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RESEARCH ARTICLE

Bacterial communities associated with anaerobic debromination of decabromodiphenyl ether from mangrove sediment

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Abstract This study evaluated decabromodiphenyl ether (BDE-209) anaerobic debromination and bacterial community changes in mangrove sediment. BDE-209 debromination rates were enhanced with zerovalent iron compared to without zerovalent iron in the sediment. BDE-209 debromination rates in microcosms constructed with sediments collected in autumn were higher than in microcosms constructed with sediments collected in spring and were higher at the Bali sampling site than the Guandu sampling site. The intermediate products resulting from the reductive debromination of BDE-209 in sediment were nona-BDE (BDE-206, BDE-207), octa-BDEs (BDE-196, BDE-197), hepta-BDEs (BDE-183, BDE-184, BDE-191), hexa-BDEs (BDE-137, BDE-138, BDE-154, BDE-157), penta-BDEs (BDE-85, BDE-99, BDE-100, BDE-126), tetra-BDEs (BDE-47, BDE-49, BDE-66, BDE-77), tri-BDEs (BDE-17, BDE-28), and di-BDEs (BDE-15). Fifty bacterial genera associated with BDE-209 debromination were identified. Overall, 12 of the 50 bacterial genera were reported to be involved in dehalogenation of aromatic compounds. These bacteria have high potential to be BDE-209 debromination bacteria. Different combinations of bacterial community composition exhibit different abilities for BDE-209 anaerobic debromination.

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Introduction

Polybrominated diphenyl ethers (PBDEs) are the most widely used brominated flame retardants (de Wit 2002). PBDEs have been dispersed into air, water, sewage sludge, sediment, and human and animal tissues (de Wit 2002; Schreiber et al. 2010; Chen et al. 2013). PBDEs disrupt thyroid hormone production, lead to hepatic toxicity, and lead to developmental neurotoxicity in humans and mammals (Tseng et al. 2008). PBDEs constitute a major concern for human health and the main targets in environmental remediation.

Microbial degradation is believed to be a major process that cleans up PBDE-contaminated sediment. The fate of decabromodiphenyl ether (BDE-209) in anoxic sediment, where it may be subject to anaerobic reductive debromination (Gerecke et al. 2005), is thus of great interest. To enhance the efficiency of biodegradation, three remedial strategies, namely, natural attenuation, bioaugmentation, and biostimulation, have been proposed (Yu et al. 2005). Higher brominated PBDEs are difficult to be debrominated by the anaerobic strains and a long reaction time is needed (Smidt and deVos 2004). The addition of brij 30, brij 35, rhamnolipid, surfactin, vitamin B_{12}, zerovalent iron, acetate, lactate, and pyruvate were shown to influence BDE-209 anaerobic debromination; zerovalent iron yielded the highest BDE-209 anaerobic debromination rate in sediment (Huang et al. 2014).

Mangrove ecosystems, the dominant intertidal estuarine wetlands along the coastlines of tropical and subtropical

regions, are closely tied to human activities and are subject to pollutants contamination. The anaerobic degradation of various pollutants in mangrove sediments has been observed (Chang et al. 2008, 2009; Li et al. 2010; Andrade et al. 2012). The Guandu and Bali mangroves are the largest mangroves in subtropical Taiwan. Both mangroves are on the bank of the Tanshui River, which is one of the most heavily contaminated rivers in northern Taiwan (Liu et al. 2000). The concentration and anaerobic degradation of nonylphenol and polycyclic aromatic hydrocarbons in mangrove sediment have been demonstrated previously (Chang et al. 2008, 2009), but little is known about anaerobic debromination of BDE-209 in mangrove sediment.

The climatic characteristics of subtropical regions foster diverse microbial communities. Metagenomic studies with next-generation sequencing (NGS) allow microbial diversity to be studied in environmental samples (Sun et al. 2015; Yang et al. 2015a, b). However, little is known about the microbial communities involved in BDE-209 anaerobic debromination in mangrove sediment. The aims of this study were to compare the debromination of BDE-209 in mangrove sediment from two sampling sites, to evaluate the effects of various factors on BDE-209 debromination, and to explore changes in the bacterial community in the mangrove sediment.

Material and methods

Chemicals

An analytical of standard solutions containing PBDE congeners (BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-137, BDE-138, BDE-153, BDE-154, BDE-157, BDE-183, BDE-184, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207, BDE-209) were purchased from Wellington Laboratories (Guelph, Canada). BDE-209 (99%) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Solvents were purchased from Mallinckrodt, Inc.

Sample collection

Sediment samples were collected from sampling sites in our previous study (Chang et al. 2009). Samples were taken from the Guandu sampling site (25.11° 6843′ N, 121.46° 4153′ E) and Bali sampling site (25.15° 8613′ N, 121.43° 5575′ E) in northern Taiwan. They were collected from deep sediment (>15 cm) during two different seasons: autumn (October 2014) and spring (March 2015) during low tide with a soil core. All sediment samples were taken randomly, in triplicate, from an area of approximately 1 m^2 at the center of each mangrove sediment site. The sediment samples were stored in glass bottles on ice in a cooler and transported back to the laboratory within 4 h of sampling. For the sediment samples taken from Guandu and Bali mangroves in the spring, temperatures are 18.1 and 20.5 °C, bacterial counts are 2.2×10^5 and 3.6×10^6 CFU/g, and salinities are 1.1 and 16.5%, respectively. For those from Guandu and Bali mangroves in the autumn, temperatures are 25.1 and 27.6 °C, bacterial counts are 9.2×10^5 and 8.6×10^6 CFU/g, and salinities are 3.1 and 28.7%, respectively. For the sediment samples taken from Guandu in the spring, the concentrations of the PBDE congeners (BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-183, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207, BDE-209) were 132, 43.9, 848, 92.6, 57.7, 242, 1.47, 138, 934, 560, 14.0, 142, 54.0, 322, 205, 195, 30.6, 387, 269, 9309, 7599, and 250,000 ng/kg, respectively. For those from Bali in the spring, the concentrations of the PBDE congeners (BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-183, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207, BDE-209) were 15.4, 26.2, 225, 143, 26.9, 3.27, 2.27, 7.28, 205, 44.3, 2.29, 0.323, 6.64, 79.6, 49.9, 211, 33.2, 542, 336, 23,563, 30,777, and 640,000 ng/kg, respectively.

Experimental design

The experiments were performed using 125-mL serum bottles containing 45 mL river water, 5 g sediment, and 10 mg/kg of BDE-209. We measured BDE-209 anaerobic debromination with or without zerovalent iron (1 g/L) from the Guandu and Bali sampling sites in the spring and autumn. Inoculated controls were incubated without zerovalent iron with shaking at 30 °C in the dark. Sterile controls were autoclaved at 121 °C for 30 min on three consecutive days.

