

## **Supplementary Information**

**Title:** Investigating bacterial populations in styrene-degrading biofilters by 16S rDNA tag pyrosequencing

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**Table S1.** Eleven nucleotide sequence barcodes used in PCR for pyrosequencing. Replicate 1 (R1) and replicate 2 (R2) samples were used in separate pyrosequencing runs.

Sample (days of biofilter operation)	Barcode Sequence
105 R1	ACGTACACACT
105 R2	ACGTACACACT
142 R1	ACGTACTGTGT
142 R2	ACGTACTGTGT
156 R1	ACTACGTCTCT
156 R2	ACTACGTCTCT
239 R1	AGTACGAGAGT
239 R2	AGTACGAGAGT

**Table S2.** Percentages of replicate 1 (R1) and 2 (R2) of total sequences from classified taxonomic groups at each sampling time point that could not be further classified at the class, order, family, genera levels (<50% cutoff of RDP Classifier).

<b>Bacterial phyla unclassified at class level</b>	<b>105 days</b>		<b>142 days</b>		<b>156 days</b>		<b>239 days</b>	
	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>
<i>Proteobacteria</i>	0.3121	0.2469	1.1930	1.0437	1.2407	0.7239	0.5307	0.6094
<i>Chloroflexi</i>	0.3277	0.1710	0.5086	0.5510	0.4913	0.4607	0.1628	0.3522
<i>Firmicutes</i>	0.2575	0.1456	0.1665	0.1748	0.0833	0.0376	0.0425	0.1901
<i>Bacteroidetes</i>	0.2107	0.2026	0.0555	0.1960	0.0874	0.1504	0.0212	0.0783
<i>Verrucomicrobia</i>	0.0390	0.0570	0.0000	0.0318	0.0125	0.0188	0.0000	0.0335

<b>Bacterial classes unclassified at order level</b>	<b>105 days</b>		<b>142 days</b>		<b>156 days</b>		<b>239 days</b>	
	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>
<i>Deltaproteobacteria</i>	2.9728	1.9946	2.8022	1.6953	4.2510	1.2739	2.4202	2.0965
<i>Alphaproteobacteria</i>	3.1445	1.7413	1.8219	1.4781	1.1158	0.4137	0.8280	1.9064
<i>Gammaproteobacteria</i>	1.8258	1.2157	1.7756	1.4516	0.8993	0.3337	0.9412	2.1971
<i>Clostridia</i>	0.0312	0.1456	0.1387	0.1801	0.0250	0.0423	0.3468	0.5926
<i>Spartobacteria</i>	0.3121	0.0886	0.0555	0.1218	0.0416	0.0141	0.0283	0.0335
<i>Betaproteobacteria</i>	0.0312	0.0127	0.0185	0.0212	0.0333	0.0141	0.0283	0.0559
<i>Actinobacteria</i>	0.0000	0.0000	0.0092	0.0053	0.0125	0.0047	0.0000	0.0000
<i>Acidobacteria Gp1</i>	0.0000	0.0000	0.0092	0.0053	0.0000	0.0000	0.0000	0.0000

<b>Bacterial orders unclassified at family level</b>	<b>105 days</b>		<b>142 days</b>		<b>156 days</b>		<b>239 days</b>	
	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>	<b>R1</b>	<b>R2</b>
<i>Rhizobiales</i>	0.8505	1.8363	3.5790	3.7084	4.7673	3.3421	2.7245	4.1874
<i>Gammaproteobacteria incertae sedis</i>	0.1717	0.3862	0.3977	0.4503	0.4372	0.4137	0.4883	0.6988
<i>Rhodospirillales</i>	0.2185	0.0760	0.3977	0.2755	0.1457	0.1551	0.1698	0.2180
<i>Chromatiales</i>	0.0156	0.0000	0.1202	0.1113	0.0416	0.0423	0.0354	0.4976
<i>Burkholderiales</i>	0.1561	0.1963	0.0555	0.0212	0.0458	0.0141	0.0495	0.2069
<i>Bacillales</i>	0.0078	0.0380	0.0462	0.1324	0.0541	0.1034	0.0071	0.0391

