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ORIGINAL PAPER

Manure amendment reduced plant uptake and enhanced rhizodegradation of 2,2′,4, 4′-tetrabrominated diphenyl ether in soil

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Abstract

To test whether manure amendment in soil reduces plant uptake of persistent organic pollutants, carrot (*Daucus carota L.*) was used as a model plant and 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) was selected as a model persistent organic pollutant to conduct a pot experiment with contaminated soil amended by composted pig manure. The results showed that the concentration and bioconcentration factors (BCFs) of BDE-47 in the edible part of carrot significantly decreased from 229.7 ± 28.2 to 43.4 ± 20.4 ng g^{-1} and from 1.86 ± 0.5 to 0.15 ± 0.03, respectively, with increasing composted pig manure dose from 0 to 4%. Organic matter (OM) derived from composted pig manure played a dominant role in reducing persistent organic pollutant bioavailability in soil. Composted pig manure amendment and carrot cultivation jointly altered the bacterial community composition in soil, especially the rhizosphere. Rhizodegradation of BDE-47 was enhanced from 8.6 to 28.5% with increasing composted pig manure dose from 0 to 4%, corresponding to increased soil microbe diversity and polybrominated diphenyl ether-degrading bacteria (*Sphingomonas*, etc.) abundance in the rhizosphere. This study is the first, to the best of our knowledge, to provide an effective agronomic strategy of manure amendment to reduce plant uptake and simultaneously enhance rhizodegradation of persistent organic pollutants in soil, and thus potentially reduce human health risks through dietary intake.

Keywords Manure · Polybrominated diphenyl ethers · Uptake · Reduction · Rhizodegradation · Bacterial community composition

Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of semi-volatile, persistent, and bioaccumulative contaminants. Their toxicity and tendency for global transport have led to international bans or restrictions on their use (Ockenden et al. 2003) as illustrated by the inclusion of some polybrominated diphenyl ethers as persistent organic pollutants under the Stockholm Convention (Wang et al. 2014). As an additive-type flame retardant, polybrominated diphenyl ethers could be released into the surroundings during their production, usage, and disposal, and then be spread in the environment through atmospheric dry and wet deposition, and farm application of sewage sludge (Hites 2004; Li et al. 2014b; Robinson 2009), and therefore have frequently been detected in the environment (Elliott et al. 2015; Guo et al. 2007; Peng et al. 2007; Vrkoslavová et al. 2010; Wang et al. 2011c; Yu et al. 2015; Zhang et al. 2013a; Zhu et al. 2015). Soil has been considered a major sink for organic pollutants due to its high sorption capacity. E-waste disassembling and farm application of sewage sludge may bring a number of organic pollutants, including polybrominated diphenyl ethers, to soil (Li et al. 2015). Polybrominated diphenyl ethers can be adsorbed in soil, degraded by microorganisms, or taken up by plant (Chow et al. 2015; Huang et al. 2010, 2011; Wang et al. 2015), further bio-accumulate through the food chain with

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potentially harmful effects to human health (Bocio et al. 2003; Hu et al. 2010; Mahmood et al. 2015; Vrkoslavová et al. 2010). Toxicological studies have demonstrated that polybrominated diphenyl ethers have endocrine-disrupting properties and may cause liver and thyroid toxicity in humans (Darnerud et al. 2005; Jiang et al. 2012). The lipophilicity and ubiquitous presence of polybrominated diphenyl ethers may also pose a threat to the health of aquatic or terrestrial ecosystem (Crosse et al. 2012; Zhang and Liu 2014). Meanwhile, slightly contaminated soil are still in use to ensure agricultural product supply due to cultivated land shortage (Chow et al. 2015; Zhang et al. 2013a). Due to the potential environmental risk associated with accumulation of polybrominated diphenyl ethers in plant-soil systems, feasible solutions for inhibiting plant uptake of contaminants need to be carefully considered in order to obtain safe agricultural products grown in contaminated soils.