All experiments were conducted in an anaerobic glove box (Forma Scientific, Model 1025S/N, USA) filled with N_2 (85%), H_2 (10%), and CO_2 (5%) gas. Bottles were capped with butyl rubber stoppers and crimp seals, wrapped in aluminum foil to prevent photolysis, and then incubated without shaking at 30 °C in the dark. Each treatment was performed in triplicate. Samples were periodically collected to measure residual BDE-209 and the bacterial community.

BDE-209 analysis

BDE-209 extraction and analysis were performed as described in our previous study (Huang et al. 2014). BDE-209 was extracted twice from whole bottles by use of hexane and acetone (9:1) and then extracted again for 20 min with use of a

Branson 5200 ultrasonic cleaner. Extracts were analyzed using a gas chromatograph (Hewlett Packard 6890) equipped with an electron capture detector (GC-ECD) and Stx-500 capillary column (0.25 mm i.d. × 0.1 μm film thickness × 30 m, Restek). The initial column temperature was set at 170 °C, increased by 10 °C/min to 300 °C, and then increased by 2.5 °C/min to 340 °C. Injector and detector temperatures were set at 350 and 370 °C, respectively. The recovery percentage for BDE-209 was 94.9% and the detection limit was 0.05 mg/L.

Intermediate products of BDE-209 in the sediment samples were measured, as previously described (Chen et al. 2013). All collected sediment samples were mixed, evaporated to near dryness under a gentle stream of nitrogen, and measured by high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) (Agilent GC 6890/VG) with a 15-m DB-5HT column (0.25 mm i.d. × 0.1 μm film thickness; J&W Scientific, Folsom, CA). The PBDE congeners (BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-137, BDE-138, BDE-153, BDE-154, BDE-157, BDE-183, BDE-184, BDE-191, BDE-196, BDE-206, BDE-207, BDE-209) were selected for calculating total PBDEs in sediment samples (Chen et al. 2013). The limits of detection for all the congeners ranged from 0.01 to 0.2 mg/mL. The recovery percentages for the PBDE congeners ranged between 91.7 and 99.5%.

DNA extraction, PCR, and pyrosequencing

Total DNA was extracted by using the PowerSoil DNA Isolation Kit (MO BIO Laboratories) for each experimental sample. Partial sequences containing the V5-V8 variable regions of the 16S ribosomal RNA (rRNA) gene were amplified from the extracted DNA. The sequence of the 5′ primer included a 454 pyrosequencing adaptor, a unique 4-mer tag for each sample and 787F (5′-ATTAGATACCCNGGTAG-3′) for 16S rRNA genes. The sequence for the 3′ primer included a 454 pyrosequencing adaptor and 1391R (5′-ACGG GCGGTGWGTRC-3′) for 16S rRNA genes. PCR was performed as previously described (Yang et al. 2015a). Pyrosequencing was performed at the Genome Center, National Yang-Ming University, Taiwan, with the GS Junior System (Roche Diagnostics Corp., CT, USA).

Data analysis

The BDE-209 remaining percentage [%] = (residue substrate concentration / initial substrate concentration) × 100 was calculated, as was the BDE-209 debromination rates [%] = [1 − (residue substrate concentration/initial substrate concentration)] × 100.

The 16S rRNA gene sequences from pyrosequencing were analyzed using the RDPipeline in the Ribosomal Database Project web site (Cole et al. 2009). Firstly, the Ribosomal Database Project (RDP) Pipeline Initial Processing steps were used to match the raw reads to experimental samples, trimming off the tag and primer portions and removing sequences of low quality. Second, the trimmed sequences over 400 bp were applied to the UCHIME (Edgar et al. 2011) to remove chimeric sequences. Third, sequences pass the preprocessing steps were used to perform Aligner (sequence alignment), complete linkage clustering (sequence clustering), Shannon index and Chao1 estimator (α-diversity evaluation), and RDP classifier (assignment of taxonomic groups). Ninety-five percent similarities (the most stringent condition provided by RDPipeline) were used for all of these analyses. Spearman correlation coefficients of sequence frequencies and BDE-209 remaining percentage data were computed by R. For testing H0, $\rho = 0$, bacterial genera with $p < 0.05$ were considered BDE-209 degradation-associated bacteria. Cluster analysis was performed by the heatmap3 package of R. Differences of bacterial diversity between experimental settings were compared by the Wilcoxon rank sum test in R.

Results and discussion

Comparison of BDE-209 concentrations in mangrove sediments

The BDE-209 concentrations in the spring and autumn were 0.25 and 0.22 mg/kg at the Guandu sampling site; they were 0.64 and 0.32 mg/kg at the Bali sampling site. The BDE-209 concentrations in the sediments of the two sampling sites were higher in the spring than in the autumn. The temperature of the sediments was higher in autumn than spring. Temperature is the key factor affecting the seasonal variation of pollutant concentrations in water environments (Xu et al. 2006). The Bali sampling site had higher BDE-209 concentrations than the Guandu sampling site. The Bali sampling site was in the Bali mangrove, which was likely affected by vehicle exhaust deposition and the discharge of industrial, livestock, and household wastewater (Chang et al. 2009).

Comparison of BDE-209 anaerobic debromination in mangrove sediments

The BDE-209 concentrations in sterile controls were first examined at the end of a 75-day incubation period. The remaining percentage of BDE-209 in sediment ranged from 89.1 to 97.6%. It revealed that the BDE-209 anaerobic debromination occurring in all of the following experiments

was due to microbial action. The effects of the addition of zerovalent iron on anaerobic debromination of BDE-209 in the sediment are shown in Fig. 1. The debromination rates of BDE-209 without zerovalent iron in spring and autumn were 40.1 and 51.3% in the Guandu sediments and 71.2 and 80.9% in the Bali sediments, respectively. The debromination rates of BDE-209 with zerovalent iron in spring and autumn were 58.1 and 74.3% in the Guandu sediments and 85.1 and 95.5% in the Bali sediments, respectively. BDE-209 debromination rates were higher with zerovalent iron than without it in the sediments. The addition of zerovalent iron could reduce PBDEs to less brominated compounds by anaerobic microbes (Kim et al. 2011). As well, sorption could play a role in the BDE-209 debromination process with the addition of zerovalent iron (Shih and Tai 2010).

The results also revealed that BDE-209 debromination rates in microcosms constructed with sediments collected in autumn were higher than in microcosms constructed with sediments collected in spring, and higher in Bali sediments than in Guandu sediments. The salinity at the Guandu sampling site in spring and autumn were 1.1 and 3.1%, respectively. The salinity at the Bali sampling site in spring and autumn were 16.5 and 28.7%, respectively. Salinity may affect organic pollutant degradation in environments (Castillo-Carvajal et al. 2014). Also, the concentration of BDE-209 was detected in the sediments of the two sampling sites. The adaptation process enhanced BDE-209 anaerobic debromination (Huang et al. 2014). The BDE-209 could be debrominated without zerovalent iron. Microorganisms adapt to site-specific conditions, resulting in varied but optimal biodegradation capacity (Chang et al. 2009).