<i>Chlamydiales</i>	0.0156	0.0760	0.0277	0.1113	0.0291	0.0423	0.0283	0.0391
<i>Clostridiales</i>	0.0390	0.0380	0.0092	0.0636	0.0000	0.0047	0.0354	0.0671
<i>Myxococcales</i>	0.0000	0.0000	0.0277	0.0159	0.1457	0.0423	0.0000	0.0000
<i>Sphingobacteriales</i>	0.0234	0.0633	0.0092	0.0212	0.0000	0.0282	0.0000	0.0000
<i>Actinomycetales</i>	0.0078	0.0127	0.0000	0.0000	0.0000	0.0235	0.0000	0.0224
<i>Bacteroidales</i>	0.0156	0.0063	0.0092	0.0000	0.0000	0.0000	0.0000	0.0112
<i>Lactobacillales</i>	0.0078	0.0000	0.0092	0.0053	0.0042	0.0047	0.0000	0.0056
<i>Alphaproteobacteria incertae sedis</i>	0.0000	0.0000	0.0000	0.0106	0.0167	0.0000	0.0000	0.0000
<i>Solirubrobacterales</i>	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000	0.0112
<i>Flavobacteriales</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0056
<i>Caulobacterales</i>	0.0000	0.0000	0.0000	0.0053	0.0000	0.0000	0.0000	0.0000
<i>Nitrosomonadales</i>	0.0000	0.0000	0.0000	0.0053	0.0000	0.0000	0.0000	0.0000

**Bacterial families unclassified at genus level**

	105 days		142 days		156 days		239 days	
	R1	R2	R1	R2	R1	R2	R1	R2
<i>Pseudomonadaceae</i>	4.9860	4.7173	7.1581	4.5031	3.7514	3.0037	1.6206	4.2489
<i>Sphingomonadaceae</i>	6.0627	19.3567	0.9988	1.0066	0.3830	0.6722	0.0566	0.2516
<i>Anaerolineaceae</i>	2.4423	0.7155	3.4126	2.7760	3.0269	1.8332	0.2972	0.5199
<i>Planctomycetaceae</i>	0.5930	0.5066	1.2670	1.9443	1.7737	2.2516	0.4741	1.0622
<i>Rhodospirillaceae</i>	0.7335	0.9814	2.1178	2.3257	1.1366	0.8790	0.5520	0.7939
<i>Bradyrhizobiaceae</i>	0.3199	0.5762	1.0728	1.2344	1.8236	0.7051	0.6511	1.1629
<i>Beijerinckiaceae</i>	0.3979	0.4116	0.7861	0.9801	0.3622	0.2632	0.3114	0.6038
<i>Erythrobacteraceae</i>	0.3589	0.3926	0.3699	0.4503	0.5496	0.5923	0.3963	0.7995
<i>Xanthobacteraceae</i>	0.1092	0.2659	0.4532	0.5986	0.4663	0.4278	0.3821	0.5311
<i>Acetobacteraceae</i>	0.1326	0.1520	0.5549	0.7788	0.2706	0.3055	0.3680	0.4305
<i>Rhodocyclaceae</i>	0.0702	0.0570	0.2312	0.3020	0.3039	0.2209	0.1981	0.4417
<i>Opitutaceae</i>	0.3277	0.2026	0.3514	0.1748	0.1041	0.0564	0.2265	0.3466
<i>Chitinophagaceae</i>	0.1873	0.3356	0.1480	0.2066	0.1457	0.2867	0.0354	0.1677
<i>Rhodobacteraceae</i>	0.3355	0.2216	0.2220	0.2437	0.1207	0.1504	0.0212	0.0280
<i>Clostridiaceae 4</i>	0.0858	0.1393	0.0370	0.0848	0.0333	0.0047	0.0283	0.0224
<i>Caulobacteraceae</i>	0.0468	0.0886	0.0555	0.0689	0.0125	0.0188	0.0000	0.0112
<i>Hyphomicrobiaceae</i>	0.0546	0.0063	0.0740	0.0053	0.0583	0.0094	0.0495	0.0056