Manure is widely used in traditional agronomic practices as fertilizer to improve crop quality and yield (e.g., production per unit of agricultural land) by returning nutrients to the soil (Choi et al. 2001; Zhang et al. 2018). Appropriate application of manure can develop a sustainable and productive agricultural system owing to its improvement of edaphic properties, especially soil organic matter (SOM) (Wen et al. 1992; Liu et al. 2010). SOM is a key component for many biogeochemical processes and a determining factor for the behavior of organic contaminants in soil ecosystems (Liu et al. 2012b). Application of glomalin-related soil protein led to reduced SOM in soil, which enhanced the availability of polycyclic aromatic hydrocarbons (PAHs) (Gao et al. 2017). Thus, we hypothesized that application of manure may increase SOM and thus reduce plant uptake of contaminants in soil. Recently, in terms of reducing plant uptake of persistent organic pollutants, biochar and cationic surfactant have been studied (Hurtado et al. 2016; Khan et al. 2013; Lu and Zhu 2009; Song et al. 2016; Yu et al. 2009). Although manure is more commonly used in agricultural production than biochar and cationic surfactant, few studies have investigated the effect of manure on plant uptake of polybrominated diphenyl ethers. On the one hand, manure application is likely to increase the content of SOM, which might enhance the adsorption of polybrominated diphenyl ethers in soil and consequently reduce uptake by plants. On the other hand, microbial activities play a very important role in affecting the behavior of organic pollutants in soils, such as degradation and formation of non-extractable residues (Macleod and Semple 2003). Polybrominated diphenyl ethers could be biodegraded by *Sphingomonas*, *Pseudomonas*, *Burkholderia*, and *Rhodococcus* under aerobic or anaerobic conditions (Liu et al. 2012a; Shi et al. 2013; Stiborova et al. 2015). Manure may improve soil porosity and nutrient availability, resulting in improved soil microbial biomass, soil enzyme activity, and soil microbial diversity (Liu et al. 2010; Luo et al. 2009), which might potentially lead to enhanced biodegradation of polybrominated diphenyl ethers in soil. However, to the best of our knowledge, there is no literature analyzing the effect of manure on the biodegradation of polybrominated diphenyl ethers in soil. Meanwhile, there is evidence supporting rhizosphere effects on the degradation of persistent organic pollutants (Lu et al. 2015; Song et al. 2016; Wang et al. 2011a; Zhao et al. 2017), but none specifically addressing the influence of manure amendment on rhizodegradation of persistent organic pollutants. Therefore, it is necessary to investigate the impact of manure on the bioavailability and biodegradation of persistent organic pollutants in plant-soil systems.

The present study tests the hypothesis that the application of manure will increase the soil organic matter content and therefore increase the microbial activity to enhance the degradation of organic pollutants in soil and reduce the uptake of pollutants by plant. Therefore, a pot experiment was conducted where BDE-47, a typical congener of polybrominated diphenyl ethers, was selected as a model compound because of its high toxicity and extensive occurrence in the soil environment due to exposure, intense absorption in soil, or degradation of more highly brominated polybrominated diphenyl ethers (Hakk et al. 2010; Li et al. 2014b). Carrot (*Daucus carota L.*), a tuberous vegetable, was used as the model plant because the edible part is in direct contact with the contaminated soil raising concerns over contamination (Chow et al. 2015; Wang et al. 2011b). Soil was amended with compost prepared from pig manure and it is used as an agronomical practice since it is a nutrient source for plants (Choi et al. 2001).

Materials and methods

Chemicals

Standards of BDE-47 (2, 2′, 4, 4′-tetrabrominated diphenyl ether), 2, 4, 4′-tribrominated diphenyl ether (BDE-28), 2, 2′, 4-tribrominated diphenyl ether (BDE-17), 4, 4′-dibrominated diphenyl ether (BDE-15), 4-monobrominated diphenyl ether (BDE-4) were obtained from AccuStandard Inc. (New Haven, Connecticut, USA). The main physicochemical characteristics of BDE-47 are: molecular formula, $C_{12}H_6Br_4O$; formula weight, 485.79; water solution, 94.7 μg/L; logKow, 6.39 (Darnerud et al. 2001). All solvents used (n-hexane and acetone) were of HPLC grade and purchased from Merk (Darmstadt, Hesse, Germany). Kieselguhr (0.074–0.15 mm) was purchased from Sigma-Aladdin (Los Angeles, California, USA). Florisil (0.15–0.3 mm) was purchased from TEDIA (Fairfield, Ohio, USA). Anhydrous sodium sulfate, potassium dihydrogen phosphate (KH_2PO_4), ammonium nitrate (NH_4NO_3), potassium sulphate (K_2SO_4), silica gel (0.074–0.15 mm), quartz sand, and concentrated sulfuric acid (analytical reagent grade) were purchased from Sinopharm chemical reagent company (Nanjing, Jiangsu, China).

Soil and composted manure preparation

A loamy soil without detectable polybrominated diphenyl ethers was collected from suburbs of Nanjing, China, dried at ambient temperatures (1.5 ± 0.3% of water content), ground, and passed through a 2-mm nylon sieve. The basic physicochemical characteristics of the soil (dry matter content) are: pH (H_2O), 7.43; total organic C, 4.16 g kg^{-1}; total N, 0.48 g kg^{-1}; total P, 0.57 g kg^{-1}; total K, 19.19 g kg^{-1}; CEC, 17.32 cmol kg^{-1}; and clay 32.8%, silt 56.8%, and sand 10.4%. Composted pig manure without detectable polybrominated diphenyl ethers was collected from suburbs of Yingtan, China. The basic physicochemical characteristics of the composted pig manure are: pH (H_2O), 8.7; total organic C, 175.4 g kg^{-1}; total N, 28.2 g kg^{-1}; total P, 32.9 g kg^{-1}; total K, 45.3 g kg^{-1}.

