

# Bacterial communities associated with sulfonamide antibiotics degradation in sludge-amended soil

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**Abstract** This study investigated the degradation of sulfonamide antibiotics (SAs) and microbial community changes in sludge-amended soil. In batch experiments, SA degradation was enhanced by addition of spent mushroom compost (SMC), SMC extract, and extract-containing microcapsule, with SMC showing higher SA degradation rate than the other additives in soil-sludge mixtures. In bioreactor experiments, the degradation of SAs in soil-sludge mixtures was in the order of sulfamethoxazole > sulfadimethoxine > sulfamethazine during four times of SA addition. SA removal was higher in soil-sludge mixtures than in soil alone. The bacterial composition differed in soil-sludge mixtures with and without SMC. In total, 44 differentially distributed bacterial genera were identified from different experimental settings and stages. Four bacterial genera, *Acinetobacter*, *Alcaligenes*, *Brevundimonas*, and *Pseudomonas*, were previously found involved in SA degradation, and 20 of the 44 bacterial genera were previously found in aromatic hydrocarbon degradation. Therefore, these bacteria have high potential to be SA degradation bacteria in this study.

**Keywords** Sulfonamide antibiotics · Degradation · Bacterial community · Spent mushroom compost · Sludge-amended soil

## Introduction

Sulfonamide antibiotics (SAs) are synthetic antimicrobials derived from sulfanilic acid. They inhibit the multiplication of

bacteria by acting as competitive inhibitors of *p*-aminobenzoic acid in the folic acid metabolism cycle (Sukul and Spitteller 2006). SAs have been detected in environmental media such as soils, sediment, and sludge (Kolpin et al. 2002; Accinelli et al. 2007; Nieto et al. 2010). Three SAs, sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamethazine (SMZ), were detected in the sludge of wastewater treatment plants (WWTP) in Taiwan (Yang et al. 2011). SAs are characterized with high water solubility, low chelating ability, and low binding constants; they can potentially enter soils (Accinelli et al. 2007). The risk of enhancing microbial resistance by prolonged exposure of microorganisms to SAs is also of great concern (Allen et al. 2010).

Microbial degradation is believed to be one of the major processes involved in cleaning up SAs-contaminated soil. Some studies have investigated the biodegradation of SAs such as SMZ, sulfadiazine (SDZ), and sulfachloropyridazine (SCP) in soils (Wang et al. 2006; Yang et al. 2009), but little is known about the degradation of SAs by the addition of sludge to soil. The addition of sludge can facilitate the degradation of organic pollutants because sludge adds supplemental nutrients and carbon into the contaminated soil (Semple et al. 2001). However, sludge tends to concentrate potential pollutants from wastewater such as organic toxic chemical residues (Clarke and Smith 2011).

*Pleurotus eryngii* is one of the most widespread mushrooms in the world (Lee et al. 2004). Spent mushroom compost (SMC), a waste product of the mushroom industry, contains many residual enzymes and can be used to degrade many organic toxic chemicals (Lau et al. 2003; Li et al. 2010). SMC contains bacteria that can degrade many organic toxic chemicals such as nonylphenol, tetrabromobisphenol-A, and tetracycline (Hsu et al. 2013; Chang and Ren 2015).

The diversity of the microbial community in soil is an important issue in modern soil microbiology. Metagenomic

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studies with next-generation sequencing allow for studying microbial diversity in environmental samples (Xu et al. 2012; Yang et al. 2015a, b). However, little is known about the microbial communities involved in SAs removal in sludge-amended soil. Here, we aimed to evaluate the degradation of SAs and bacterial community change in sludge-amended soil. With batch experiments, we evaluated SAs degradation with SMC, SMC extract (SE), and extract-containing microcapsule (MC) in soil-sludge mixtures. In bioreactor experiments, we compared SAs removal rates in soil-sludge mixtures with SMC and revealed the bacterial community composition associated with SAs removal in soil-sludge mixtures with SMC in a bioreactor. The target SAs compounds were SMX, SDM, and SMZ.

## Materials and methods

### Chemicals

SMX, SDM, and SMZ of 99.0 % purity were from Sigma Chemical Co. (St. Louis, MO). Solvents were from Mallinckrodt (Paris, KY); all other chemicals were from Sigma Chemical Co. Individual stock solutions of SMX, SDM, and SMZ were dissolved in 0.03 M NaOH, established at a concentration of 5000 mg/L, then diluted with water before use.

### Sampling and medium

Sludge is a semi-solid slurry and was produced as sewage sludge from the Dihus wastewater treatment plant in Taipei, Taiwan. The soil was collected from Taoyuan areas in northern Taiwan. SMX, SDM, and SMZ concentrations in sludge were 0.059, 0.094, and 0.052 mg/kg, respectively, and the three SAs in soil were below the detection limit (ND). The basal medium contained the following (in mg/L):  $K_2HPO_4$ , 65.3;  $KH_2PO_4$ , 25.5;  $Na_2HPO_4 \cdot 12H_2O$ , 133.8;  $NH_4Cl$ , 5.1;  $CaCl_2$ , 82.5;  $MgSO_4 \cdot 7H_2O$ , 67.5; and  $FeCl_3 \cdot 6H_2O$ , 0.75. The pH of the basal medium was adjusted to 7.2 before autoclaving at 121 °C for 20 min.

### Preparation of SMC, SMC extract, and SMC extract-containing microcapsule

The SMC of *P. eryngii* was produced at a mushroom cultivation factory in Chiayi, Taiwan. The SMC extract (SE) was extracted from 120 g SMC by using 600 mL sodium acetate buffer (pH 5.0) for 3 h at 4 °C. The samples were centrifuged (10,000g × 10 min), and the supernatants were partially purified by precipitation with ammonium sulfate and dialysis. SMC is a potential source of ligninolytic enzymes (Li et al. 2010). Most ligninolytic fungal species constitutively produce at least

one laccase isoenzyme and laccases are also dominant among ligninolytic enzymes in the environment (Baldrian 2006). We measured laccase activity in soil-sludge mixtures with SMC, SE, and MC. Laccase analysis was performed as we previously described (Liao et al. 2012). The material was stored at −20 °C.

Alginate solution was made by dissolving sodium alginate (4 wt%) in 0.9 wt% sodium chloride with stirring for 1 h at room temperature. SE was then added to the alginate solution. The final concentration of alginate was 2.0 wt% in the mixture solution. An electrostatic droplet generator was used to fabricate the capsules (Hsu et al. 2013). The mixture solution was drawn into a 10-mL syringe fitted with a 23 G needle and attached to a syringe pump that provided a steady solution flow rate. The solution was pumped through the needle at a fixed feed rate (25.2 mL/h) and fixed voltage (12 kV) into a gently agitated aqueous solution of calcium chloride (1.5 wt%) to form an MC of 250 μm in diameter for the experiments.

### Experimental design

The batch experiments involved 125-mL serum bottles containing 35 mL medium, 5 g sludge, 5 g soil, 5 g SMC, and 200 mg/kg (20 mg/L) SMX. Inoculated controls were incubated with soil-sludge mixtures with shaking at 30 °C in the dark. Sterile controls were prepared by autoclaving soil-sludge mixtures with SMC, MC, or SE at 121 °C for 20 min on three consecutive days. Each treatment was performed in triplicate. Samples were periodically collected to measure residual SMX.