<i>Veillonellaceae</i>	0.0312	0.0317	0.0277	0.0053	0.0208	0.0235	0.0283	0.0559
<i>Phyllobacteriaceae</i>	0.0078	0.0380	0.0185	0.0159	0.0208	0.0282	0.0283	0.0391
<i>Paenibacillaceae 1</i>	0.0156	0.0063	0.0370	0.0265	0.0167	0.0141	0.0000	0.0447
<i>Streptosporangiaceae</i>	0.0000	0.0063	0.0092	0.0000	0.0000	0.0047	0.0000	0.1398
<i>Porphyromonadaceae</i>	0.0546	0.0633	0.0092	0.0000	0.0000	0.0094	0.0000	0.0168
<i>Xanthomonadaceae</i>	0.0078	0.0253	0.0277	0.0000	0.0208	0.0000	0.0071	0.0224
<i>Thermomonosporaceae</i>	0.0000	0.0127	0.0092	0.0053	0.0042	0.0094	0.0000	0.0503
<i>Rhizobiales incertae sedis</i>	0.0156	0.0317	0.0000	0.0053	0.0083	0.0141	0.0071	0.0056
<i>Ruminococcaceae</i>	0.0156	0.0063	0.0000	0.0053	0.0042	0.0282	0.0000	0.0224
<i>Hyphomonadaceae</i>	0.0156	0.0000	0.0000	0.0000	0.0000	0.0094	0.0000	0.0391
<i>Cytophagaceae</i>	0.0078	0.0000	0.0185	0.0212	0.0000	0.0141	0.0000	0.0000
<i>Clostridiaceae 2</i>	0.0390	0.0127	0.0000	0.0000	0.0000	0.0000	0.0071	0.0000
<i>Erysipelotrichaceae</i>	0.0312	0.0253	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Sphingobacteriaceae</i>	0.0156	0.0190	0.0000	0.0000	0.0000	0.0094	0.0000	0.0112
<i>Lachnospiraceae</i>	0.0000	0.0000	0.0185	0.0053	0.0000	0.0094	0.0000	0.0112
<i>Polyangiaceae</i>	0.0000	0.0000	0.0000	0.0000	0.0291	0.0141	0.0000	0.0000
<i>Cystobacteraceae</i>	0.0000	0.0127	0.0092	0.0000	0.0042	0.0000	0.0071	0.0056
<i>Oxalobacteraceae</i>	0.0312	0.0000	0.0000	0.0000	0.0000	0.0000	0.0071	0.0000
<i>Peptococcaceae 2</i>	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0142	0.0056
<i>Flavobacteriaceae</i>	0.0156	0.0000	0.0000	0.0053	0.0000	0.0000	0.0000	0.0168
<i>Rhodobiaceae</i>	0.0000	0.0000	0.0000	0.0000	0.0208	0.0000	0.0071	0.0056
<i>Alcaligenaceae</i>	0.0000	0.0000	0.0000	0.0053	0.0125	0.0094	0.0000	0.0000
<i>Comamonadaceae</i>	0.0078	0.0000	0.0000	0.0000	0.0000	0.0094	0.0000	0.0000
<i>Methylocystaceae</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0112
<i>Thermoanaerobacteraceae</i>	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000	0.0000
<i>Clostridiaceae 1</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0056
<i>Neisseriaceae</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0056
<i>Parachlamydiaceae</i>	0.0000	0.0000	0.0000	0.0053	0.0000	0.0000	0.0000	0.0000
<i>Enterobacteriaceae</i>	0.0000	0.0000	0.0000	0.0000	0.0042	0.0000	0.0000	0.0000

**Table S3.** Significant correlations ( $P < 0.05$ ) between biofilter operational/functional parameters and the percentages of total sequences from the top 40 most abundant detected genera.