Pot experiment

An aliquot of 1000 g soil was spiked with 100 mL, 4 mg L^{-1} BDE-47 dissolved in acetone and mixed thoroughly followed by solvent volatilization under a fume hood for 24 h to prepare BDE-47 spiked soil. An aliquot of 960 g spiked soil was mixed homogeneously with 40 g un-spiked soil, 10 g composted pig manure and 30 g un-spiked soil, 20 g composted pig manure and 20 g un-spiked soil, and 40 g composted pig manure, respectively, to give 0 (serve as the control), 1%, 2%, and 4% composted pig manure (*w*/*w*) amended and 384.5 ng g^{-1} BDE-47 contaminated soil with three replicates each. Two layers of dense nylon mesh were placed in the bottom of each porcelain pot to prevent soil components from leeching. Each porcelain pot received a total of 3000 g prepared soil. All soil received mineral nutrients at rates of 100 mg P (KH_2PO_4), 300 mg N (NH_4NO_3), and 200 mg K (K_2SO_4) kg^{-1} soil as base fertilizer in preparation for plant cultivation. Pots of parallel treatments without seeding (plant-free) were set up as blank control.

Seeds of carrot (*Daucus carota L.*) purchased from Ganxin Seeds Company (Ganxin, Jiangxi, China) were sterilized in 5% H_2O_2 (*w*/*w*) solution for 30 min, washed with deionized water thoroughly, and germinated on clean moist filter paper in the dark at 20 °C. In each pot, approximately ten pre-germinated seeds were sown, and 7 days after germination, the seeds were thinned to three. All pots were positioned randomly once every 3 days in the greenhouse. Deionized water was added as required to maintain 30–35% of soil moisture content, corresponding to 60–70% of water holding capacity. The temperature in the greenhouse fluctuated between 25–35 °C in the daytime and 15–25 °C in the nighttime. The moisture in the greenhouse fluctuated between 50 and 75%.

After 90 days, carrots were harvested and separated into aboveground tissue (stem and leaf) and root (edible part). They were fully rinsed with deionized water to remove adhering dust or soil, and then dried with filter paper. All carrot samples were cut into small pieces and individually stored in glass tubes at − 20 °C prior to the determination of BDE-47.

Soil samples were collected from each pot. Rhizosphere soil was collected from the soil adhered tightly to roots and bulk soil was collected from the residual soil (Cui and Yang 2011). In detail, carrot roots were pulled from cultivated soil, shook continuously until the roots were free of large soil particles, then the soil adhered tightly to the root was collected as the rhizosphere soil. Aliquots of samples were stored at − 80 °C for microbial analysis. The rests were ground, sieved with 0.25 mm nylon sieve, and stored at − 20 °C prior to the determination of polybrominated diphenyl ethers, soil properties.

Analysis of BDE-47 and its metabolites

Analysis of BDE-47 and its debrominated metabolites was performed according to a previous study (Xiang et al. 2016) with minor modification. Briefly, BDE-47 and its metabolites were extracted from soil and plant samples by accelerate solvent extraction (ASE200, Dionex, USA) using a mixture of n-hexane and acetone (4:1, *v*/*v*). Afterwards, a purification process was conducted using solid phase extraction (SPE) for separation of interfering co-extracts. A sulfuric acid silica gel SPE column was filled with 0.5 g anhydrous sodium sulfate then 1 g sulfuric acid silica gel and 1 g anhydrous sodium sulfate to purify soil samples. A multi-layer Florisil/sulfuric acid silica gel SPE column was filled, first with 0.5 g anhydrous sodium sulfate and then followed by 0.5 g Florisil, 0.5 g sulfuric acid silica gel and 1 g anhydrous sodium sulfate to purify plant samples. An Agilent 6890A gas chromatograph (Agilent Technologies, USA) coupled with electron capture detector (ECD) was used to quantify the amount of target compounds. A DB-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA) was used for the separation of polybrominated diphenyl ethers. The detector temperature was set at 298 °C. The injection was performed in the splitless mode with 265 °C of injector temperature and 1-μL injection volume.

Microbial diversity analysis

To evaluate bacterial community composition, samples from the rhizosphere soil and bulk soil were analyzed using 16s rRNA sequencing (Vestergaard et al. 2017; Schöler et al. 2017). Genomic DNA was extracted from 1 g soil using a PowerSoil DNA Isolation Kit (MoBio Labs, Solana Beach, CA, USA) following the manufacturer's instruction. The DNA concentration was quantified with a Nanodrop Spectrophotometer (Thermo Scientific). For 16s rRNA analysis, the hypervariable V4-V5 region was amplified by polymerase chain reactions (PCRs) using the universal primer pair 515F (5′-GTGCCAGCMGCCGCGG-3′) and reverse primer 907R (5′-CCGTCAATTCMTTTRAGTT-3′) with unique

12 nt barcode (Angenent et al. 2005). PCR amplification was performed with a 25-μL mixtures, which contained 1× PCR buffer, 1.5 mM $MgCl_2$, each primer at 1.0 μM and 0.5 U of Ex Taq (TaKaRa, Dalian, China), each deoxynucleoside triphosphate at 0.4 μM and 10 ng soil genomic DNA. Triplicate PCR products were pooled for electrophoresis. The band with correct size was excised and then purified by TaKaRa MiniBEST Agarose Gel DNA Extraction Kit 66 (TaKaRa, Dalian, China) and also quantified by Nanodrop Spectrophotometer. The purified amplicons were pooled by equal molar amount from each sample. The sequencing samples were conducted with TruSeq DNA kit following manufacture's instruction.