Identification of intermediate products from BDE-209 debromination in the sediment

Firstly, bromide ion release was assessed in the BDE-209 debromination process after incubation for 75 days in the sediments. As shown in Fig. 2, the bromide ion concentrations increased to 1.5 and 2.4 mg/L without zerovalent iron and to 4.0 and 5.2 mg/L with zerovalent ion in the Guandu and Bali sediments collected in spring, respectively. The bromide ion concentrations increased to 3.3 and 4.8 mg/L without zerovalent iron and to 5.6 and 7.7 mg/L with zerovalent ion in the Guandu and Bali sediments collected in autumn, respectively. The results indicated that bromide ions were produced from BDE-209 debromination. The bromide ion concentrations were higher with zerovalent iron than without zerovalent iron and higher from the Bali sediments than the Guandu sediments.

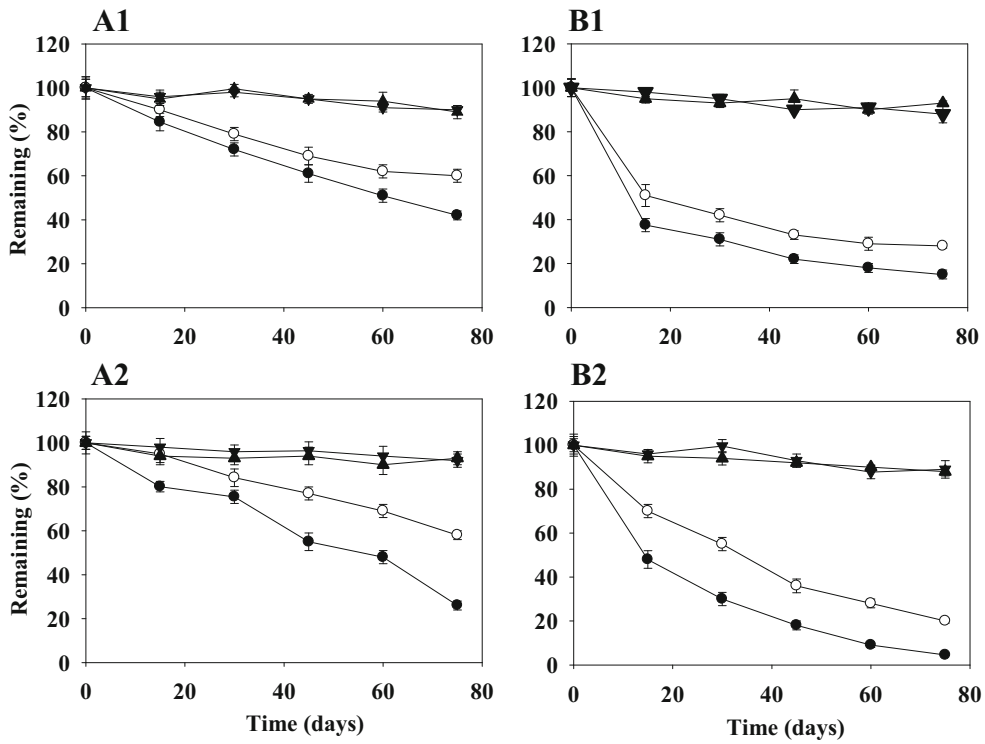

Fig. 1 Comparison of BDE-209 anaerobic debromination in the sediments from the Guandu and Bali sampling sites after incubation for 75 days in the sediments. **a** Guandu spring. **b** Bali spring. **c** Guandu autumn, **d** Bali autumn. Symbols: *filled triangles*, sterile sediment with zerovalent iron; *inverted filled triangles*, sterile sediment without zerovalent iron; *filled circles*, non-sterile sediment with zerovalent iron; *empty circles*, non-sterile sediment without zerovalent iron

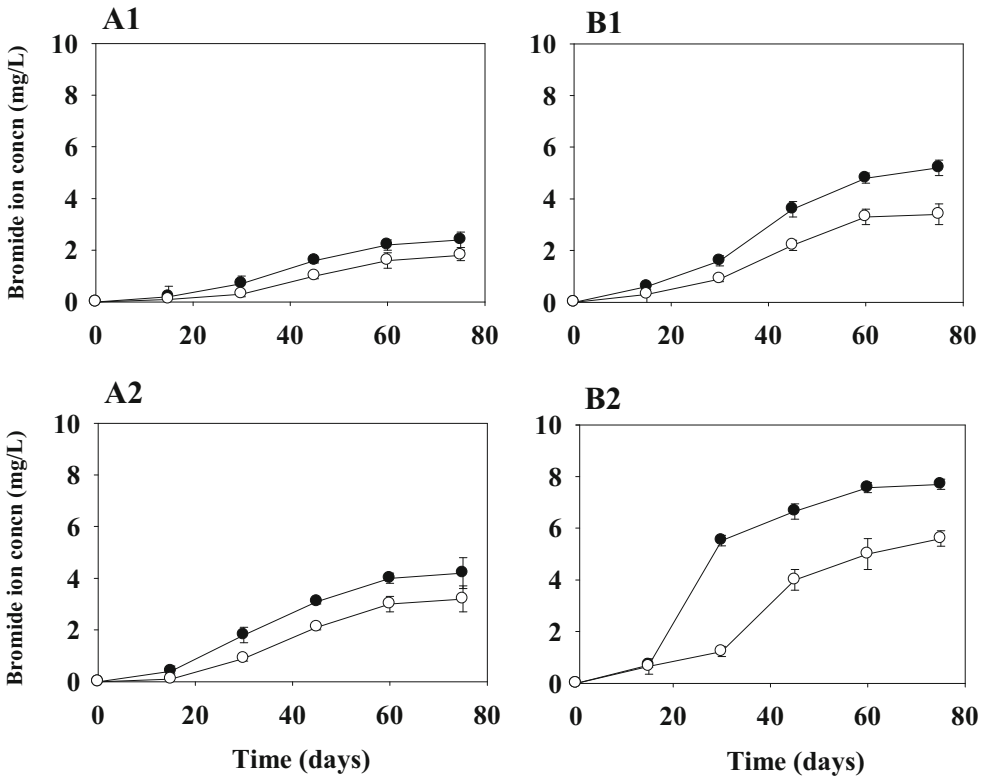

Fig. 2 The change of bromide ion concentration on BDE-209 anaerobic debromination from the Guandu (**a**) and Bali (**b**) sampling sites within the 75 days of incubation in the sediments. *1*, spring; *2*, autumn. Symbols: *filled circles*, with zerovalent iron; *empty circles*, without zerovalent iron