The bioreactor was previously described (Chang and Ren 2015). It was aerated by an air diffuser, and materials were agitated by use of a stirrer. Bioreactor A was filled with 1800 mL medium and 200 g soil; bioreactor B was filled with 1800 mL medium and 200 g sludge; bioreactor C was filled with 1600 mL medium, 200 g sludge, and 200 g SMC. Bioreactor A added three SAs one time, and bioreactors B and C added three SAs three times. When SMX decreased to below ND, then the three SAs were re-added. The SAs concentrations were 200 mg/kg (20 mg/L) (each of SMX, SDM, and SMZ) for each SA addition. After 45 days incubations, 200 g soil was added to bioreactors B and C. The three SAs were re-added when SMX decreased to below ND. The SAs concentrations were 500 mg/kg (50 mg/L) (each of SMX, SDM, and SMZ) for each SA addition. The soil-sludge mixtures experiments lasted 38 days. Samples were taken periodically to measure residual SAs, total organic content (TOC), and the bacterial community.

### Chemical analysis

Residual SAs determination involved use of an Agilent 1260 HPLC equipped with a 4.6 × 250 mm column (Zorbax eclipse plus C18, Agilent) with photodiode array detector monitoring

at 270 nm. The mobile phase was acetonitrile and water (with 0.1 % formic acid), 70 %:30 %. Flow rate was 1 mL/min. The 0.5-mL aqueous phase samples were collected, and an equal volume of extraction solution was added with water (with 0.1 % formic acid): acetonitrile/methanol (10:3:1), vortexed well, and centrifuged at 10,000 rpm for 5 min. The supernatant was filtrated with a 0.22- $\mu$ m filter into a vial for high-performance liquid chromatography. The recovery percentages for SMX, SDM, and SMZ were 97.5, 97.4, and 96.4 %, respectively. The detection limits for SMX, SDM, and SMZ were all 0.1 mg/L. TOC was measured by using the TOC Analyzer (OI Analytical 1030 W, USA).

### 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 V5–V8 variable regions of the 16S rRNA gene were amplified from the extracted DNA. The sequence for 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'-ACGGGCGGTGWGTRC-3') for 16S rRNA genes. PCR reactions were performed as we previously described (Yang et al. 2015a). Pyrosequencing was performed at the Genome Center, National Yang-Ming University, Taiwan, ROC, with the GS Junior System (Roche Diagnostics Corp., CT, USA).

### Data analysis

Calculations were for SAs or TOC remaining percentage (%) = (residue substrate concentration/initial substrate concentration)  $\times$  100. SAs removal rate (%) = [1 - (residue substrate concentration/initial substrate concentration)]  $\times$  100. The degradation data for SAs were calculated with first-order kinetics:  $S = S_0 \exp(-k_1 t)$ ,  $t_{1/2} = \ln 2/k_1$ , where  $S_0$  is the initial concentration,  $S$  is the substrate concentration,  $t$  is the time, and  $k_1$  is the degradation rate constant.

The 16S rRNA gene sequence data were analyzed by using Chimera Check to remove the chimeric sequences. The RPD classifier in the Ribosomal Database Project (RDP) web site RDPipeline (<http://pyro.cme.msu.edu/>) was used to analyze 16 S rRNA gene sequences passing the Chimera Check for phylogenetic classification. The highest similarity (95 %) provided by the classifier software was used for grouping operational taxonomic units (OTUs) of bacteria. Comparison of bacterial community compositions between the samples was performed by the heatmap3 package of R. Spearman correlation coefficients were computed using the cor.test function of R. Differences in the distribution of the bacterial genera were identified with Mann-Whitney  $U$  tests using the wilcox.

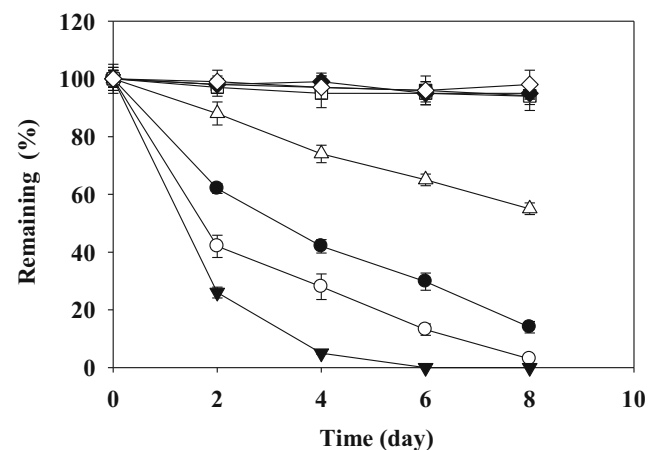
test function of R. Non-parametric ANOVA (the Kruskal-Wallis test) was computed by the posthoc.kruskal.nemenyi.test function of R. Non-parametric pairwise comparisons using Tukey and Kramer (Nemenyi) test were computed by the posthoc.kruskal.nemenyi.test function in the PMCMR package of R.  $p < 0.05$  was considered as statistically significant.

## Results and discussion

### SMX degradation in batch experiments

The SMX remaining percentage in sterile controls was examined to be ranged from 94.5 to 98.1 % in soil-sludge mixtures during 8 days incubation. Therefore, the SMX degradation in the following experiments was due to microbial actions. A comparison of the effects of SMC, MC, and SE addition in soil-sludge mixtures on SMX degradation was shown in Fig. 1. At 8 days, SMX in soil-sludge mixtures with SMC was completely degraded, but remained at 3.1, 14.4, and 55.2 % for soil-sludge mixtures with MC, SE, and the inoculated control (soil-sludge mixtures without SMC, MC, and SE), respectively (Fig. 1). The degradation data for the SMX fit well with the first-order kinetics. The degradation half-life ( $t_{1/2}$ ) in soil-sludge mixtures were 0.69, 1.8, and 3.0 days for SMC, MC, and SE, respectively. The order of SMX degradation efficiency in soil-sludge mixtures with the three additives was SMC > MC > SE.

SMC contains ligninolytic enzymes that can enhance SMX degradation (Lau et al. 2003). The enzyme activity in the soil-sludge mixtures with SMC, MC, and SE were initially  $856.4 \pm 18.1$ ,  $850.1 \pm 13.8$ , and  $840.1 \pm 19.2$  U/L, respectively, and  $850.4 \pm 19.6$ ,  $808.4 \pm 17.9$ , and  $611.9 \pm 16.2$  U/L, after



**Fig. 1** Remaining percentages of SMX by the addition of SMX for SMC, MC, and SE in soil-sludge mixtures. *Black down-pointing triangle* non-sterile SMC, *white circle* non-sterile MC, *black circle* non-sterile SE, *white triangle* non-sterile inoculated control, *black square* sterile SMC, *white square* sterile MC, *black diamond* sterile SE, *white diamond* sterile inoculated control

**Table 1** The removal of the three sulfonamide antibiotics (SAs) in sludge with or without spent mushroom compost (SMC) in bioreactors with three times additions of SAs after 45 days of incubation