Processing of the raw sequences was performed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0-dev pipeline (Caporaso et al. 2010). The pair-end reads were merged with FLASH (Magoč and Salzberg 2011). Reads with ambiguous bases, improper primers, and quality score < 20 were discarded before clustering. The resultant high-quality sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE algorithm, version 7.1 (http:// drive5/uparse/). Taxonomic classification of the representative sequence from individual OTU was performed by RDP classifier, version 2.2 (Wang et al. 2007). The downstream analysis was performed in QIIME and R v3.2.1 (Dixon 2003): (a) alpha diversity index expressed as Shannon index was calculated; (b) heatmap analysis was performed using vegan package depending on vegdist and hclust function.

Quality control and statistics

To estimate BDE-47 recoveries in soil and carrot samples, a recovery study was conducted by spiking 0.1 μg of BDE-47 to either 1 g of soil or 1 g of carrot sample (combined aboveground tissue and root). The average recovery of triplicate samples was 92.4 ± 1.8% in the soil samples and 99.1 ± 1.4% in the carrot samples. Together with each extraction batch (12 samples), one procedure blank was carried out. Data were analyzed using a one-way ANOVA (LSD) with a significance level of $p < 0.05$. All statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., USA).

Results and discussion

Uptake of BDE-47 in carrot and dissipation of BDE-47 in soil

After 90 days of carrot planting, BDE-47 concentrations in roots simultaneously decreased with increasing amount of the amended composted pig manure in soil (Fig. 1a, $p < 0.05$). The 4% composted pig manure treatment was particularly effective for reducing uptake of BDE-47 in roots with the reduction of 81.1% compared to the control, due to a difference ascribed to higher amount of SOM than the control (Table S1). Bioconcentration factor (BCF)—the quotient between the plant and soil concentrations—(Bizkarguenaga et al. 2016; Song et al. 2010) of BDE-47 was calculated to further assess the uptake of BDE-47 in the roots (Fig. 1b). Bioconcentration factor values for carrot roots in the composted pig manure treatments decreased from 1.86 ± 0.5 to 0.15 ± 0.03 with increasing dose of composted pig manure (Fig. 1b, $p < 0.05$), indicating that composted pig manure amendment reduced the uptake of BDE-47 in carrot root. Similar result was reported by Bizkarguenaga et al. (2016)

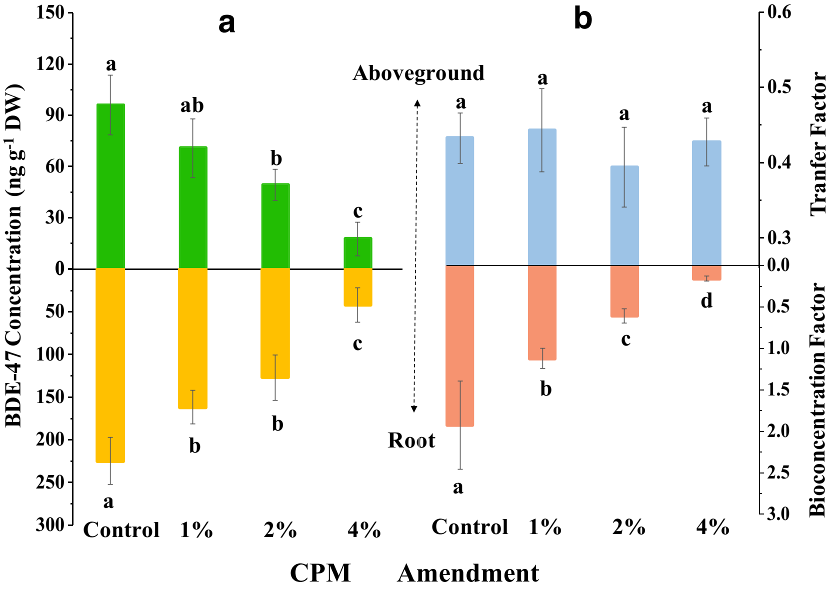

Fig. 1 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) concentrations in the carrot tissues (roots and aboveground tissues: stems and leaves) grown in soil amended with 0 (serve as the control), 1, 2, and 4% composted pig manure (ng g^{-1}, dry weight, **a**). Translocation factors (TF, $C_{\text{aboveground tissue}}/C_{\text{root}}$) and bioconcentration factors (BCF, $C_{\text{root}}/C_{\text{soil}}$) of BDE-47 for carrots amended with 0 (serve as the control), 1, 2, and 4% composted pig manure (**b**). Values are the means ± standard deviation ($n = 3$). Bars with different lowercase letters within the same region indicate significant differences at $p < 0.05$

that lower bioconcentration factor values of polybrominated diphenyl ethers were obtained from carrots grown in compost-amended soils.