The BDE-209 biotransformation in the sediment was also monitored by HRGC/HRMS. The concentrations of the PBDE congeners (BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-77, BDE-85, BDE-99, BDE-100, BDE-126, BDE-137, BDE-138, BDE-154, BDE-157, BDE-183, BDE-184, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207, BDE-209) were 4.7, 3.0, 28.9, 17.1, 3.5, 2.2, 1.3, 1.74, 14.7, 4.7, 1.5, 2.2, 2.4, 7.2, 0.99, 33.5, 12.7, 8.4, 263.6, 223.9, 5574, 7663, and 175,882 μg/kg, respectively. Compared to the concentrations of PBDE congeners in original sediments, the PBDE congeners (BDE-15, BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-77, BDE-85, BDE-99, BDE-100, BDE-126, BDE-137, BDE-138, BDE-154, BDE-157, BDE-183, BDE-184, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207) may be the intermediate from the reductive debromination of BDE-209 in the sediment. BDE-209 parent starting material was converted into nona-BDE (BDE-206 and BDE-207). BDE-207 was converted by *meta*-substitution into octa-BDE (BDE-197), by *meta*-substitution into BDE-184, by *ortho*-substitution into BDE-137, by *ortho*-substitution into BDE-85, by *ortho*-substitution into BDE-66, by *meta*-substitution into BDE-28, and by *ortho*-substitution into BDE-15. BDE-206 was converted by *meta*-substitution into BDE-196; BDE-196 was converted by *meta*-substitution into BDE-183 and by *ortho*-substitution into BDE-191. BDE-191 was converted by *ortho*-substitution into BDE-157, by *ortho* substitution into BDE-126, and by *meta*-substitution into BDE-77. BDE-183 was converted into BDE-138 and BDE-154. BDE-138 was converted by *meta*-substitution into BDE-85, BDE-66, BDE-28, and BDE-15. BDE-154 was converted into BDE-99 and BDE-100. BDE-99 was converted by *para*-substitution into BDE-49 and by *meta*-substitution into BDE-17. BDE-100 was converted by *ortho*-substitution into BDE-47. BDE-47 was converted by *ortho*-substitution into BDE-28 and by *para*-substitution into BDE-17.

From the experiments, BDE-209 with *ortho*, *meta*, and *para* specificity dominated the debromination in the sediment. BDE-209 could be biotransformed via a debromination process to nona-BDE (BDE-206, BDE-207), octa-BDEs (BDE-196, BDE-197), hepta-BDEs (BDE-183, BDE-184, BDE-191), hexa-BDEs (BDE-137, BDE-138, BDE-154, BDE-157), penta-BDEs (BDE-85, BDE-99, BDE-100, BDE-126), tetra-BDEs (BDE-47, BDE-49, BDE-66, BDE-77), tri-BDEs (BDE-17, BDE-28), and di-BDE (BDE-15) in the sediment. A proposed reductive debromination pathway for the biotransformation of BDE-209 in the sediment is simulated in Fig. 3.

Keum and Li (2005) revealed that BDE-209 was debrominated to di-BDE (BDE-7, BDE-8, BDE-15) by zerovalent iron treatment. Kim et al. (2011) showed that reductive debromination of BDE-209 with zerovalent iron

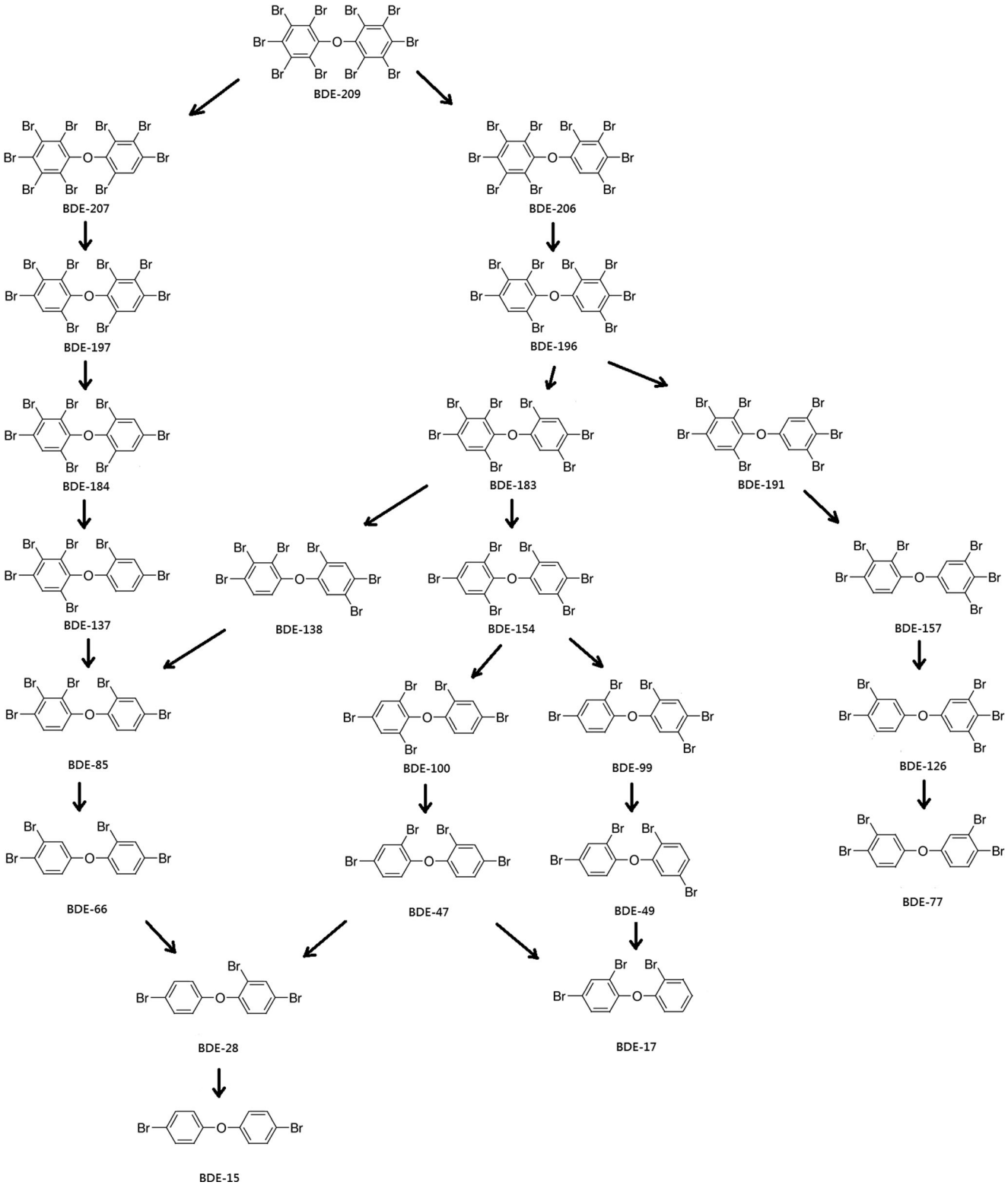

Fig. 3 Proposed debromination pathways for biotransformation of BDE-209 in sediment

