Supplementary Information

MATERIALS AND METHODS

Media

Media were made individually for each column by spiking an aliquot of a stock solution containing all PPCPs (except sodium diclofenac and 5-flurouracil) in acetone into the bottom of an empty 1 L glass flask. The spike for the 0.25 μg L^{-1} column medium came from a 0.5 mg L^{-1} stock, whereas the spike for all other columns came from a 500 mg L^{-1} stock. Volumes added for each column's medium are as follows: 0.020 mL of 500 mg L^{-1} stock for both 10 μg L^{-1} columns (abiotic and active), 0.50 mL of 0.5 mg L^{-1} stock for the 0.25 μg L^{-1} column, 0.20 mL of 500 mg L^{-1} stock for the 100 μg L^{-1} column, and 2.0 mL of 500 mg L^{-1} stock for the 1000 μg L^{-1} column. A volume of pure acetone equal to the difference between 2.0 mL and the PPCP spike was also added so that each flask contained the same total volume of acetone. A gentle stream of nitrogen gas was directed into the flask until the acetone evaporated to dryness. The PPCP residue remaining on the bottom of the flask was reconstituted in water by adding 1 L of mineral medium (100 μg L^{-1} acetate as sodium acetate, 8.5 mg L^{-1} KH_2PO_4, 22 mg L^{-1} K_2HPO_4, 33 mg L^{-1} Na_2HPO_4, 28 mg L^{-1} $CaCl_2$, 0.25 mg L^{-1} $FeCl_3$, 2.7 mg L^{-1} NH_4Cl, and 23 mg L^{-1} $MgSO_4$ in ultrapure water) and stirring with a Teflon-coated magnetic stirbar for 15 min. The media were also supplemented with sodium diclofenac and 5-fluorouracil from a 5 mg L^{-1} aqueous stock to match the final concentrations of the other PPCPs in each medium. These two PPCPs were supplied separately due to their low solubility in acetone.

Column sample collection and PPCP analysis. Influent and effluent column samples were collected and compared to determine the extent of biological removal of PPCPs in the columns. Influent samples were withdrawn aseptically from the influent media flasks, and effluent samples were collected into amber, foil-covered glass vessels. For the abiotic, 10 μg L^{-1}, 100 μg L^{-1}, and 1000 μg L^{-1} columns, effluent samples were collected for approximately three hours to yield enough volume for triplicate subsampling. Sample volumes were different for each column to facilitate analytical detection (i.e. smaller sample volumes for higher PPCP concentrations and vice versa), but volumes were always identical for each column's influent and effluent samples at any given time point. The sample volumes were as follows: 0.2 mL for the 1000 μg L^{-1} column, 1 mL for the 100 μg L^{-1} column, 5 mL for both of the 10 μg L^{-1} columns (abiotic and active). Due to decreased analytical sensitivity later in the experiment when analyses were switched to a different GC-MS, sample volumes were later increased slightly to 0.25, 4.0, and 7.0 mL for the 1000, 100, and 10 μg L^{-1} PPCP columns, respectively.

Samples from the 0.25 μg L^{-1} PPCP column were prepared for analysis differently than their higher concentration counterparts from the other columns. Duplicate samples (150 mL) were collected over a period of approximately 42-48 h, during which time mercuric chloride was periodically spiked into the effluent collection vessel to prevent biotransformation from occurring after passage through the column. The solid phase extraction (SPE) method of Yu et al. (1) was followed using Phenomenex Strata-X cartridges (500 mg, Torrance, CA) to concentrate the PPCP analytes before derivatization. Our method differed slightly from Yu et al.'s method in that our sample volumes were 150 mL for both controls and experimental samples, no surrogate standards were used as differences in matrix effects in the influent and effluent were assumed to be negligible, and the final cartridge-washing step using 5 mL of 10%

46 methanol was omitted due to the relative cleanliness of our laboratory system as compared to the
47 complexity of the WWTP samples for which the method was developed. Lab fortified samples
48 (150 mL of 0.25 μg L^{-1} PPCPs in ultrapure water) were also periodically processed along with
49 the column samples to assess method recoveries. SPE samples were eluted with 7.0 mL of
50 acetonitrile and combined with 7.0 mL of water, after which they were derivatized along with the
51 samples from the higher concentration columns that did not undergo SPE.
52 In preparation of the subsequent derivatization step prior to GC-MS analysis, aliquots of
53 5 mL and 4 mL ultrapure water were added to the 1000 μg L^{-1} and 100 μg L^{-1} samples,
54 respectively, so that samples could be analyzed at similar volumes. The derivatization procedure
55 also required a 1:1 ratio of aqueous sample to acetonitrile, so aliquots of 5 mL of acetonitrile
56 were added to each of the aqueous samples collected. All samples and calibration standards
57 (ranging in concentration from 0 to 2480 μg L^{-1}) underwent a pentafluorobenzylation
58 derivatization procedure, followed by GC-MS analysis as described elsewhere (1). Our method
59 differed from Yu et al.'s only in our use of unlabeled 4,4-di-tert-butylbiphenyl (Sigma-Alrich-
60 Fluka) as the internal standard. Analyses for samples collected prior to day 266 were analyzed
61 on a ThermoQuest Trace 2000 GC coupled to a quadrupole MS (San Jose, CA) with an Rtx-5
62 column (Restek, Bellefonte, PA; 30 m length x 0.32 mm i.d. x 1 μm phase thickness). Due to
63 loss of functionality of the ThermoQuest GC-MS, samples from day 266 and later were analyzed
64 using a Shimadzu GC-MS (GC17A/QP5050A, Columbia, MD) with a DB5 column (Agilent
65 J&W, Santa Clara, CA; 30 m length, 0.25 mm ID, 0.25 film thickness). The method employed
66 was identical for both GC-MS instruments.
67

Due to the increased analytical variability observed in the SPE step, samples from the 0.25 μg L^{-1} column underwent an additional quality control step to determine if influent concentrations were within 50 to 150% of the supplied concentration. Samples that did not meet this requirement were dropped from further data analysis.