Biofilter functional/operational parameter	Genera	Spearman's Rank Correlation Coefficient ( $\rho$ )	Significance ( $P$ value)
<b>EBRT</b>	<i>Xanthobacter</i>	-0.772	0.025
<b>Elimination Capacity</b>	<i>Asticcacaulis</i>	-0.781	0.022
	<i>Subdivision3 genera incertae sedis</i>	-0.732	0.039
<b>Evenness (E)</b>	<i>Sphaerobacter</i>	0.762	0.028
	<i>Haloferula</i>	0.881	0.004
	<i>Sphingomonas</i>	0.81	0.015
	<i>Mesorhizobium</i>	0.81	0.015
	<i>Rhodoplanes</i>	0.786	0.021
	<i>Brucella</i>	0.786	0.021
<b>Evenness (J)</b>	<i>Sphaerobacter</i>	0.929	0.001
	<i>Haloferula</i>	0.905	0.002
	<i>Sphingomonas</i>	0.857	0.007
	<i>Rhodoplanes</i>	0.714	0.047
	<i>Brucella</i>	0.81	0.015
<b>Inverse Simpson index</b>	<i>Gp3</i>	0.714	0.047
	<i>Sphaerobacter</i>	0.905	0.002
	<i>Haloferula</i>	0.952	<0.001

	<i>Xanthobacter</i>	0.714	0.047
	<i>Sphingomonas</i>	0.929	0.001
	<i>Brucella</i>	0.786	0.021
<b>Moisture content</b>	<i>Rhodobacter</i>	0.732	0.039
	<i>Pasteuria</i>	0.878	0.004
	<i>Opitutus</i>	0.927	0.001
	<i>Pseudonocardia</i>	-0.781	0.022
<b>Observed OTUs</b>	<i>Sphaerobacter</i>	0.881	0.004
	<i>Haloferula</i>	0.881	0.004
	<i>Xanthobacter</i>	0.738	0.037
	<i>Sphingomonas</i>	0.976	<0.001
	<i>Rhodoplanes</i>	0.714	0.047
<b>pH</b>	<i>Gemmatimonas</i>	-0.878	0.004
	<i>Ohtaekwangia</i>	0.781	0.022
	<i>Rhodoplanes</i>	-0.781	0.022
<b>Removal efficiency</b>	<i>Brevundimonas</i>	-0.732	0.039
	<i>Asticcacaulis</i>	0.781	0.022
	<i>Hydrogenophaga</i>	-0.732	0.039
	<i>Achromobacter</i>	-0.781	0.022
	<i>Gemmatimonas</i>	0.878	0.004
	<i>Subdivision3 genera incertae sedis</i>	0.732	0.039
	<i>Steroidobacter</i>	0.878	0.004
	<i>Gp6</i>	0.878	0.004
	<i>Stenotrophomonas</i>	-0.83	0.011
	<i>Kaistia</i>	0.878	0.004