produced various intermediates ranging from nona-BDEs to tri-BDEs (BDE-28, BDE-30) which were then treated with *Sphingomonas* sp. PH-07 strain to mono-BDEs. Huang et al. (2014) showed successive biotransformation of BDE-209 to BDE-3 via a reductive debromination process by BDE-adapted sediment. Our results revealed that different ecosystems present different microbial communities, resulting in various debromination rates and intermediate

products from BDE-209 in the sediment. As degradation rates are expected to vary greatly between individual anaerobic systems and prevailing redox conditions (sludge, sediment, soil) (Gerecke et al. 2005). The capability of degradation appears to be dependent on several factors such as microbial community structure, salinity, redox potential, total organic carbon, and presence of other pollutants (Stiborova et al. 2015).

Diversity of microbial community in BDE-209 anaerobic debromination experiments

In total, 126,933 16S rRNA gene sequences were produced from 28 experimental samples. Analysis with an RDP classifier revealed 31 phyla, 50 classes, 74 orders, 166 families, and 379 genera of known bacteria in the samples. The number of sequences, OTUs, and biological classification groups of the bacteria as well as the diversity indexes are in Table 1. Comparison of diversity indexes by the Wilcoxon rank sum test indicates that the microbial diversity of the sediment sample at day 0 was higher than that of the experimental samples at days 15, 45, and 75 ($p = 0.0064$). The microbial diversity of the experiment samples from the Guandu sampling site in autumn was higher than that in spring ($p = 0.0081$). However, the microbial diversity of experimental samples from the Bali sampling site in autumn and the Bali sampling site in spring exhibited no difference. The microbial diversity of the experimental samples with and without zerovalent iron from both the Guandu and Bali sampling sites exhibited no difference. These results suggest that the addition of zerovalent iron did not change microbial diversity in the experiments. Table 1 shows that there is a huge decline in overall diversity after addition of BDE-209. The effects of organic pollutants on microbial community structure in natural communities are complex. This complexity is reflected by the diverse physical, chemical, and biological factors (Battin et al. 2008). BDE-209 entering the sediment may affect microbial communities not only by reducing the diversity, but they may also change the composition of the microbial community (Zhang et al. 2012).

The major bacterial classes identified in BDE-209 anaerobic debromination were Gammaproteobacteria (62.8% of 126,933 sequences), Clostridia (5.2%), Flavobacteriia (4.3%), Alphaproteobacteria (3.5%), Betaproteobacteria (3.2%), Deltaproteobacteria (2.4%), Actinobacteria (1.3%), and Bacilli (1.2%) (Fig. 4a). The five archaeal classes (1.1% of 126,933 sequences) found in the sediment samples at day 0 were largely decreased in the experimental samples at days 15, 45, and 75 (Fig. 4b). Cluster analysis revealed that the microbial community compositions are highly diverse between sampling sites, sampling seasons, and experimental settings (Fig. 5).

Table 1 Number of sequences, operational taxonomic units (OTUs), classification information, and diversity indexes for each sample

Exp.	Seq. no	OTUs	Phy	Cla	Ord	Fam	Gen	Shann	Chao1
GS0	4660	823	27	40	63	103	166	5.79	1136
GA0	4960	880	25	41	64	98	127	5.43	1180
BS0	5549	378	28	35	47	48	67	6.08	989
BA0	3442	277	23	30	46	62	72	3.23	406
GSF15	4272	134	15	22	34	40	51	1.78	212
GAF15	5358	490	23	36	56	82	103	3.66	647
BSF15	3569	265	15	23	41	55	63	2.83	462
BAF15	3473	198	19	27	45	53	60	3.36	247
GSF45	6076	355	17	30	43	74	92	2.58	642
GAF45	5875	445	18	26	44	52	67	2.77	640
BSF45	8708	251	15	20	34	49	56	2.04	449
BAF45	5147	270	16	22	40	52	53	3.14	390
GSF75	3510	123	14	20	29	33	43	1.68	178
GAF75	3362	216	22	27	42	60	63	3.45	376
BSF75	2425	140	14	19	31	38	41	1.80	234
BAF75	3558	373	13	19	26	43	45	2.68	470
GSN15	3180	112	13	16	24	31	42	1.57	156
GAN15	3646	292	19	32	48	73	87	3.45	365
BSN15	2561	192	11	15	30	41	48	2.70	312
BAN15	5268	170	14	20	36	42	41	2.41	219
GSN45	2900	102	13	18	25	30	27	1.12	171
GAN45	4031	232	19	31	45	61	67	2.36	330
BSN45	2198	167	13	20	32	49	44	2.06	316
BAN45	5320	273	16	22	42	56	66	2.46	394
GSN75	2724	109	12	11	22	31	33	1.49	160
GAN75	5210	512	26	33	55	84	104	4.11	734
BSN75	7241	311	17	31	51	91	97	2.66	574
BAN75	8710	364	21	27	42	57	68	3.42	622

G and B represent Guandu and Bali sampling sites, respectively. S and A represent spring and autumn, respectively. F and N represent with Fe^0 and without Fe^0, respectively. The numbers 0, 15, 45, and 75 represent days 0, 15, 45, and 75, respectively

Bacteria associated with BDE-209 anaerobic debromination

To identify bacteria associated with anaerobic *debromination* of PBDEs, the proportion of the 16S rRNA gene sequences from each bacterial genus in each sample and the BDE-209 remaining percentages in the debromination experiments were used to compute Spearman correlation coefficients. Bacteria with sequence proportions exhibiting a negative correlation ($p < 0.05$) with the BDE-209 remaining percentages (increasing with BDE-209 debromination) were selected. Twelve, 12, 9, and 12 bacterial genera in samples with zerovalent iron from the Guandu sediment in spring, Bali sediment in spring, Guandu sediment in autumn, and Bali sediment in autumn