RESULTS

Column characterization. The biodegradation of the sodium acetate supplied to the columns as an easily degradable carbon source was measured using ^{14}C-labeled acetate and liquid scintillation counting. The "Total ^{14}C" and "^{14}C Acetate" concentrations were determined for the influent and effluent samples from the abiotic control, 100 μg/L PPCP, and 1000 μg/L PPCP columns (Table S4). The "% in effluent" column was calculated by dividing the effluent concentration by the influent concentration. Recovery of the radioactive label was found to be 95% in the abiotic control column, with 89% measured as acetate. Because additional experiments have shown that our acid volatilization method for acetate measurement is reasonably accurate and precise without production or loss of acetate (± 3%), the data seem to suggest that either some small $HgCl_2$-resistant population is degrading acetate within the abiotic column or that roughly 6% of acetate can falsely measure as "other products." Much less recovery was observed in the two active columns, in which 68-69% recovery was found. Previous experiments and calculations reveal that this loss is likely due to assimilation of ^{14}C label into biofilm biomass. Acetate concentration measurements for the active columns show only 2.0% ^{14}C acetate remaining in the effluent of the 100 μg/L PPCP column, and only 5.0% of the ^{14}C acetate remaining in the effluent of the 1000 μg/L PPCP column. This acetate

91 measurement may include not only acetate, but also any other non-acid-volatilized metabolites or
92 byproducts of degradation and biofilm decay. In any case, these low concentrations remaining in
93 the effluent of the active columns suggest that the majority of the acetate supplied to the columns
94 in the influent media is being degraded in the columns.

95 Another indication of active biofilms present in the columns is depletion of dissolved
96 oxygen in the media. For the abiotic control, 0.25 μg/L PPCP, 10 μg/L PPCP, 100 μg/L PPCP,
97 and 1000 μg/L PPCP columns, influent media dissolved oxygen concentrations were 8.7, 8.7,
98 8.8, 8.9, and 8.8 mg/L and effluent dissolved oxygen concentrations were 2.7, 2.7, 2.8, 4.7, and
99 3.3 mg/L, respectively. Depletion of oxygen in the abiotic column suggests that there is some
100 population of $HgCl_2$ resistant bacteria in the column, as also indicated by the minor acetate
101 degradation observed, or that one or more abiotic oxidation reactions are occurring. Measured
102 pH values of influent (7.2 – 7.3) and effluent (6.6 – 6.8) were similar among the columns.

103 Tritiated water tracer tests performed on days 291-293 provide insight into the
104 hydrodynamic conditions in the columns. Breakthrough curves for each column were modeled
105 using CXTFIT software, which fits the data to the convection dispersion equation in order to
106 calculate the average porewater velocity (cm/day) and dispersion coefficient (cm^2/day) (2)
107 (Table S5). The average pore-water velocities ranged from 85 to 100 cm/d, and dispersion
108 coefficients ranged from 6.1 to 94 cm^2/d. Overall, the columns' hydrodynamic properties were
109 similar enough to each other that they should not confound any findings based on the single
110 experimental variable of PPCP concentration.

Primer	Roche primer	Library key	Multiplex identifier (MID)	16S target
Column Low F	5'-CGT ATC GCC TCC CTC GCG CCA	TCA G	ACG AGT GCG T	GTG CCA GCM GCN GCG G-3'
Column Low R	5'-CTA TGC GCC TTG CCA GCC CGC	TCA G	ACG CTC GAC A	GGG TTN CGN TCG TTG-3'
Column High F	5'-CGT ATC GCC TCC CTC GCG CCA	TCA G	AGA CGC ACT C	GTG CCA GCM GCN GCG G-3'
Column High R	5'-CTA TGC GCC TTG CCA GCC CGC	TCA G	AGC ACT GTA G	GGG TTN CGN TCG TTG-3'
SAT Day 1 F	5'-CGT ATC GCC TCC CTC GCG CCA	TCA G	CGT GTC TCT A	GTG CCA GCM GCN GCG G-3'
SAT Day 1 R	5'-CTA TGC GCC TTG CCA GCC CGC	TCA G	CTC GCG TGT C	GGG TTN CGN TCG TTG-3'
SAT Day 2 F	5'-CGT ATC GCC TCC CTC GCG CCA	TCA G	TAG TAT CAG C	GTG CCA GCM GCN GCG G-3'
SAT Day 2 R	5'-CTA TGC GCC TTG CCA GCC CGC	TCA G	TCT CTA TGC G	GGG TTN CGN TCG TTG-3'
SAT Day 3 F	5'-CGT ATC GCC TCC CTC GCG CCA	TCA G	TGA TAC GTC T	GTG CCA GCM GCN GCG G-3'
SAT Day 3 R	5'-CTA TGC GCC TTG CCA GCC CGC	TCA G	TAC TGA GCT A	GGG TTN CGN TCG TTG-3'