Treatment	Removal rates (%)		P value <sup>a</sup>
	Without SMC	With SMC	
<b>First addition of SAs</b>			
SMX	99.2 ± 1.9	99.4 ± 1.0	>0.05
SDM	93.9 ± 2.1	97.1 ± 1.1	<0.05
SMZ	91.8 ± 3.1	94.5 ± 2.8	>0.05
<b>Second addition of SAs</b>			
SMX	99.9 ± 1.6	99.9 ± 1.4	>0.05
SDM	95.0 ± 2.2	98.1 ± 2.3	<0.05
SMZ	92.9 ± 2.1	95.9 ± 1.1	<0.05
<b>Third addition of SAs</b>			
SMX	99.9 ± 1.9	99.9 ± 0.7	>0.05
SDM	97.8 ± 2.1	99.9 ± 1.2	>0.05
SMZ	95.9 ± 3.5	99.9 ± 1.1	<0.05

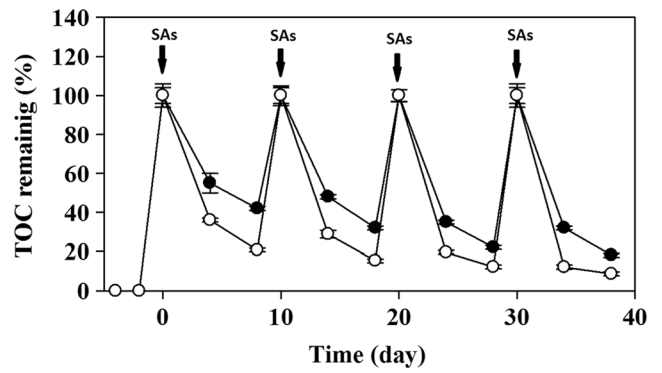
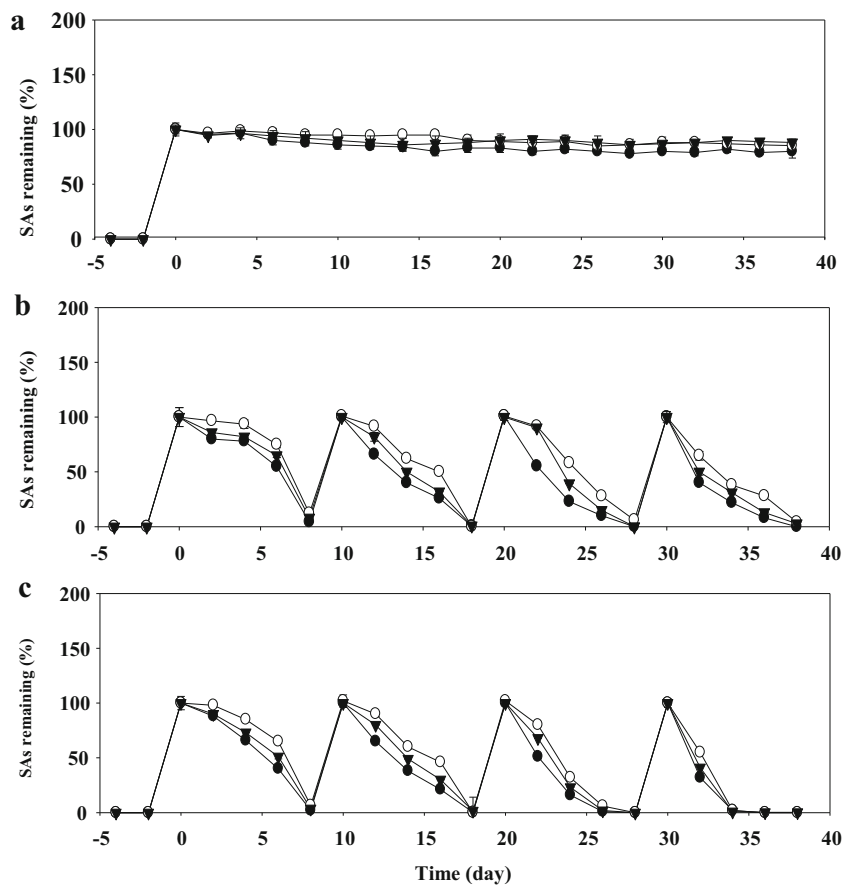
Data are mean ± SD from three repeated tests

SMX sulfamethoxazole, SDM sulfadimethoxine, SMZ sulfamethazine

<sup>a</sup>p values are results from Mann-Whitney U tests

8 days of incubation, respectively. The order of enzyme activity of the three additives was SMC > MC > SE. SMC contains

**Fig. 2** Sulfonamide antibiotics (SAs) remaining percentages during four times additions of three SAs in the bioreactor experiments of soil alone (a), soil-sludge mixtures without spent mushroom compost (SMC) (b), and soil-sludge mixture with SMC (c). Black circle sulfamethoxazole (SMX), white circle sulfamethazine (SMZ), black down-pointing triangle sulfadimethoxine (SDM)



**Fig. 3** Total organic content (TOC) remaining percentages in the soil-sludge mixtures during four times re-addition of three SAs in the bioreactor experiments. Black circle without spent mushroom compost (SMC), white circle with SMC

enzymes, bacteria, and fungi that all might play roles in the degradation of SMX. Additionally, the SMX degradation rate was higher with MC than with SE in the soil-sludge mixtures. SMX removal was better with MC than SE in terms of improving tolerance to the environmental loadings. Similar trends were revealed in our previous study, which demonstrated more efficient removal of nonylphenol with immobilized SE than the suspended form (Hsu et al. 2013).

### SAs removal in bioreactor experiments

The removal rates of three SAs in sludge with or without SMC in bioreactors are in Table 1. SA removal rates were significantly higher ( $p < 0.05$ ) with than without SMC for SDM during the first and second SA additions in sludge. It is also true ( $p < 0.05$ ) also for SMZ in the second and third addition (Table 1). The removal of the three SAs in soil-sludge mixture with or without SMC in bioreactors is in Fig. 2. The removal rates of SMX, SDM, and SMZ were 15.1, 5.1, and 12.9 % in soil alone,

respectively, on day 38 (Fig. 2a). SA-degrading bacteria may be present in soil, and only 5–15 % SAs were removed. In soil-sludge mixtures without SMC, the removal rates of SMX, SDM, and SMZ were 95.7, 87.9, and 92.0 % on day 8 after the first addition, and 99.9, 95.8, and 97.2 % on day 38 after the fourth addition (Fig. 2b). However, with SMC, the removal rates of SMX, SDM, and SMZ were 97.8, 93.4, and 96.4 % on day 8 after the first addition, and three SAs removal rates were all 99.9 % on day 38 after the fourth addition (Fig. 2c). The order of removal rates in sludge was SMX > SDM > SMZ.