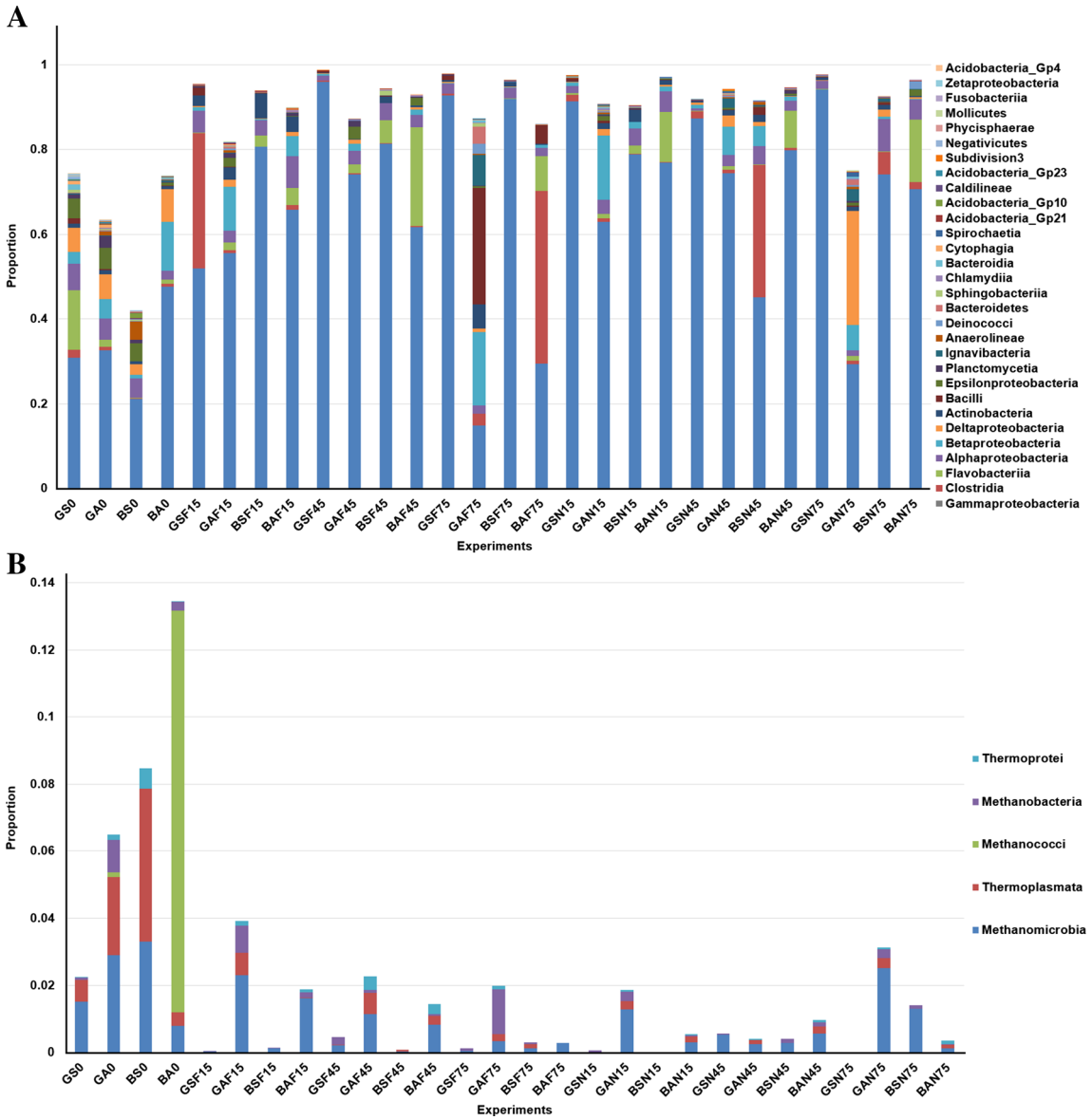

Fig. 4 Major bacterial (**a**) and archaea (**b**) classes in each experiment. *G* and *B* represent the Guandu and Bali sampling sites, respectively. *S* and *A* represent spring and autumn, respectively. *F* and *N* represent with zerovalent iron and without zerovalent iron, respectively. The numbers *0*, *15*, *45*, and *75* represent days 0, 15, 45, and 75, respectively

exhibiting negative correlations ($p < 0.05$) with BDE-209 debromination were identified (Fig. 6a, c, e, g). Seven, 10, 12, and 11 bacterial genera in samples without zerovalent iron from the Guandu sediment in spring, Bali sediment in spring, Guandu sediment in autumn, and Bali sediment in autumn exhibiting negative correlations ($p < 0.05$) with BDE-209 debromination were identified (Fig. 6b, d, f, h). Overall, 50 bacterial genera associated with BDE-209 debromination were identified in the eight experimental settings. Twelve of the 50 bacterial genera were previously found to be involved in dehalogenation of aromatic compounds, and six of the 50 bacterial genera including *Bacillus*, *Brevibacillus*, *Clostridium XI*, *Clostridium XlVa*, *Clostridium_ss*, and *Pseudomonas* were reported to be involved in debromination of aromatic compounds (Shih et al. 2012; Lu et al. 2013; Shi et al. 2013; Tang et al. 2014). Another six of the 50 bacterial genera, *Anaeromyxobacter*, *Burkholderia*, *Mycobacterium*, *Rhodobacter*, *Sedimentibacter*, and *Shewanella*, were reported to be involved in dechlorination or deiodination of aromatic compounds (Jesenska et al. 2000; He and Sanford 2002; Redwood et al. 2008; Pandey et al. 2011; Lohner and Spormann 2013; Oba et al. 2014). These results suggest that these bacteria have high potential to be BDE-209 debromination bacteria. Different combinations of bacterial community compositions exhibit different abilities for BDE-209 anaerobic debromination.