Table S1. Target 16S sequences and the Roche primers, library keys, and multiplex identifiers used for PCR amplification and tagging

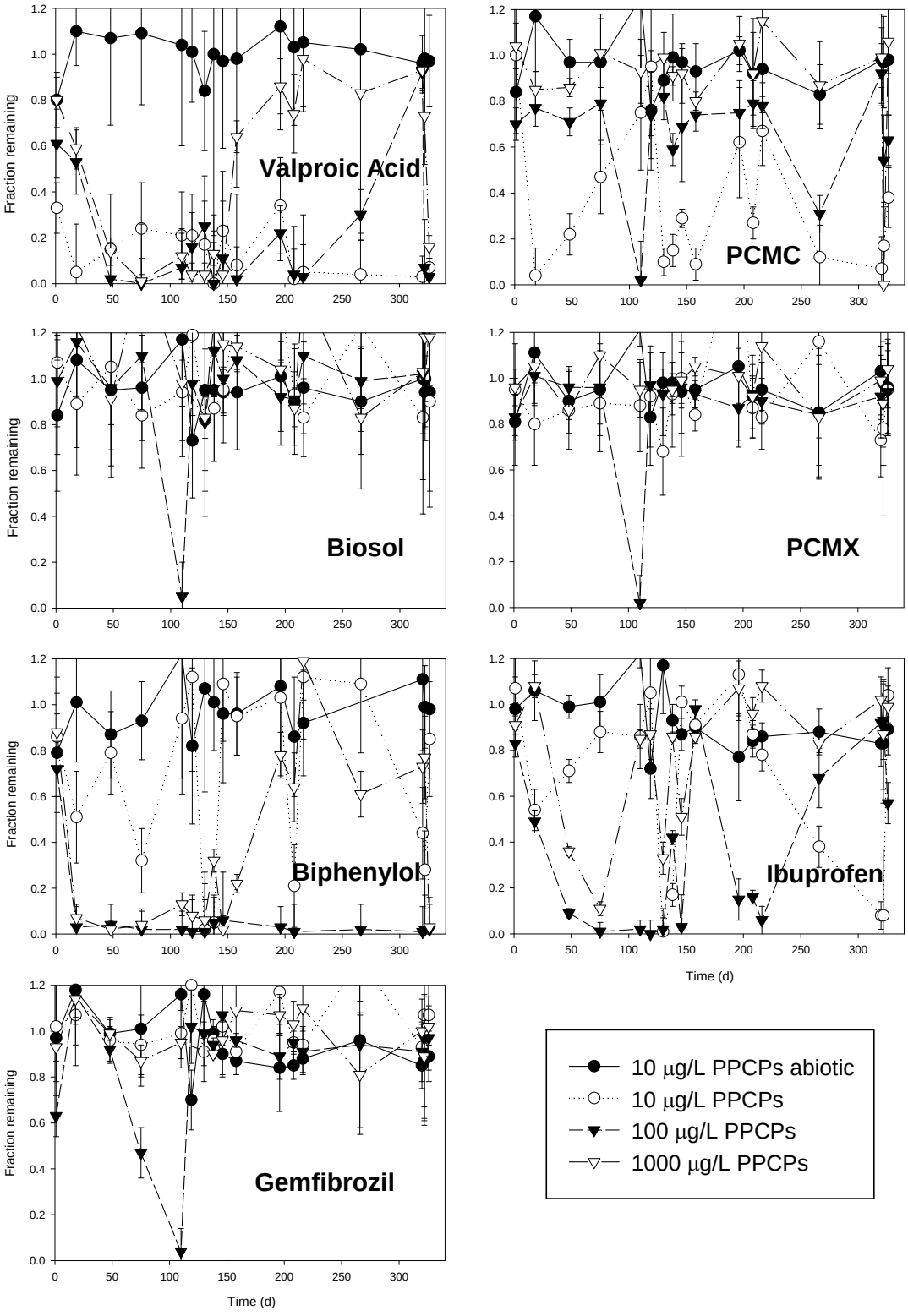
Valproic Acid
PCMC
Biosol
PCMX
Biphenylol
Ibuprofen
Gemfibrozil
Fraction remaining
Time (d)
10 μg/L PPCPs abiotic
10 μg/L PPCPs
100 μg/L PPCPs
1000 μg/L PPCPs