BDE-47 concentrations in aboveground carrot tissues decreased with increasing amount of composted pig manure in soil compared with the control (Fig. 1a), indicating that composted pig manure treatments inhibited the transfer of BDE-47 to the aboveground tissues. Translocation factor (TF)—the quotient between aboveground tissue and root concentrations of xenobiotics—is often used to assess the plant's capability to accumulate and translocate xenobiotics (Lu et al. 2015; Wang et al. 2015). Translocation factor values of BDE-47 ranged from 0.40 ± 0.05 to 0.45 ± 0.03 (Fig. 1b), which was consistent with Li's study (Wang et al. 2011b). No difference was observed between the treatment's translocation factor values, which were confirmed by a strong linear correlation between BDE-47 concentrations in the carrot roots and those in the aboveground tissues (Fig. 2, $R^2 = 0.96$, $p < 0.001$). It demonstrated that accumulation of BDE-47 in aboveground carrot tissues may result mainly from the root-to-aboveground transport as it is reported that root uptake and translocation are the main source of BDE-47 in shoots of radishes (Huang et al. 2010). Our results further support lowered human exposure risks to soil contaminations in produce via manure amendment by documenting the clear inhibition of BDE-47 accumulation in both above and below ground carrot tissues with the addition of composted pig manure.

After 90 days of greenhouse cultivation, the BDE-47 concentrations in the composted pig manure amended soil were significantly higher than in the control (Table 1), which might be due to that composted pig manure amendment facilitated adsorption and consequently inhibited the dissipation of BDE-47 in soil. Compared to the plant-free soil, the dissipation of BDE-47 in the bulk soil was enhanced by carrot cultivation (Table 1). The increased dissipation of BDE-47 might be attributed to carrot uptake as well as enhanced microbial degradation in majority soil (Kim et al. 2007; Zhang et al. 2013b). However, carrot uptake was not a dominant factor in the dissipation of BDE-47 in the cultivated soil because the amount of BDE-47 in whole carrot was less than 1% of the initial BDE-47 amount in one pot (Table S3) based on mass balance. Hence, microbial degradation was the main contributor to the BDE-47 dissipation in majority of the cultivated soil (Stiborova et al. 2015). BDE-47 dissipation percentages in the rhizosphere soil were greater than in the bulk soil (Table 1), indicating that the degradation of polybrominated diphenyl ethers was enhanced by rhizosphere microorganisms. This is congruent with previous studies that have found that the degradation of polybrominated diphenyl ethers could be enhanced in the rhizosphere of mangrove and ryegrass (Chen et al. 2015; Wang et al. 2011a). Compared to the bulk soil, the BDE-47 dissipation percentages in the rhizosphere soil increased by 8.6, 13.4, 20.9, and 28.5% for the control, 1, 2, and 4% composted pig manure treatments, respectively (Table 1), suggesting that the rhizodegradation of BDE-47 could be enhanced by composted pig manure amendment. The increased concentrations of degradation product: 2, 4, 4′-tribrominated diphenyl ether in rhizosphere soil (Table S4) also confirmed the result. These observations suggest that, in the presence of carrot, composted pig manure amendment enhanced microbial degradation of polybrominated diphenyl ethers in the rhizosphere.

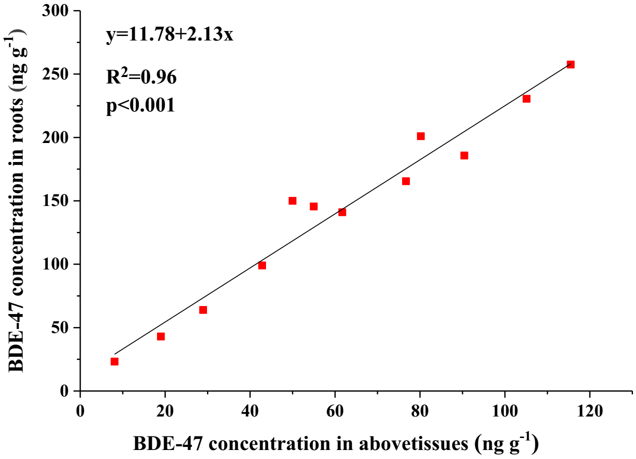

Fig. 2 Relationship between the 2, 2′, 4, 4′-tetrabrominated diphenyl ether (BDE-47) concentrations in aboveground carrot tissues and in roots (ng g^{-1}, dry weight)

Effect of composted pig manure on the fate of BDE-47 in the plant-soil system

As expected, composted pig manure amendment significantly increased the SOM content (Table S1), which is in congruence with former studies that indicate that the application of exogenous organic matter can increase SOM content (Liu et al. 2010; Rivero et al. 2004). Furthermore, positive correlations between the SOM contents and corresponding BDE-47 residues in plant-free soil or bulk soil were observed ($R^2 = 0.99$, 0.98, respectively, $p < 0.01$, Fig. S1), indicating that the OM derived from composted pig manure played an important role in adsorbing polybrominated diphenyl ethers in soil no matter cultivated carrot or not. In contrast, a negative linear relationship between the SOM contents and corresponding BDE-47 concentration in carrot roots was found ($R^2 = 0.90$, $p < 0.05$, Fig. S2), indicating that the OM derived from composted pig manure played an important role in reducing polybrominated diphenyl ether bioavailability in soil.