**Table 2** Number of sequences, operational taxonomic units (OTUs), classification information, and diversity indexes for each sample

Exp.	No. of sequences	OTUs	Phy	Cl	Ord	Fam	Gen	Shann	Chao1
A1	3225	569	16	24	32	38	51	2.13	182.1
A2	5774	200	13	18	20	28	41	1.34	140.0
A3	4114	120	10	12	13	20	23	1.04	110.8
A4	3648	143	9	12	12	18	27	1.16	109.7
A5	5247	192	11	18	21	35	48	2.15	179.4
A6	4312	213	11	14	21	32	34	2.55	179.6
A7	3257	187	10	14	19	31	31	2.37	133.0
A8	5321	250	11	15	21	36	37	2.18	178.4
A9	3016	113	10	13	15	20	18	1.74	112.0
A10	2654	106	10	11	12	13	12	1.78	101.5
A11	3120	182	13	15	19	31	27	1.58	144.9
A12	3073	239	12	16	23	41	37	1.49	210.1
B1	4321	468	17	26	29	44	63	2.90	257.5
B2	5085	299	13	19	23	38	42	2.78	241.0
B3	5933	299	12	17	22	31	39	2.59	224.4
B4	4379	270	12	20	26	35	35	2.53	207.9
B5	4158	206	9	14	20	39	39	1.75	172.0
B6	3400	184	11	14	21	31	33	1.91	118.0
B7	3554	239	11	14	18	34	37	2.15	164.0
B8	3387	188	11	15	20	32	35	1.82	176.3
B9	3396	237	11	15	23	35	40	2.38	154.5
B10	5503	211	13	18	23	31	40	1.54	176.1
B11	3080	149	10	15	18	22	23	1.42	145.8
B12	5481	310	13	17	27	38	42	2.41	270.4
C1	2648	262	11	17	23	31	43	3.00	239.0
C2	5126	376	14	21	27	38	57	3.22	298.0
C3	6326	512	14	22	29	45	63	3.78	517.3
C4	4426	275	12	16	19	24	33	2.87	233.6
C5	4107	451	15	25	32	54	87	3.64	416.3
C6	5846	288	11	16	22	31	36	3.01	216.3
C7	4627	263	11	16	24	32	38	2.80	179.1
C8	4982	209	11	15	21	32	41	3.06	141.1
C9	6750	397	11	16	23	40	56	3.40	326.1
C10	4792	276	12	15	20	30	41	3.26	167.7
C11	4322	186	10	14	19	21	26	2.25	158.0
C12	4777	212	11	15	21	30	33	1.80	162.0

A, B, and C represent soil, soil-sludge mixtures, and soil-sludge-SMC mixtures, respectively. 1–12 represent days 0, 4, 8, 10, 14, 18, 20, 24, 28, 30, 34, and 38, respectively

The SA removal rates were not significantly enhanced by the addition of SMC. As a conclusion, the SA removal rates were not always significantly enhanced by the addition of SMC. The addition of organic supplements may increase the rate of contaminant degradation (Semple et al. 2001).

Functional groups may contribute electronegativity effects that hinder degradation by affecting the interaction between the contaminant and microbes. With increasing electronegativity of the substituents, SA degradation rates decrease (Pepper et al. 2015). SA-degrading bacteria may be present in sludge and SMC, which could enhance the SAs removal. The three re-additions of SAs might increase the SAs-degrading activity of the microbes. The solutions in the bioreactor experiments were aerated with an air diffuser and agitated with a stirrer, which enhanced the removal of SAs. These results are similar to our previous study of the effects of acclimation on degradation of three tetracyclines in river sediment (Chang and Ren 2015).

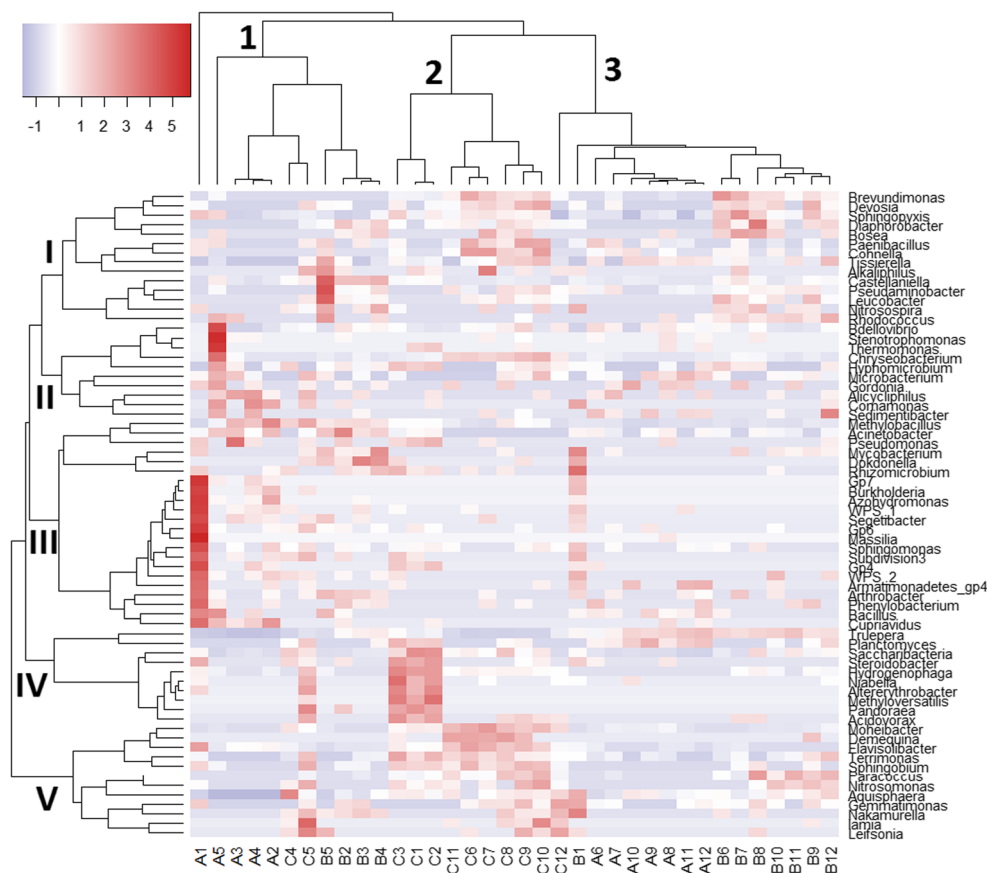
The studies were further extended with TOC measurements to demonstrate the apparent mineralization of these chemicals in the bioreactors. The TOC remaining percentages during four times re-addition of the three SAs are in Fig. 3. In the soil-sludge mixtures alone, the remaining TOC percentages were 42.1, 32.2, 22.1, and 18.5 % on days 8, 18, 28, and 38, respectively. In contrast, the remaining TOC percentages were 20.6,

15.2, 12.1, and 8.5 % on days 8, 18, 28, and 38, respectively, in the soil-sludge mixtures with SMC. The correlations between the SA removal (Fig. 2) and the TOC remaining proportions (Fig. 3) were examined. In the soil-sludge mixtures alone, the Spearman correlation coefficients between the SMX, SDM, and SMZ removal and the TOC remaining proportions were 0.958 ( $p = 8.741e-07$ ), 0.947 ( $p = 2.91e-06$ ), and 0.911 ( $p = 9.559e-05$ ), respectively. In the soil-sludge mixtures with SMC, the Spearman correlation coefficients between the SMX, SDM, and SMZ removal and the TOC remaining proportions were 0.965 ( $p = 3.623e-07$ ), 0.971 ( $p = 1.535e-07$ ), and 0.951 ( $p = 1.894e-06$ ), respectively. These results reveal strong correlations between the TOC remaining percentages and SAs removal in the soil-sludge mixtures. Higher proportion of SAs was mineralized in the soil-sludge mixtures with SMC than in the soil-sludge mixtures alone. The addition of SMC affected bacterial communities and thus slightly enhanced the SA removal in soil-sludge mixtures.