Figure 1 continued

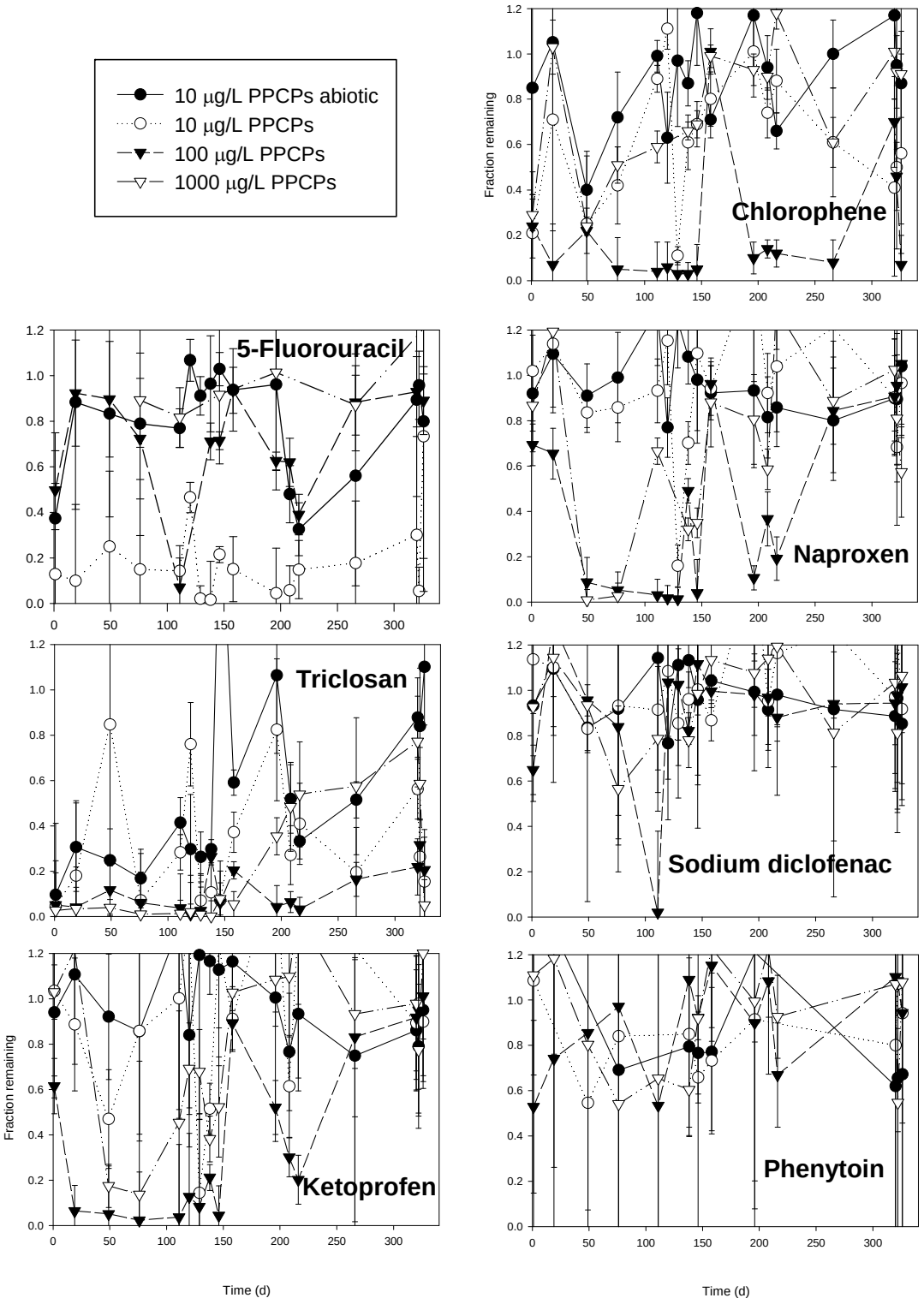

Figure S1 The fraction of the applied concentration remaining in the column effluent for each time point sampled from day 0 to day 326 for each PPCP.

PPCP	P-values (all columns)	P-values (active columns)
Biosol	0.16	0.52
Biphenylol	< 0.0010	< 0.0010
p-Chloro-*m*-cresol	< 0.0010	< 0.0010
p-Chloro-*m*-xylenol	0.89	0.83
Chlorophene	<0.0010	< 0.0010
Diclofenac (sodium)	0.0060	0.011
5-Fluorouracil	< 0.0010	< 0.0010
Gemfibrozil	0.0090	0.27
Ibuprofen	0.0020	0.0061
Ketoprofen	0.011	0.015
Naproxen	0.028	0.054
Phenytoin	0.19	0.48
Triclosan	< 0.0010	0.12
Valproic Acid	< 0.0010	< 0.0010

Table S2. P-values from ANOVA testing of mean PPCP removals between days 138 and 326. Values less than or equal to 0.05 indicate that the mean removals in the five columns were not all equivalent at a 95% confidence interval.

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Columns Compared		**Valproic Acid**	***p*-Chloro-*m*-cresol**	**Biphenylol**	**Ibuprofen**	**Chlorophene**	**5-Flurouracil**	**Diclofenac**	**Ketoprofen**
10 µg/L abiotic	0.25 µg/L	0.750	**0.000**	0.997	0.686	0.892		**0.034**	0.809
10 µg/L abiotic	10 µg/L	**0.000**	**0.000**	0.264	0.467	0.259	**0.000**	0.279	0.990
10 µg/L abiotic	100 µg/L	**0.000**	**0.030**	**0.000**	**0.048**	**0.000**	0.962	1.000	0.083
10 µg/L abiotic	1000 µg/L	**0.013**	0.886	**0.013**	0.997	0.982	0.341	0.974	1.000
0.25 µg/L	10 µg/L	**0.000**	0.081	0.640	0.063	0.073		**0.004**	0.953
0.25 µg/L	100 µg/L	**0.000**	0.533	**0.000**	**0.004**	**0.000**		**0.034**	**0.019**
0.25 µg/L	1000 µg/L	0.494	**0.006**	0.106	0.849	0.644		**0.018**	0.765
10 µg/L	100 µg/L	0.941	**0.001**	**0.000**	0.750	**0.030**	**0.000**	0.279	**0.027**
10 µg/L	1000 µg/L	**0.000**	**0.000**	0.630	0.279	0.562	**0.000**	0.622	0.979
100 µg/L	1000 µg/L	**0.000**	0.230	**0.007**	**0.020**	**0.000**	0.195	0.974	0.103

Table S3. P-values from Tukey analysis performed using R software. P-values suggesting statistically significant differences (< 0.05) between the two columns compared are bolded, while those that are not significant are shaded.