Table 1 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) concentrations (ng g^{-1}, dry weight) and dissipation percentages (%) in the initial soil (the soil collected at the beginning of cultivation), plant-free soil (FS), bulk soil (BS), and rhizosphere soil (RS) amended with 0 (serve as the control), 1, 2, and 4% composted pig manure (CPM) after 90-day cultivation

Treatments	Concentration ng g^{-1}				Dissipation percentage %		
	Initial	FS	BS	RS	FS	BS	RS
Control	384.5^{aA}	125.2^{dB}	121.5^{dB}	88.4^{bC}	67.4^{aB}	68.4^{aB}	77.0^{aA}
1% CPM	384.5^{aA}	160.4^{cB}	152.9^{cB}	101.4^{aC}	58.3^{bB}	60.2^{bB}	73.6^{bA}
2% CPM	384.5^{aA}	232.2^{bB}	196.0^{bC}	115.6^{aD}	39.6^{cC}	49.0^{cB}	69.9^{bA}
4% CPM	384.5^{aA}	324.4^{aB}	269.0^{aC}	121.1^{aD}	15.6^{dC}	30.0^{dB}	68.5^{bA}

Values with uppercase letters in the same line of "Concentration" or "Dissipation percentage" indicate significant differences of BDE-47 concentrations or dissipation percentages of soil samples in one treatment at $p < 0.05$. Values with lowercase letters in the same column indicate significant differences of BDE-47 concentrations or dissipation percentages in soils of different treatments at $p < 0.05$

Bacterial community composition in soil

Relative abundance of the microbial community was measured in the rhizosphere soil and bulk soil using the Shannon index and indicated that diversity in the control was lower than in the composted pig manure treatments (Fig. 3), demonstrating that soil microbial diversity was modified starting from the first dose of composted pig manure. Meanwhile, microbial diversity in the rhizosphere soil was lower than the bulk soil (Fig. 3, $p < 0.05$). Increased B-glucosidase activity has been shown to decrease microbial diversity in the rhizosphere (Pathan et al. 2015). The lowest microbial diversity with the fastest dissipation of hexachlorobenzene is observed within 2 mm of the rhizosphere of ryegrass (Song et al. 2016). In this study, lower microbial diversity and higher BDE-47 dissipation percentage were found in the rhizosphere soil (Fig. 3, Table 1) which indicates faster microbial degradation of BDE-47 relative to the bulk soil.

At the genus level, a heatmap coupled with cluster analysis was performed (Fig. 4). The bacterial communities in the control soil, composted pig manure amended bulk soil, and composted pig manure amended rhizosphere soil were individually clustered together, indicating that composted pig manure amendment and carrot cultivation jointly altered the bacterial community composition. The predominant bacteria were *Anaerolineaceae*, *Ohtaekwangia*, *Betaproteobacteria*, and *Gemmatimonas*, and their abundance was improved by composted pig manure amendment (Fig. 4). Among these bacteria, *Ohtaekwangia* were considered eutrophic bacteria since they could utilize easily degradable organic compounds and multiplied rapidly (Li et al. 2014a). *Betaproteobacteria*, as a class of gram-negative *Proteobacteria*, have significant contributions to nitrogen fixation in various kinds of plants (Ishii et al. 2011) and can produce nitrate by oxidizing ammonium which is important for plant growth (Bouskill et al. 2011).

Bacterial degradation of BDE-47 in the rhizosphere

Compared with the bulk soil, higher concentrations of debrominated degradation product: 2, 4, 4′-tribrominated diphenyl ether (BDE-28) were observed in the rhizosphere soil (Table S4), confirming a faster microbial degradation of BDE-47 in the rhizosphere. Samples taken from the rhizosphere soil revealed that several types of bacteria benefited from the addition of composted pig manure (Fig. 4); specifically, linear correlations were observed between the dissipation of BDE-47 in the rhizosphere and the relative abundance of bacteria (e.g., *Sphingomonas*, *Arthrobacter*, and *Pseudomonas* etc., with R^2 of 0.99, 0.78, and 0.94, respectively (Fig. 5, Table S7)). Among these bacteria, three