**Bacteria associated with SA degradation in the bioreactor experiments**

A total of 160,167 16S rRNA gene sequences were produced from 36 bioreactor experimental samples. Analysis with the RDP classifier revealed 22 phyla, 39 classes, 50 orders, 103

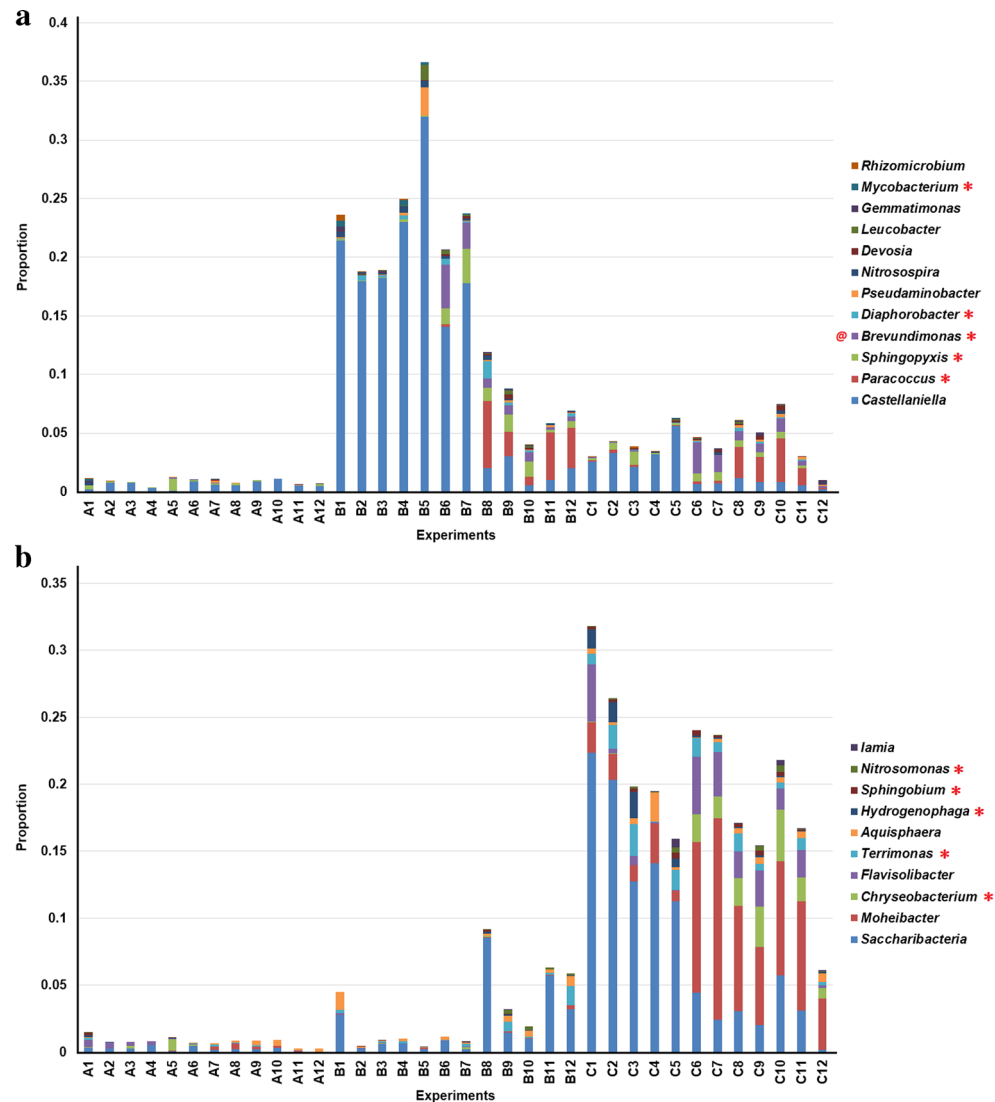
**Fig. 4** Cluster analysis of bacterial community compositions in bioreactor experiments. A, B, and C represent soil, soil-sludge mixtures, and soil-sludge-SMC mixtures, respectively. 1–12 represent days 0, 4, 8, 10, 14, 18, 20, 24, 28, 30, 34, and 38, respectively



families, and 212 genera of known bacteria in the samples. The number of sequences and biological classification groups of bacteria as well as the diversity index for each experimental sample are in Table 2. The Shannon diversity indexes (mean  $\pm$  SE) are  $1.79 \pm 0.14$ ,  $2.18 \pm 0.14$ , and  $3.00 \pm 0.16$  for samples from A, B, and C bioreactors, respectively. The Chao1 diversity indexes (mean  $\pm$  SE) are  $148.5 \pm 10.5$ ,  $192.3 \pm 13.7$ , and  $254.5 \pm 33.5$  for samples from A, B, and C bioreactors, respectively. The  $p$  values of non-parametric ANOVA (the Kruskal-Wallis test) for Shannon and Chao1 diversity indexes are  $1.05E-4$  and  $0.016$ , respectively. The Tukey-type non-parametric multiple comparison analysis indicates the Shannon diversity indexes are significantly different between samples of bioreactors A and C ( $p = 7.4E-5$ ) and between samples of bioreactors A and B ( $p = 0.016$ ). The  $p$  value of the Tukey-type non-parametric multiple comparison for the Chao1 diversity indexes between samples of bioreactors A and C is  $0.012$ . Overall, the order of diversity of bacterial community composition for samples from the three bioreactors was  $C > B > A$ .

The differences of bacterial community compositions between bioreactor samples were analyzed by cluster analysis (Fig. 4). Three clusters were identified according to experimental samples. The first cluster included early stages of bioreactors A and B samples (A1–A5, B2–B5). The second cluster included samples from bioreactor C samples. The third cluster included late stages of bioreactors A and B samples (A6–A12, B6–B12). On the other hand, four clusters of bacterial genera were identified. The first cluster included bacterial genera with high proportions in bioreactors B and C samples (B2–B12, C6–C12). The second cluster included bacterial genera with high proportions in early stages of bioreactor A samples (A1–A5). The third cluster included bacterial genera with high proportions in early stages of bioreactors A and B samples (A1–A5, B1–B5). The fourth cluster included bacterial genera with high proportions in early stages of bioreactor C samples (C1–C5). The fifth cluster included bacterial genera with high proportions in all stages of bioreactor C samples (C1–C12) and late stages of bioreactor B samples (B8–B12).