Column	Total ^{14}C			^{14}C Acetate		
	Influent (dpm/g)	Effluent (dpm/g)	% in effluent	Influent (dpm/g)	Effluent (dpm/g)	% in effluent
Abiotic Control	14500 ± 350	13800 ± 800	95	13700 ± 230	12100 ± 150	89
100 µg/L PPCP	14000 ± 130	9500 ± 520	68	13500 ± 150	276 ± 28	2.0
1000 µg/L PPCP	14300 ± 140	9800 ± 260	69	13900 ± 200	699 ± 180	5.0

Table S4. Total ^{14}C and ^{14}C-acetate activities in dpm/g for influent and effluent samples for three columns, along with the percent of the influent acetate concentration remaining in the effluent of each. The mean of four discrete sampling points is presented plus or minus one standard deviation.

Column	Average pore-water velocity (cm/d)	Hydraulic residence time (h)	D (cm^2/d)
Abiotic Control	100	7.1	6.1
0.25 μg/L PPCP	85	8.5	14
10 μg/L PPCP	91	7.9	9.6
100 μg/L PPCP	100	6.9	8.9
1000 μg/L PPCP	94	7.6	9.4

Table S5. Results of tritiated water tracer test modeled with CXTFIT software. The software uses the convection dispersion equation to estimate average pore-water velocity (cm/d) and D (cm^2/d), the dispersion coefficient. The column length (30 cm) was divided by the average pore-water velocity to calculate the hydraulic residence time for each column (h).

Parameter	Measurement
Bromide	0.52 mg/L
Chloride	161 mg/L
Fluoride	0.58 mg/L
Nitrate as N	2.6 mg/L
Nitrite as N	1.1 mg/L
Orthophosphate as P	3.1 mg/L
Sulfate	152 mg/L
Total organic carbon	16.8 mg/L
TKN	27 mg/L
Dissolved oxygen	5.66 mg/L
Temperature	27.1 °C
pH	7.60
Conductivity	1376 µmho/cm

Table S6. Water quality parameters for the water applied to the SAT spreading basin. Sample analyses were performed by MWH Labs for all but the last four parameters.

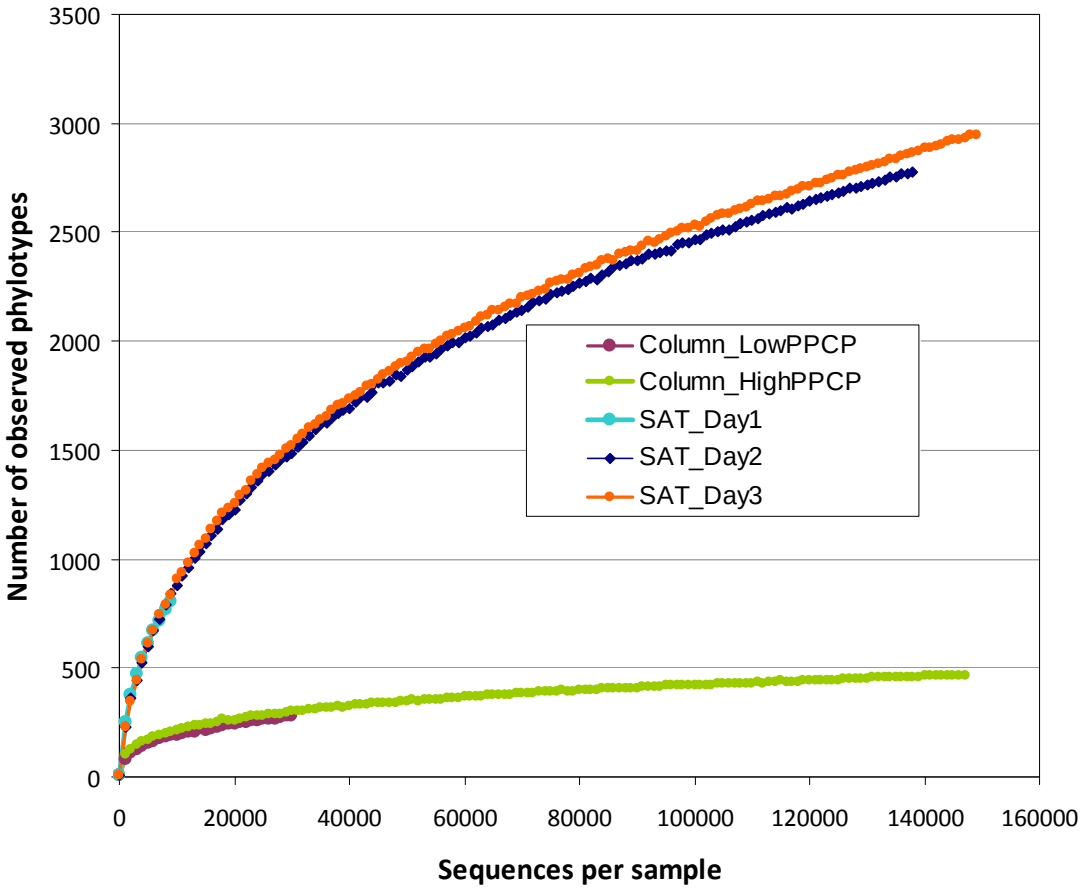

Figure S2. Rarefaction curve showing the number of observed phylotypes as a function of the number of sequences sampled.