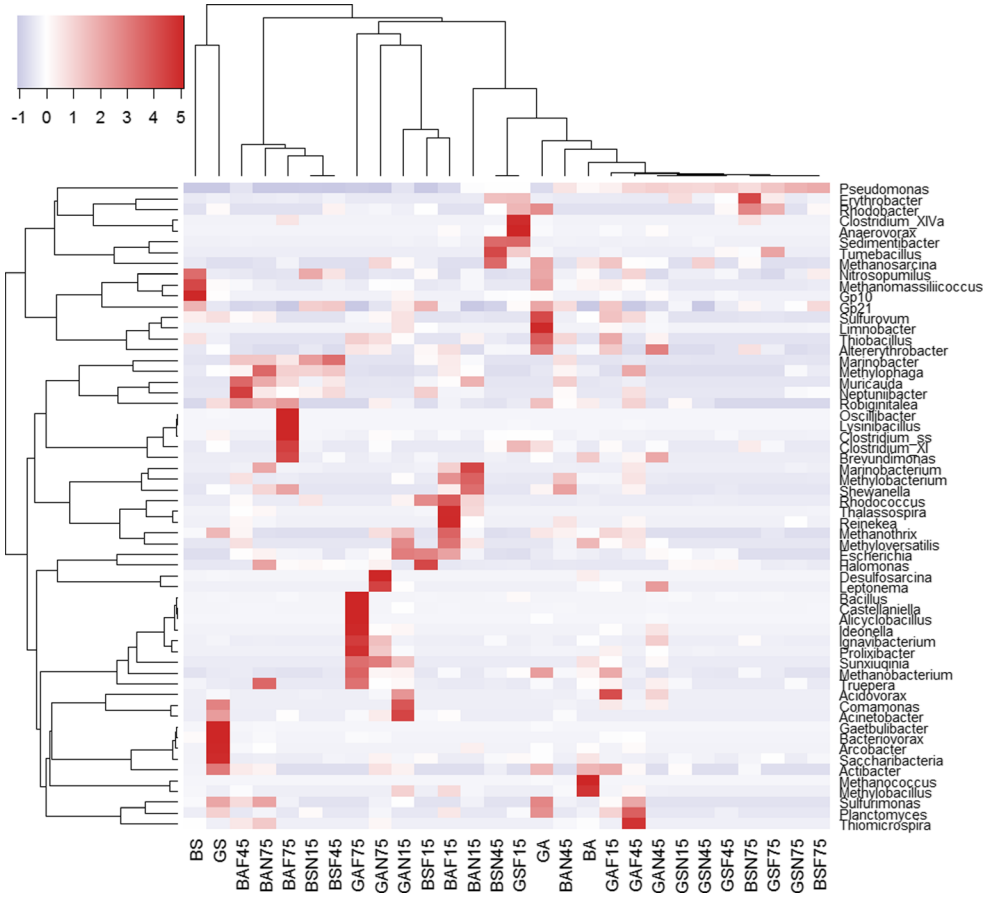

Fig. 5 Cluster analysis of bacterial community compositions between experiments. *G* and *B* represent the Guandu and Bali sampling sites, respectively. *S* and *A* represent spring and autumn, respectively. *F* and *N* represent with zerovalent iron and without zerovalent iron, respectively. The numbers *0*, *15*, *45*, and *75* represent days 0, 15, 45 and 75, respectively

Commonly and differentially distributed bacteria associated with BDE-209 anaerobic debromination in different settings

The results in Fig. 1 indicate that BDE-209 debromination rates were higher in autumn than in spring, and higher with zerovalent iron addition than without zerovalent iron. To identify commonly and differentially distributed bacteria associated with BDE-209 anaerobic debromination in different settings, Venn diagram analysis was conducted. For the sediments from the Guandu sampling site (Fig. 7a), 32 bacterial genera were divided into 9 groups. Eight, four, two, and eight bacterial genera were differentially distributed in the spring sample with zerovalent iron, autumn sample with zerovalent iron, spring sample without zerovalent iron, and autumn sample without zerovalent iron, respectively. For the sediments from the Bali sampling site (Fig. 7b), 31 bacterial genera were divided into 9 groups. Six, five, five, and three bacterial genera were differentially distributed in the spring sample with zerovalent iron, autumn sample with zerovalent iron, spring sample without zerovalent iron, and autumn sample without zerovalent iron, respectively. These differentially distributed bacteria groups provide insights into the different BDE-209 debromination rates in different experimental settings. Overall, 30 of the 50 bacterial genera in sediments from Guandu and Bali sampling sites, such as *Ensifer*, *Gracilimonas*, *Ideonella*, *Marinobacter*, *Prolixibacter*, and *Sulfurimonas* (Li et al. 2012; Hausler et al. 2014; Yousuf et al. 2014; Al-Mailem et al. 2015; Li et al. 2016; Vavourakis et al. 2016) were reported to be salinity tolerant extremophiles (Fig. 7). Eleven of the 30 bacterial genera including *Bacillus*, *Brevibacillus*, *Clostridium XI*, *Clostridium XlVa*, *Clostridium_ss*, *Pseudomonas*, *Burkholderia*, *Mycobacterium*, *Rhodobacter*, *Sedimentibacter*, and *Shewanella* were reported to be involved in dehalogenation of aromatic compounds. Therefore, study of the BDE-209 anaerobic debromination ability of bacteria from mangrove sediments may be useful for applications in bioremediation of saline environments.

Conclusions

Microbial degradation of BDE-209 is a major process that results in the decontamination of mangrove sediments. The