**Fig. 5** Differentially distributed bacterial genera in bioreactor experiments. **a** Bacterial genera with higher proportions in both B and C than A bioreactor experiments. **b** Bacterial genera with higher proportions in C than in A and B bioreactor experiments. A, B, and C represent soil, soil-sludge mixtures, and soil-sludge-SMC mixtures, respectively. 1–12 represent days 0, 4, 8, 10, 14, 18, 20, 24, 28, 30, 34, and 38, respectively. The red at sign indicate bacterial genera that have been reported to be involved in SAs degradation. The red asterisk indicate bacterial genera that have been reported to be involved in the degradation of aromatic hydrocarbons

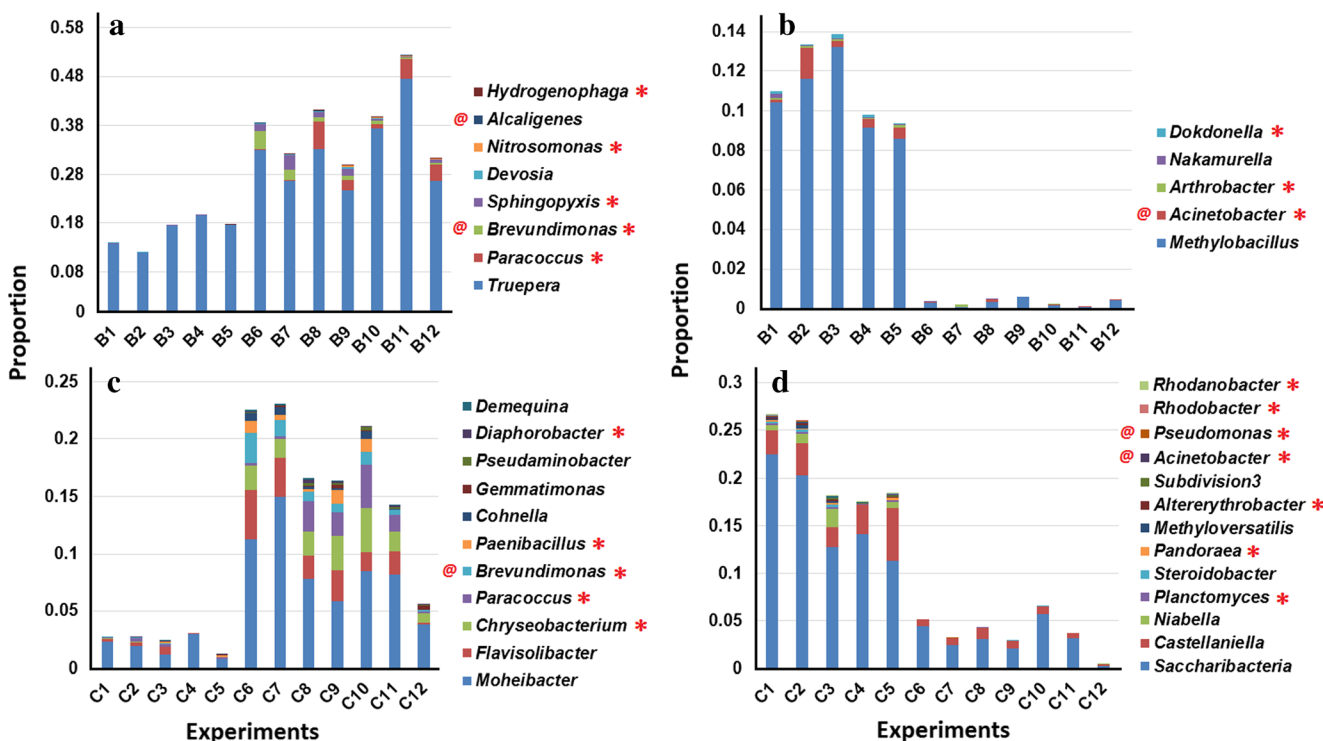


These results suggest that the bacterial community composition substantially differed between the experiments of soil-sludge mixtures alone and soil-sludge mixtures with SMC. Moreover, the major bacterial genera composition differed greatly between the early and late stages of the experiments. Two major factors may have affected the bacterial composition during the experimental periods. First, the nutrient or carbon sources differed between the experiments of soil-sludge mixtures alone and soil-sludge mixtures with SMC. The second factor was the addition of SAs. The bacterial genera identified in the late stage of the experiments might represent bacteria that adapted or resistant to SAs.

Differentially distributed bacterial genera in different bioreactor experiments were identified by Mann-Whitney *U* tests (Fig. 5). Twelve bacterial genera with high proportion in bioreactor B were found (Fig. 5a). Five of the 12 bacterial genera (i.e., *Brevundimonas*, *Diaphorobacter*, *Mycobacterium*, *Paracoccus*, and *Sphingopyxis*) have been reported to be associated with the degradation of aromatic hydrocarbons (Teng et al. 2010; Xiao et al. 2010; Gallego et al. 2014; Kweon et al. 2015; Muangchinda et al. 2015). On the other hand, ten bacterial genera with high proportion in bioreactor C were found (Fig. 5b). Five of the 10 bacterial genera (i.e., *Chryseobacterium*, *Hydrogenophaga*, *Nitrosomonas*,

*Sphingobium*, and *Terrimonas*) have been reported to be associated with the degradation of aromatic hydrocarbons (Chang et al. 2002; Hou et al. 2015; Oberoi et al. 2015; Song et al. 2015; Chen et al. 2016).

Results in Fig. 6 indicate the SAs degradation efficiency and bacterial community composition differed between early (B1–B5, C1–C5) and late (B6–B12, C6–C12) stages of bioreactor samples. Differentially distributed bacterial genera in different stages of bioreactor experiments were identified by Mann-Whitney *U* tests (Fig. 6). Eight bacterial genera with high proportion in the late stage (B6–B12) of bioreactor B were found (Fig. 6a). Five of the eight bacterial genera (i.e., *Brevundimonas*, *Hydrogenophaga*, *Nitrosomonas*, *Paracoccus*, and *Sphingopyxis*) have been reported to be associated with the degradation of aromatic hydrocarbons (Chang et al. 2002; Teng et al. 2010; Xiao et al. 2010; Gallego et al. 2014; Hou et al. 2015). Five bacterial genera with high proportion in early stage (B1–B5) of bioreactor B were found (Fig. 6b). Three of the five bacterial genera (i.e., *Acinetobacter*, *Arthrobacter*, and *Dokdonella*) have been reported to be associated with the degradation of aromatic hydrocarbons (Ferreira et al. 2013; Yuan et al. 2014; Bacosa and Inoue 2015). On the other hand, 11 bacterial genera with high proportion in the late stage (C6–C12) of bioreactor C were