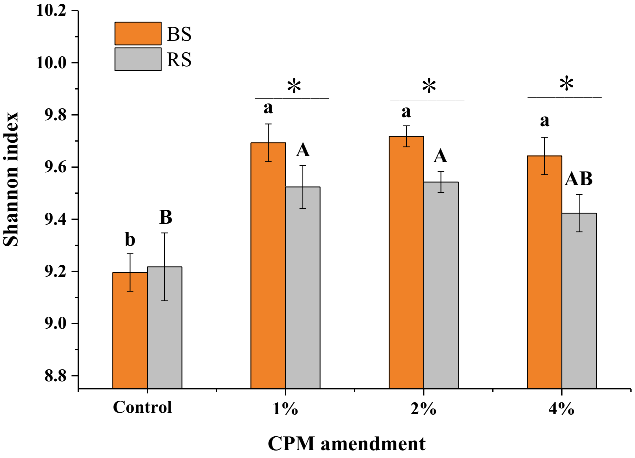

Fig. 3 Shannon index in the rhizosphere soil (RS) and bulk soil (BS) amended with 0 (serve as the control), 1, 2, and 4% composted pig manure (CPM). Values are the means ± standard deviation ($n = 3$). Bars with letters indicate significant differences in microbial diversity in the BS (lowercase) or RS (uppercase) at $p < 0.05$. Bars with asterisks indicate significant differences in microbial diversity within the same treatment at $p < 0.05$

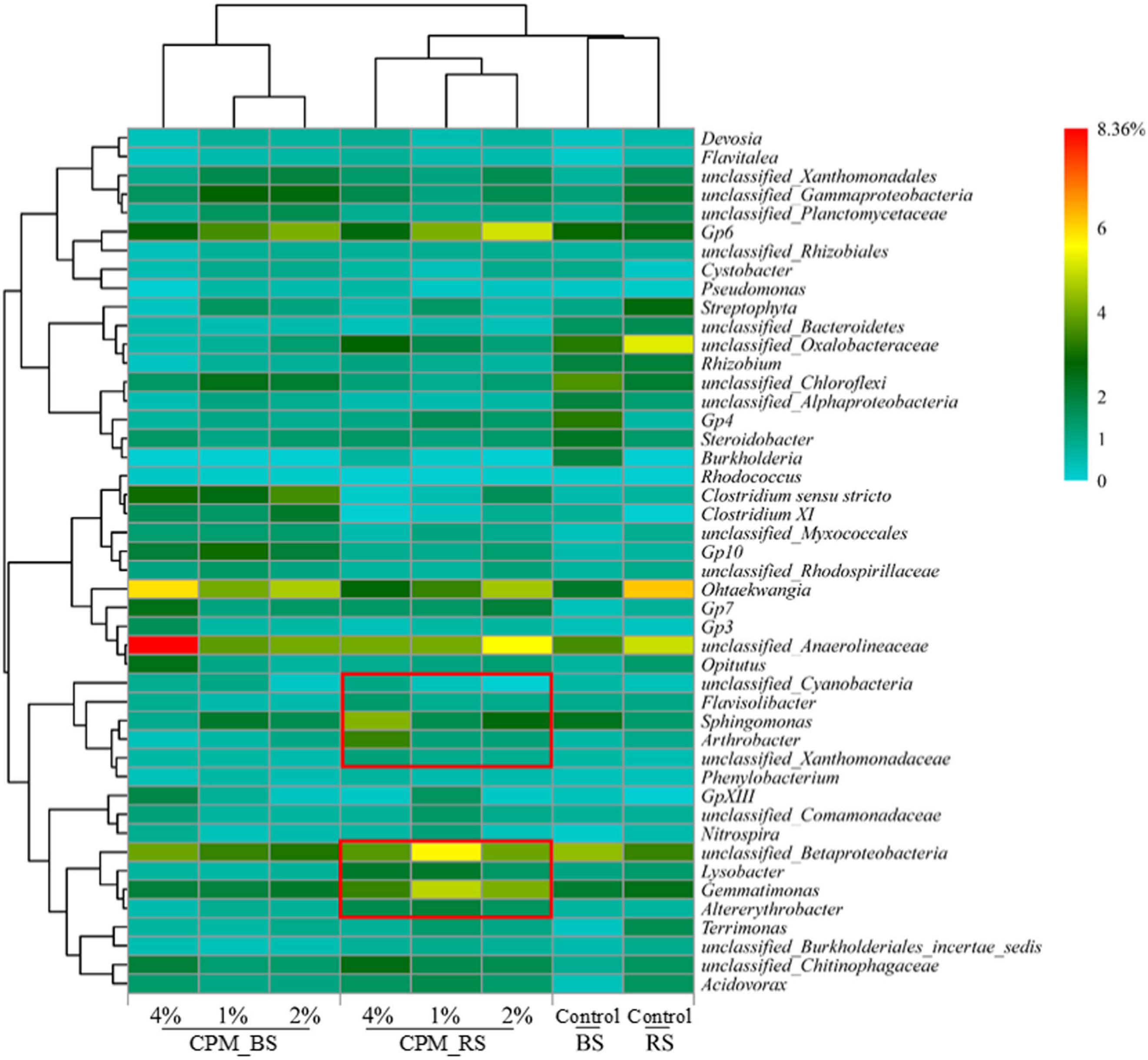

Fig. 4 Heatmap of bacterial community composition coupled with cluster analysis in the rhizosphere soil (RS) and bulk soil (BS) amended with 0 (serve as the control), 1, 2, and 4% composted pig manure (CPM) at the genus level

