the correlation factor size of the factor and the ordination axes, and the smaller the angle is, the greater the correlation is (Deng et al. 2015). Thus, the analysis results showed that the correlation of the process factor and the second ordination axes was significantly higher than that of the process factor and the first ordination axes in general, which indicated that the second ordination axes could better reflect the relationship between microbial communities and compost factor. In addition, the projection of samples in connecting arrowed vector lines testified that the value of the samples in pile B corresponding BDE-47 increased first then decreased roughly (Fig. 4), which was consistent with the trend of dehydrogenase activity. However, this obvious phenomenon was not found in Fig. 5. Therefore, the addition of BDE-47 played an important role on the bacterial community composition (Tian et al. 2014) but may have little effect in fungal community composition.

Clustering analysis was employed to reveal the relationship among the composting samples depending on bacterial and fungal DGGE fingerprints. Cluster analysis showed that those samples from piles A and B clustered together with similarity (Figs. 6 and Fig. 7). There was no clear relation among samples collected at different times. This may be due to the rapid change of microbial population composition in the composting process (Klamer and Bååth 1998). The comparison of the same collection time samples from composts A and B indicated that the earlier stage samples (days 1, 3, and 10) showed lower bacterial community similarity than that of fungi, while in the later stage (days 15, 20, 30, and 45), bacterial community similarity was higher. It was probably because bacteria in earlier stage was more sensitive to BDE-47 than fungi; thus, the concentration of BDE-47 may be the main factor that resulted in the change of bacterial community composition in pile A (Xu et al. 2012). However, in the later stage, when BDE-47 concentration decreased and bacteria adaptability was improved, the influence of BDE-47 decreased.

The fungal band intensities in A1, A45, B1, and B45 were apparently higher than other samples. This may be because the majority of fungi were mesophiles, which grew between 5 and 37 °C, with an optimum temperature of 25–30 °C (Tuomela et al. 2000). Along with the composting, fungal population reduced gradually, and there was a substantial reduction in fungal population as pile temperature reached about 50 °C due to the inhibition of temperature (Pietikäinen et al. 2005). The fungal community would almost disappear entirely when the pile temperature was more than 60 °C (Tuomela et al. 2000). But, the population of fungi recovered as the temperature dropped below 45 °C. This was also compatible with the result of RDA analysis, in which temperature was a main effect factor for the distribution of fungal community.

In conclusion, the present study showed a suitable method by using agricultural wastes composting to degrade BDE-47 in contaminated soil. The experiments indicated that BDE-47 could be effectively degraded in the compost compared to natural conditions. There were different environmental factors affecting composting bacterial and fungal community compositions. The predominant effect factors for the distribution of bacterial and fungal community compositions were the addition of BDE-47 and temperature, respectively. Furthermore, the addition of BDE-47 had stronger influences on bacterial community than fungal community, and high temperature may inhibit the growth of fungi.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The article is an original paper, is not under consideration by another journal, and has not been published previously. All authors read and approved the final manuscript.

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