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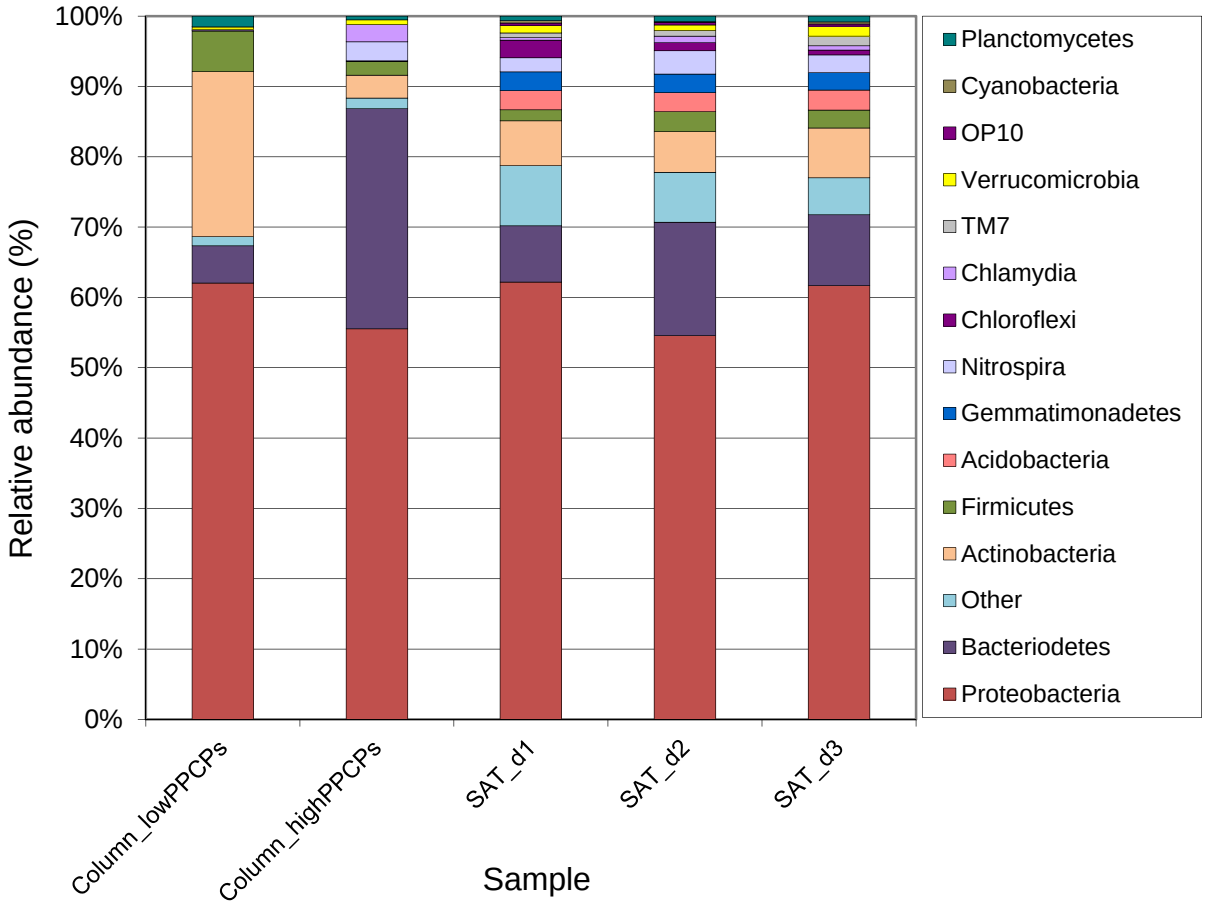

Figure S3. Relative abundance of each phylum for column and SAT samples. Only phyla that were present at an abundance of at least 0.1% in one or more samples were included.

Consensus taxonomy	Closest named species	% Identity	Column low PPCP	Column high PPCP	SAT d1	SAT d2	SAT d3	RSD (SAT)
Xanthobacteraceae	*Xanthobacter flavus* (JN592464)	99	14.69	14.81	17.84	17.38	19.25	5
Xanthobacteraceae	*Xanthobacter flavus* (JN592464)	99	6.62	5.42	6.10	6.44	6.61	4
Mycobacterium	*Mycobacterium sp. R5* (JN110434)	99	13.93	3.04	3.22	4.01	5.63	29
Xanthobacteacea	*Xanthobacter flavus* (JN592464)	99	14.77	12.52	0.93	1.05	0.53	32
Xanthobacteraceae	*Xanthobacter flavus* (JN592464)	99	5.44	2.95	3.97	4.37	4.91	11
Burkholderiales	*Methylibium sp. LCB69* (JN650585)	98	4.33	1.60	2.54	2.49	3.28	16
Azospira	*Dechlorosoma suillum* (CP003153)	99	1.79	2.14	3.36	3.09	3.15	4
Methylibium	*Piscinibacter aquaticus* (AB681749)	99	1.86	2.51	2.41	2.35	2.52	4
Pseudoxanthomonas	*Pseudoxanthomonas mexicana* (JQ660737)	99	0.30	0.22	3.68	1.66	3.25	37
Ferruginibacter	*Ferruginibacter alkalilentus* (NR_044588)	98	0.30	3.44	1.08	2.47	1.59	41
Mycobacterium	*Mycobacterium gilvum* (JN590246)	99	7.09	0.02	0.17	0.08	0.14	36
Euglenaceae	*Trachelomonas volvocinopsis* (FJ719709)	97	0.87	3.66	0.44	1.55	0.94	57
Sphingobium	*Sphingobium yanoikuyae* (JF681288)	98	1.89	3.49	0.38	0.48	0.95	50
Lacibacter	*Lacibacter cauensis* (AB682227)	97	0.23	2.54	0.74	1.52	0.93	39