types of bacteria were associated with polybrominated diphenyl ethers degradation in the rhizosphere: *Sphingomonas*, the predominant polybrominated diphenyl ether-degrading bacteria in contaminated sewage sludge (Stiborova et al. 2015), *Pseudomonas*, a kind of 4-chlorobenzoate-degrading bacteria (Löffler et al. 1995), which can degrade BDE-209 with certain amount of co-metabolic substrates (Shi et al. 2013), and *Rhodococcus*, a kind of butachlor- and PCB-degrading bacteria, was able to degrade polybrominated diphenyl ethers on account of their similar chemical structure (Liu et al. 2012a; Robrock et al. 2009). Among the polybrominated diphenyl ether-degrading bacteria, the relative abundance of *Sphingomonas* was the highest; compared to the control of rhizosphere soil, the relative abundance of *Sphingomonas* increased by 21.7–203% in the composted pig manure amended rhizosphere soil. Therefore, we suggest that the degradation of BDE-47 is mainly attributed to *Sphingomonas* in the rhizosphere.

Environmental implications

Application of manure is a conventional agronomic technique that aids plant growth by adding nutrients to the soil,

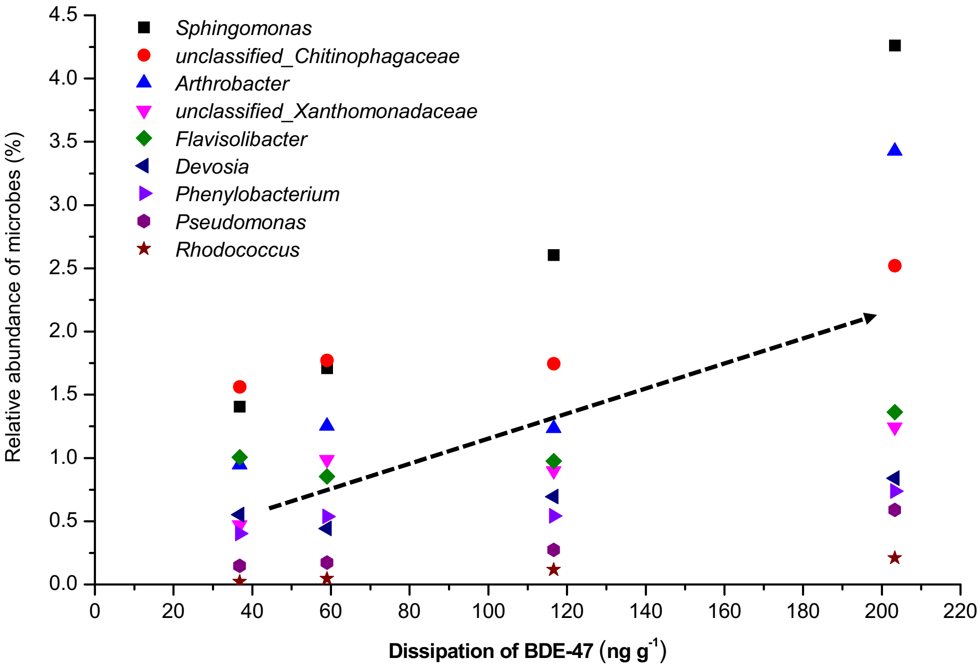

Fig. 5 Relationships between the increased dissipation of 2, 2′, 4, 4′-tetrabrominated diphenyl ether (BDE-47, ng g^{-1}) and the relative abundance of bacteria (%) in the rhizosphere soils within the outlined red box regions in Fig. 4

promoting healthy soil communities and recycling a valuable waste product. In this study, effects of composted manure amendment on the degradation and bioavailability of BDE-47 in soil are demonstrated. BDE-47 can be absorbed from the soil by carrot and thus potentially cause harm to human health via consumption. Composted pig manure amendment could substantially absorb BDE-47 in soil and consequently reduce uptake of BDE-47 in carrot tissues, thus the reducing risks of human exposure to BDE-47 through dietary intake. This strategy might also be effective for other polybrominated diphenyl ethers or their analogs (e.g., polychlorinated biphenyls, polychlorinated dibenzofuran, etc.). Considering that plant uptake was minor in composted pig manure amended soil, microbial degradation was likely the main route of BDE-47 dissipation. The addition of composted pig manure significantly stimulated the microbial degradation of BDE-47 in the rhizosphere through improving soil nutrient cycles and increasing the abundance of polybrominated diphenyl ether-degrading bacteria (e.g., *Sphingomonas*, *Pseudomonas*, and *Rhodococcus*, etc.). This study provides a feasible agronomic strategy of manure amendment for reducing plant uptake of persistent organic pollutants and enhancing removal of persistent organic pollutants by microbial degradation in the rhizosphere and therefore, improving food safety. However, it is worth mentioning that application of manure or compost manure may also introduce contaminants such as heavy metals or antibiotic residues into the soil (Cheesanford et al. 2009; Ko et al. 2008). Future studies are needed to investigate these effects and whether this agronomic strategy of manure amendment could be used for other agriculturally important field crops and/or other types of organic pollutants.

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Compliance with ethical standards

Competing interest The authors declare that they have no competing interest.

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