**Fig. 6** Differentially distributed bacterial genera in different stages of bioreactor experiments. **a** Bacterial genera with high proportions in late stage of bioreactor B experiments. **b** Bacterial genera with high proportions in early stage of bioreactor B experiments. **c** Bacterial genera with high proportions in late stage of bioreactor C experiments. **d** Bacterial genera with high proportions in early stage of bioreactor C

experiments. A, B, and C represent soil, soil-sludge mixtures, and soil-sludge-SMC mixtures, respectively. 1–12 represent days 0, 4, 8, 10, 14, 18, 20, 24, 28, 30, 34, and 38, respectively. The red at sign indicate bacterial genera that have been reported to be involved in SAs degradation. The red asterisk indicate bacterial genera that have been reported to be involved in the degradation of aromatic hydrocarbons



found (Fig. 6c). Five of the 11 bacterial genera (i.e., *Brevundimonas*, *Chryseobacterium*, *Diaphorobacter*, *Paenibacillus*, and *Paracoccus*) have been reported to be associated with the degradation of aromatic hydrocarbons (Haritash and Kaushik 2009; Teng et al. 2010; Xiao et al. 2010; Muangchinda et al. 2015; Oberoi et al. 2015). Thirteen bacterial genera with high proportion in the early stage (C1–C5) of bioreactor C were found (Fig. 6d). Seven of the 13 bacterial genera (i.e., *Acinetobacter*, *Altererythrobacter*, *Pandoraea*, *Planctomyces*, *Pseudomonas*, *Rhodanobacter*, and *Rhodobacter*) have been reported to be associated with the degradation of aromatic hydrocarbons (Tang et al. 2010; Yuan et al. 2014; Guermouche M'rassi et al., 2015; Hou et al. 2015; Huang et al. 2015; Kaci et al. 2015; Song et al. 2016). Overall, 44 differentially distributed bacterial genera were identified from different experimental settings and stages. Twenty of the 44 bacterial genera were previously found involved in aromatic hydrocarbon degradation. Four bacterial genera (*Acinetobacter*, *Alcaligenes*, *Brevundimonas*, and *Pseudomonas*) have been reported as SA-degrading bacteria (Yan et al. 2011; Islas-Espinoza et al. 2012; Zhang et al. 2012; Herzog et al. 2013). We suggest that the 20 bacterial genera previously found in aromatic hydrocarbon or SA degradation might have high potential to involve in SA degradation in this study.

Differentially distributed bacterial genera identified among different experimental settings and stages in Fig. 6 imply multiple possibilities of bacterial community compositions for SA removal. Changes in bacterial community composition are the combined result of many factors such as symbiosis/competition among bacteria for carbon sources and adaptation of bacteria for environmental conditions.

## Conclusion

Microbial degradation is a major process of SA removal in soil-sludge mixtures. The presence of sludge may provide an important base for SA removal. In batch experiments, addition of SMC in soil-sludge mixtures exhibits highest SMX degradation efficiency than other additives. However, in bioreactor experiments, SA removal rates were not always significantly enhanced by the addition of SMC. With the high correlations between TOC and the proportions of SAs remaining, SAs may be mineralized to a greater extent with than without SMC in the soil-sludge mixtures. Forty-four bacterial genera were identified in different settings and stages of bioreactor experiments. Four bacterial genera *Acinetobacter*, *Alcaligenes*, *Brevundimonas*, and *Pseudomonas* were previously found involved in SA degradation, and 20 of the 44 bacterial genera were previously found involved in aromatic hydrocarbon degradation. Therefore, these bacteria have high potential to be SA degradation bacteria in this study.

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## Compliance with ethical standards

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

## References

- Accinelli C, Koskinen WC, Becker JM, Sadowsky MJ (2007) Environmental fate of two sulfonamide antimicrobial agents in soil. *J Agric Food Chem* 55:2677–1682. doi:10.1021/jf063709j
- Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environment. *Nat Rev Microbiol* 8:251–259. doi:10.1038/nrmicro2312
- Bacosa HP, Inoue C (2015) Polycyclic aromatic hydrocarbons (PAHs) biodegradation potential and diversity of microbial consortia enriched from tsunami sediments in Miyagi, Japan. *J Hazard Mater* 283:689–697. doi:10.1016/j.jhazmat.2014.09.068
- Baldrian P (2006) Fungal laccases occurrence and properties. *FEMS Microbiol Rev* 30:215–242. doi:10.1111/j.1574-4976.2005.00010.x
- Chang BV, Ren YL (2015) Biodegradation of three tetracyclines in river sediment. *Ecol Eng* 75:272–277. doi:10.1016/j.ecoleng.2014.11.039
- Chang SW, Hyman MR, Williamson KJ (2002) Cooxidation of naphthalene and other polycyclic aromatic hydrocarbons by the nitrifying bacterium, *Nitrosomonas europaea*. *Biodegradation* 13:373–381. doi:10.1023/A:1022811430030
- Chen C, Lei W, Lu M, Zhang J, Zhang Z, Luo C, Chen Y, Hong Q, Shen Z (2016) Characterization of Cu(II) and Cd(II) resistance mechanisms in *Sphingobium* sp. PHE-SPH and *Ochrobactrum* sp. PHE-OCH and their potential application in the bioremediation of heavy metal-phenanthrene co-contaminated sites. *Environ Sci Pollut Res* 23:6861–6872. doi:10.1007/s11356-015-5926-0
- Clarke BO, Smith SR (2011) Review of ‘emerging’ organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ Int* 37:226–247. doi:10.1016/j.envint.2010.06.004
- Ferreira L, Cobas M, Tavares T, Sanroman MA, Pazos M (2013) Assessment of *Arthrobacter viscosus* as reactive medium for forming permeable reactive biobarrier applied to PAHs remediation. *Environ Sci Pollut Res* 20:7348–7354. doi:10.1007/s11356-013-1750-6
- Gallego S, Vila J, Tauler M, Nieto JM, Breugelmans P, Springael D, Grifoll M (2014) Community structure and PAH ring-hydroxylating dioxygenase genes of a marine pyrene-degrading microbial consortium. *Biodegradation* 25:543–556. doi:10.1007/s10532-013-9680-z
- Guermouche M'rassi A, Bensalah F, Gury J, Duran R (2015) Isolation and characterization of different bacterial strains for bioremediation of *n*-alkanes and polycyclic aromatic hydrocarbons. *Environ Sci Pollut Res* 22:15332–15346. doi:10.1007/s11356-015-4343-8
- Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *J Hazard Mater* 169:1–15. doi:10.1016/j.jhazmat.2009.03.137
- Herzog B, Lemmer H, Horn H, Muller E (2013) Characterization of pure cultures isolated from sulfamethoxazole-acclimated activated sludge with respect to taxonomic identification and sulfamethoxazole biodegradation potential. *BMC Microbiol* 13:276. doi:10.1186/1471-2180-13-276
- Hou J, Liu W, Wang B, Wang Q, Luo Y, Franks AE (2015) PGPR enhanced phytoremediation of petroleum contaminated soil and