Consensus taxonomy	Closest named species	% Identity	Column low PPCP	Column high PPCP	SAT d1	SAT d2	SAT d3	RSD (SAT)
Desulfosporosinus	*Desulfosporosinus youngiae* (DQ117470)	98	0.83	1.86	0.65	1.40	1.15	36
Sphingobacteriales	*Nostoc sp. NTK29* (DQ513319)	96	0.19	2.26	0.81	1.47	0.73	41
Comamonadaceae	*Variovorax paradoxus* (JQ692086)	100	0.49	0.66	1.02	1.33	1.89	31
Nitrospira	*Nitrospira moscoviensis* (AF155155)	99	0.00	0.01	1.21	2.11	1.71	27
Lacibacter	*Lacibacter cauensis* (AB682227)	95	0.18	1.80	0.59	1.39	0.84	44
Nitrospira	*Candidatus Nitrospira defluvii* (FP929003)	99	0.00	2.54	0.54	0.91	0.60	29
Chitinophagaceae	*Chitinophaga arvensicola* (AB681053)	96	0.13	1.77	0.68	1.27	0.73	37
Desulfosporosinus	*Desulfosporosinus youngiae* (DQ117470)	97	4.36	0.00	0.02	0.08	0.07	58
Proteobacteria	*Agrobacterium tumefaciens* (JQ659544)	98	1.53	0.27	0.57	0.74	0.80	17
Chitinophagaceae	Terrimonas sp. YJ03(JN848793)	95	0.11	1.77	0.40	0.94	0.55	44
Rhodobacter	*Paracoccus carotinifaciens* strain ZM5 (HQ538757)	97	0.05	0.19	1.18	1.05	1.13	6
Gemmatimonas	*Gemmatimonas aurantiaca* (AP009153)	94	0.00	0.03	0.92	1.27	1.13	16
Flavobacterium	Flavobacterium chungangense (NR_044581)	98	0.04	3.10	0.02	0.01	0.01	54
Parachlamydiaceae	Parachlamydia acanthamoebae UV-7 (FR872580)	93	0.07	1.48	0.30	0.80	0.47	49
Mucilaginibacter	Mucilaginibacter sp. HME6636 (HM638228)	98	0.20	1.26	0.24	0.83	0.52	56
Sphingobium	*Sphingobium yanoikuyae* (JF681288)	99	0.84	1.29	0.13	0.21	0.33	45
Bosea	*Bosea thiooxidans* (JQ659580)	98	0.15	0.04	1.11	0.93	0.42	43
Flavobacterium	Flavobacterium terrigena (JQ692099)	97	0.00	2.19	0.00	0.01	0.00	108
Sediminibacterium	Sediminibacterium salmoneum (AB682145)	99	0.15	1.34	0.11	0.34	0.26	48
Nocardiaceae	*Gordonia sp. NP8-5* (NR_042664)	99	1.08	0.00	0.18	0.09	0.13	33
Rhodobacteraceae	*Rhodobacter megalophilus* (JQ692104)	99	1.17	0.03	0.03	0.04	0.03	14
Xanthobacter	*Xanthobacter autotrophicus* (AB680655)	99	0.00	1.23	0.00	0.00	0.00	n.a.

Table S7. Abundantly detected phylotypes for each sample, along with their relative abundance (%). Only phylotypes present in at least one sample at ≥ 1 % abundance are included, ranked in order of decreasing additive abundance across all samples. The "% identity" parameter refers to the similarity of the sample sequence to the named species' sequence. "RSD" is the relative standard deviation calculated for the SAT samples only.