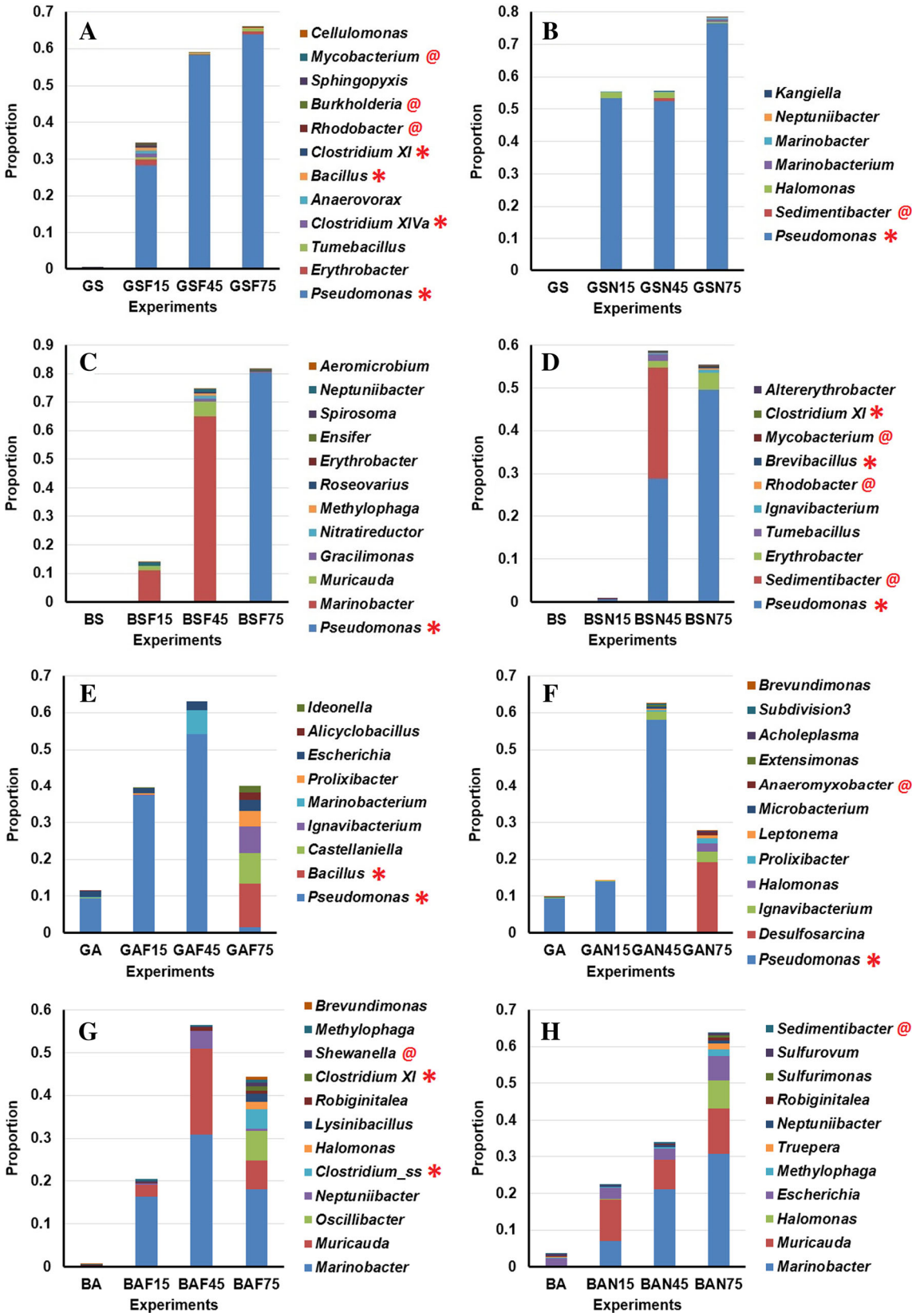

Fig. 6 Bacterial genera exhibiting a negative correlation with BDE-209 remaining percentage in the experiments. *G* and *B* represent the Guandu and Bali sampling sites, respectively. *S* and *A* represent spring and autumn, respectively. *F* and *N* represent with zerovalent iron and without zerovalent iron, respectively. The numbers *0*, *15*, *45*, and *75* represent day 0, 15, 45, and 75, respectively. The *red asterisks* and *at symbols* indicate bacterial genera that have been reported to be involved in reductive debromination and dehalogenation of aromatic hydrocarbons, respectively

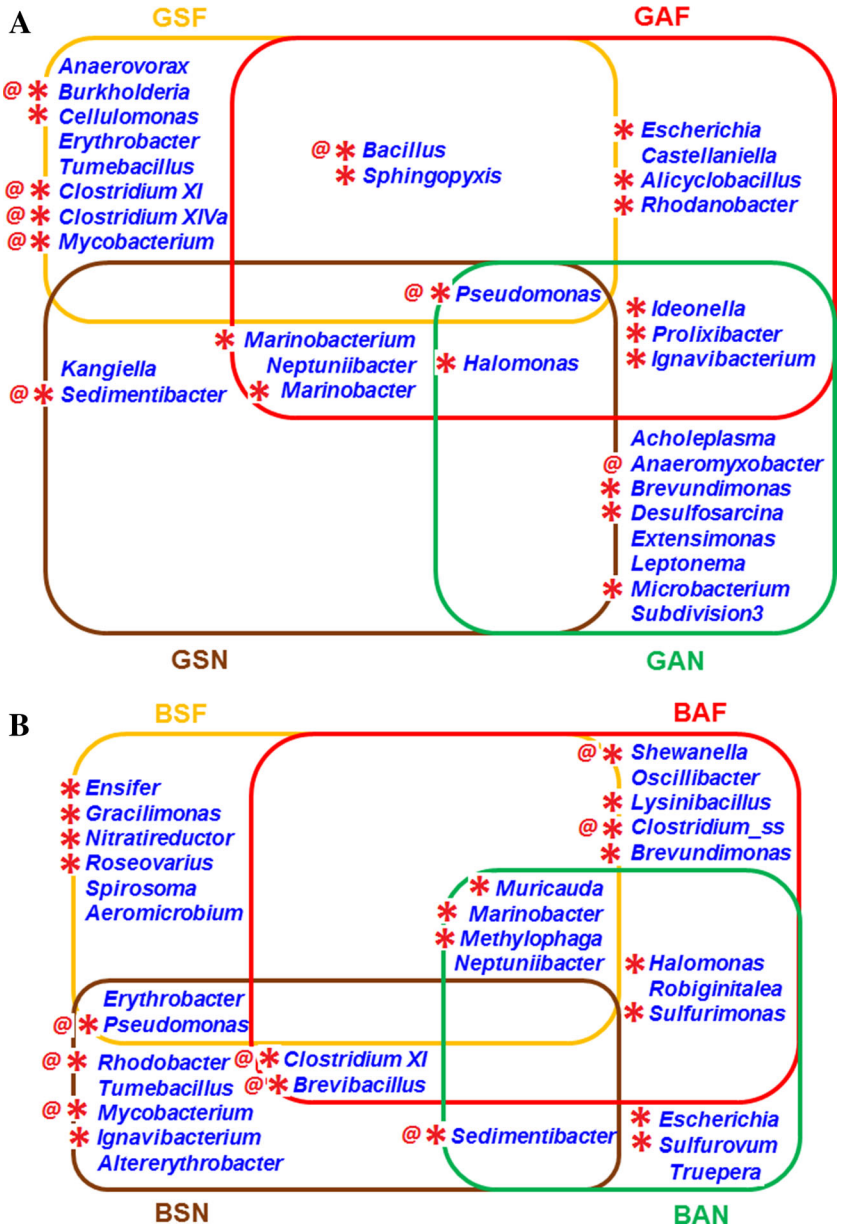

Fig. 7 Comparison of BDE-209 debromination-associated bacteria between experiments with and without zerovalent iron from the Guandu (**a**) and Bali (**b**) sampling sites. *G* and *B* represent the Guandu and Bali sampling sites, respectively. *S* and *A* represent spring and autumn, respectively. *F* and *N* represent with zerovalent iron and without zerovalent iron, respectively. The numbers *0*, *15*, *45*, and 75 represent day 0, 15, 45, and 75, respectively. The *red asterisks* indicate bacterial genera that have been reported to be salinity tolerant extremophiles. The *red at symbols* indicate bacterial genera that have been reported to be involved in reductive dehalogenation of aromatic hydrocarbons

addition of zerovalent iron enhanced BDE-209 anaerobic debromination in the sediment. Anaerobic debromination of BDE-209 could be biotransformed to di-BDEs (BDE-15) in the sediment. A reductive debromination pathway for biotransformation of BDE-209 in the sediment is proposed in Fig. 3. Fifty bacterial genera associated with BDE-209 debromination were identified, and 12 of the 50 bacterial genera were previously found to be involved in dehalogenation of aromatic compounds. These bacteria have high potential to be BDE-209 debromination bacteria. Moreover, 30 of the 50 bacterial genera were reported to be salinity tolerant extremophiles. Eleven of the 30 bacterial genera were reported to be involved in dehalogenation of aromatic compounds. Bacteria from mangrove sediments may be applicable in the bioremediation of saline environments.

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Compliance with ethical standards

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

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