- rhizosphere microbial community response. *Chemosphere* 138: 592–598. doi:10.1016/j.chemosphere.2015.07.025
- Hsu FY, Wang ZY, Chang BV (2013) Use of microcapsules with electrostatically immobilized bacterial cells or enzyme extract to remove nonylphenol in wastewater sludge. *Chemosphere* 91:745–750. doi:10.1016/j.chemosphere.2013.02.019
- Huang Y, Zeng Y, Feng H, Wu Y, Xu X (2015) *Croceicoccus naphthovorans* sp. nov., a polycyclic aromatic hydrocarbons-degrading and acylhomoserine-lactone-producing bacterium isolated from marine biofilm, and emended description of the genus *Croceicoccus*. *Int J Syst Evol Microbiol* 65:1531–1536. doi:10.1099/ijs.0.000132
- Islas-Espinoza M, Reid BJ, Wexler M, Bond PL (2012) Soil bacterial consortia and previous exposure enhance the biodegradation of sulfonamides from pig manure. *Microb Ecol* 64:140–151. doi:10.1007/s00248-012-0010-5
- Kaci A, Petit F, Fournier M, Cecillon S, Boust D, Lesueur P, Berthe T (2015) Diversity of active microbial communities subjected to long-term exposure to chemical contaminants along a 40-year-old sediment core. *Environ Sci Pollut Res* 23:4095–4110. doi:10.1007/s11356-015-4506-7
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. *Environ Sci Technol* 36:1202–1211. doi:10.1021/es011055j
- Kweon O, Kim SJ, Blom J, Kim SK, Kim BS, Baek DH, Park SI, Sutherland JB, Cerniglia CE (2015) Comparative functional pan-genome analyses to build connections between genomic dynamics and phenotypic evolution in polycyclic aromatic hydrocarbon metabolism in the genus *Mycobacterium*. *BMC Evol Biol* 15:21–44. doi:10.1186/s12862-015-0302-8
- Lau KL, Tsang YY, Chiu SW (2003) Use of spent mushroom compost to bioremediate PAH-contaminated samples. *Chemosphere* 52:1539–1546. doi:10.1016/S0045-6535(03)00493-4
- Lee SM, Koo BW, Lee SS, Kim MK, Choi DH, Hong EJ, Jeung EB, Choi IG (2004) Biodegradation of dibutyl phthalate by white rot fungi and evaluation on its estrogenic activity. *Enzym Microb Technol* 35: 417–423. doi:10.1016/j.enzmictec.2004.06.001
- Li XZ, Lin XG, Zhang J, YC W, Yin R, Feng YZ, Wang Y (2010) Degradation of polycyclic aromatic hydrocarbons by crude extracts from spent mushroom substrate and its possible mechanisms. *Curr Microbiol* 60:336–342. doi:10.1007/s00284-009-9546-0
- Liao CS, Yuan SY, Hung BH, Chang BV (2012) Removal of organic toxic chemicals using the spent mushroom compost of *Ganoderma lucidum*. *J Environ Monit* 14:1983–1988. doi:10.1039/c2em10910g
- Muangchinda C, Chavanich S, Viyakarn V, Watanabe K, Imura S, Vangnai AS, Pinyakong O (2015) Abundance and diversity of functional genes involved in the degradation of aromatic hydrocarbons in Antarctic soils and sediments around Syowa Station. *Environ Sci Pollut Res* 22:4725–4735. doi:10.1007/s11356-014-3721-y
- Nieto A, Borrull F, Pocurull E, Marce RM (2010) Occurrence of pharmaceuticals and hormones in sewage sludge. *Environ Toxicol Chem* 29:1484–1489. doi:10.1002/etc.188
- Oberoi AS, Philip L, Bhallamudi SM (2015) Biodegradation of various aromatic compounds by enriched bacterial cultures: part A—monocyclic and polycyclic aromatic hydrocarbons. *Appl Biochem Biotechnol* 176:1870–1888. doi:10.1007/s12010-015-1684-1
- Pepper LL, Gerba CP, Gentry TJ (2015) Environmental microbiology. In: Maier MM, Gentry TJ (eds) *Microorganisms and organic pollutants*. Elsevier Inc, CA, pp. 377–413
- Semple KT, Reid BJ, Fermor TR (2001) Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ Pollut* 112:269–283. doi:10.1016/S0269-7491(00)00099-3
- Song M, Luo C, Jiang L, Zhang D, Wang Y, Zhang G (2015) Identification of benzo[a]pyrene-metabolizing bacteria in forest soils by using DNA-based stable-isotope probing. *Appl Environ Microbiol* 81:7368–7376. doi:10.1128/AEM.01983-15
- Song M, Jiang L, Zhang D, Luo C, Wang Y, Yu Z, Yin H, Zhang G (2016) Bacteria capable of degrading anthracene, phenanthrene, and fluoranthene as revealed by DNA based stable-isotope probing in a forest soil. *J Hazard Mater* 308:50–57. doi:10.1016/j.jhazmat.2016.01.009
- Sukul P, Spiteller M (2006) Sulfonamides in the environment as veterinary drugs. *Rev Environ Contam Toxicol* 187:67–101. doi:10.1007/0-387-32885-8\_2
- Tang X, He LY, Tao XQ, Dang Z, Guo CL, GN L, Yi XY (2010) Construction of an artificial microalgal-bacterial consortium that efficiently degrades crude oil. *J Hazard Mater* 181:1158–1162. doi:10.1016/j.jhazmat.2010.05.033
- Teng Y, Luo Y, Sun M, Liu Z, Li Z, Christie P (2010) Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbons from an aged contaminated soil. *Bioresour Technol* 101:3437–3443. doi:10.1016/j.biortech.2009.12.088
- Wang QQ, Guo MX, Yates SR (2006) Degradation kinetics of manure-derived sulfadimethoxine in amended soil. *J Agric Food Chem* 54: 157–163. doi:10.1021/jf052216w
- Xiao J, Guo L, Wang S, Lu Y (2010) Comparative impact of cadmium on two phenanthrene-degrading bacteria isolated from cadmium and phenanthrene co-contaminated soil in China. *J Hazard Mater* 174: 818–823. doi:10.1016/j.jhazmat.2009.09.126
- Xu M, Chen X, Qiu M, Zeng X, Xu J, Deng D, Sun G, Li X, Guo J (2012) Bar-coded pyrosequencing reveals the responses of PBDE-degrading microbial communities to electron donor amendments. *PLoS One* 7:e30439. doi:10.1371/journal.pone.0030439
- Yan P, Lu M, Guan Y, Zhang W, Zhang Z (2011) Remediation of oil-based drill cuttings through a biosurfactant-based washing followed by a biodegradation treatment. *Bioresour Technol* 102:10252–10259. doi:10.1016/j.biortech.2011.08.074
- Yang JF, Ying GG, Yang LH, Zhao JL, Liu F, Tao R (2009) Degradation behavior of sulfadiazine in soils under different conditions. *J Environ Sci Health B* 44:241–248. doi:10.1080/03601230902728245
- Yang SF, Lin CF, Lin AYC, Hong PKA (2011) Sorption and biodegradation of sulfonamide antibiotics by activated sludge: experimental assessment using batch data obtained under aerobic conditions. *Water Res* 45:3389–3397. doi:10.1016/j.watres.2011.03.052
- Yang CW, Huang HW, Chao WL, Chang BV (2015a) Bacterial communities associated with aerobic degradation of polybrominated diphenyl ethers from river sediment. *Environ Sci Pollut Res* 22: 3810–3819. doi:10.1007/s11356-014-3626-9
- Yang Y, Wang Z, He T, Dai Y, Xie S (2015b) Sediment bacterial communities associated with anaerobic biodegradation of bisphenol-A. *Microb Ecol* 70:97–104. doi:10.1007/s00248-014-0551-x
- Yuan H, Yao J, Masakorala K, Wang F, Cai M, Yu C (2014) Isolation and characterization of a newly isolated pyrene-degrading *Acinetobacter* strain USTB-X. *Environ Sci Pollut Res* 21:2724–2732. doi:10.1007/s11356-013-2221-9
- Zhang WW, Wen YY, Niu ZL, Yin K, DX X, Chen LX (2012) Isolation and characterization of sulfonamide-degrading bacteria *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51. *World J Microbiol Biotechnol* 28:447–452. doi:10.1007/s11274-011-0834-z