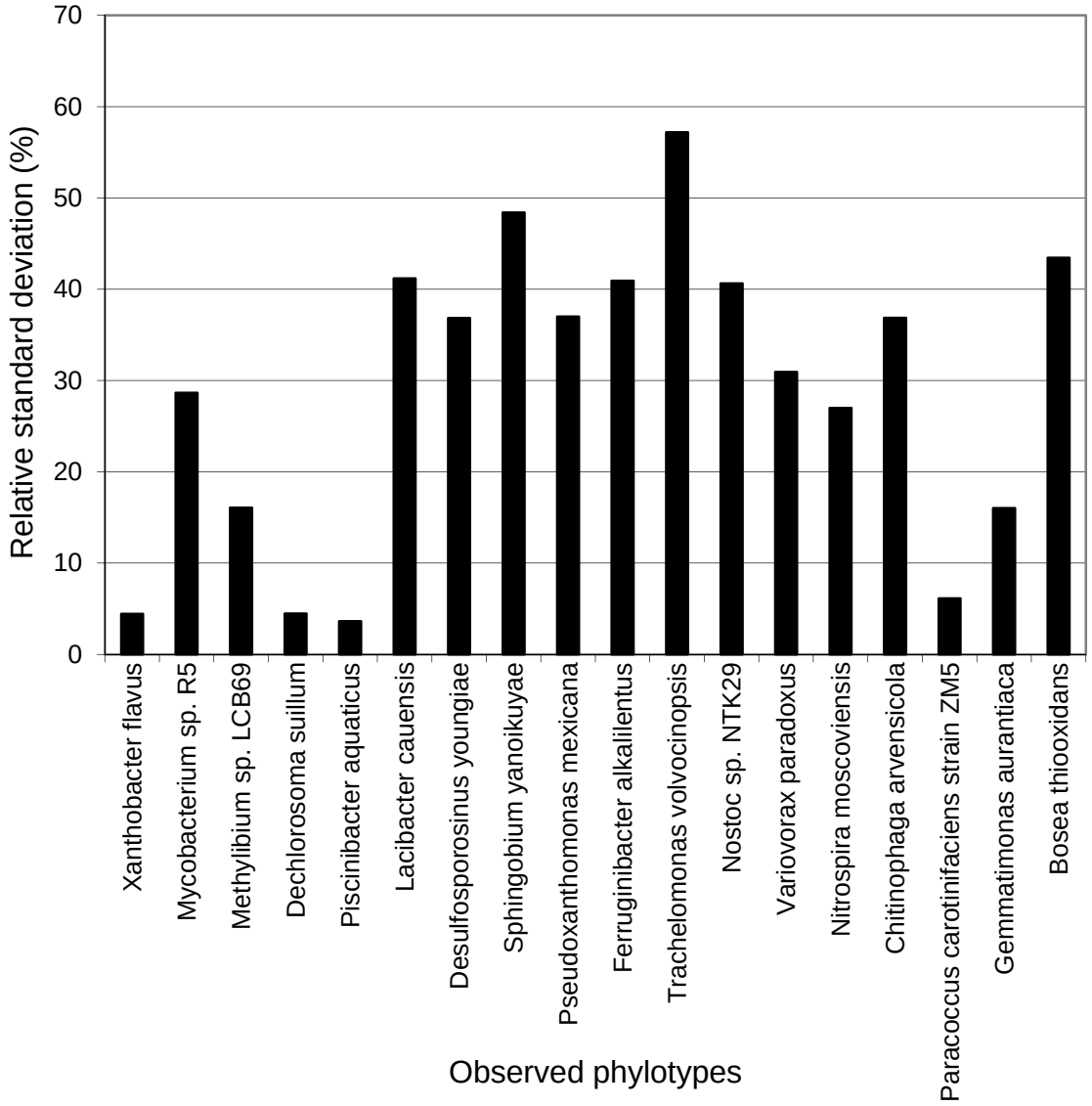

Figure S4. Relative standard deviation of phylotypes found in SAT soil samples. Only phylotypes that were present in at least one of the SAT samples at an abundance ≥ 1 % were included.

	Minimum inhibitory concentrations (μg L^{-1})			
	Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli	Klebsiella pneumoniae
Triclosan	[a]100, [b]10, [c]100, [d]16, [e]1-2 × 10^3, [f]250, [g]3.1 × 10^3	[a] > 3 × 10^5	[a]5.0 × 10^3, [b]100, [c]90-100, [g]600	[a]5.0 × 10^3
Biphenylol	[a]1.0 × 10^5	[a]1.0 × 10^6	[a]1.0 × 10^6	[a]5.0 × 10^5
PCMX	[a]2.50 × 10^5, [g]7.5 × 10^4	[a]1.0 × 10^6	[a]1.0 × 10^6 [g]2 × 10^5	[a]5.0 × 10^5
PCMC	[a]6.25 × 10^5	[a]1.25 × 10^6	[a]1.25 × 10^6	[a]6.25 × 10^5

[a]Russell and Gould (3); [b]broth test, Gomez Escalada et al. (4), [c]agar test, Gomez Escalada et al.(4); [d]results for 24 of the 31 tested clinical strains, Fan et al. 2002 (5), [e]results for 6 of the 31 strains, Fan et al. 2002 (5), [f]results for 1 of the 31 strains, Fan et al. 2002 (5), [g] Johnson et al. (6)

Table S8. Literature values for minimum inhibitory concentrations (MICs) for four antiseptics and four bacterial strains. MICs are expressed in μg L^{-1}. Superscripts denote the literature source cited.

Literature strain	Sequence ID number	% Identity	Number of sequences detected (Relative abundance, %)				
			Low PPCP column	High PPCP column	Day 1 SAT	Day 2 SAT	Day 3 SAT
KCY1 (7) DQ983313	497	97	0	0	0	4 (0.003%)	4 (0.003%)
	1387	99	0	0	1 (0.010%)	1 (0.001%)	0
	1881	97	1 (0.003%)	12 (0.008%)	7 (0.071%)	36 (0.026%)	57 (0.036%)
	2158	97	1 (0.003%)	0	3 (0.030%)	49 (0.035%)	40 (0.025%)
Sphingomonas sp. PH-07 (8) DQ185574	1407	97	587 (1.903%)	5166 (3.503%)	41 (0.414%)	678 (0.488%)	1531 (0.966%)
	199	99	261 (0.846%)	1904 (1.291%)	14 (0.141%)	303 (0.218%)	533 (0.336%)
	264	98	0	36 (0.024%)	2 (0.020%)	25 (0.018%)	22 (0.014%)
VAL (9)		97	1 (0.003%)	0	0	0	0

Table S9. Detections of strains with ≥97% similarity to two triclosan-degrading strains described in the literature and one valproic acid-degrading strain. The strain name, reference, and GenBank accession number are provided in the first column. The percent identity, the number of sequences detected, and the relative abundance are also provided.

References

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