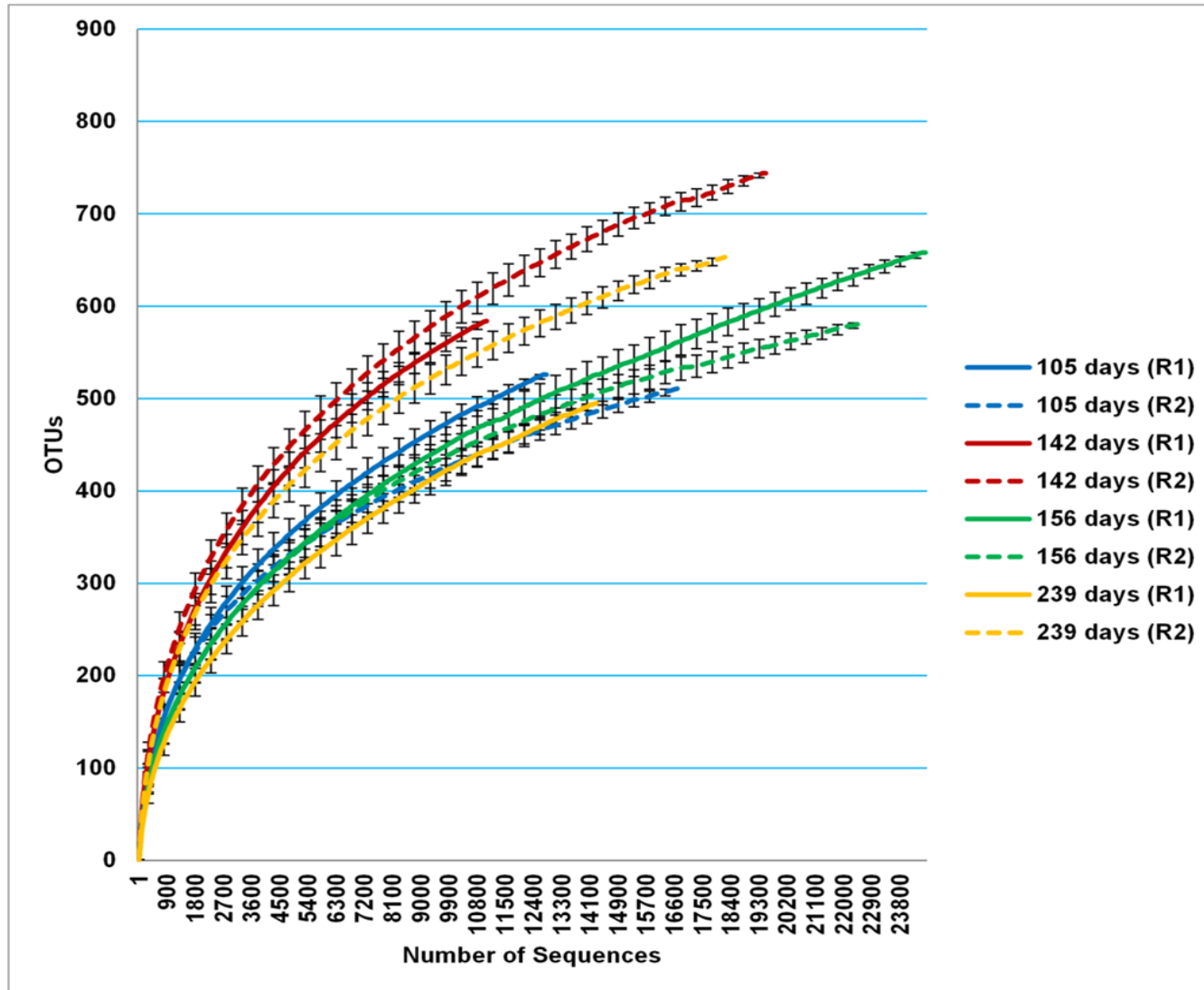
	<i>Ohtaekwangia</i>	-0.976	<0.001
	<i>Truepera</i>	0.781	0.022
	<i>Alkaliphilus</i>	-0.781	0.022
<b>Shannon index</b>	<i>Sphaerobacter</i>	0.976	<0.001
	<i>Haloferula</i>	0.929	0.001
	<i>Sphingomonas</i>	0.929	0.001
	<i>Brucella</i>	0.786	0.021
<b>Styrene inlet concentration</b>	<i>Asticcacaulis</i>	-0.976	<0.001
	<i>Subdivision3 genera incertae sedis</i>	-0.878	0.004
	<i>Gp6</i>	-0.878	0.004
	<i>Stenotrophomonas</i>	0.781	0.022
	<i>Ohtaekwangia</i>	0.781	0.022
<b>Styrene inlet load</b>	<i>Asticcacaulis</i>	-0.781	0.022
	<i>Subdivision3 genera incertae sedis</i>	-0.732	0.039
<b>Styrene outlet concentration</b>	<i>Brevundimonas</i>	0.732	0.039
	<i>Asticcacaulis</i>	-0.781	0.022
	<i>Hydrogenophaga</i>	0.732	0.039
	<i>Achromobacter</i>	0.781	0.022
	<i>Gemmatimonas</i>	-0.878	0.004
	<i>Subdivision3 genera incertae sedis</i>	-0.732	0.039
	<i>Steroidobacter</i>	-0.878	0.004



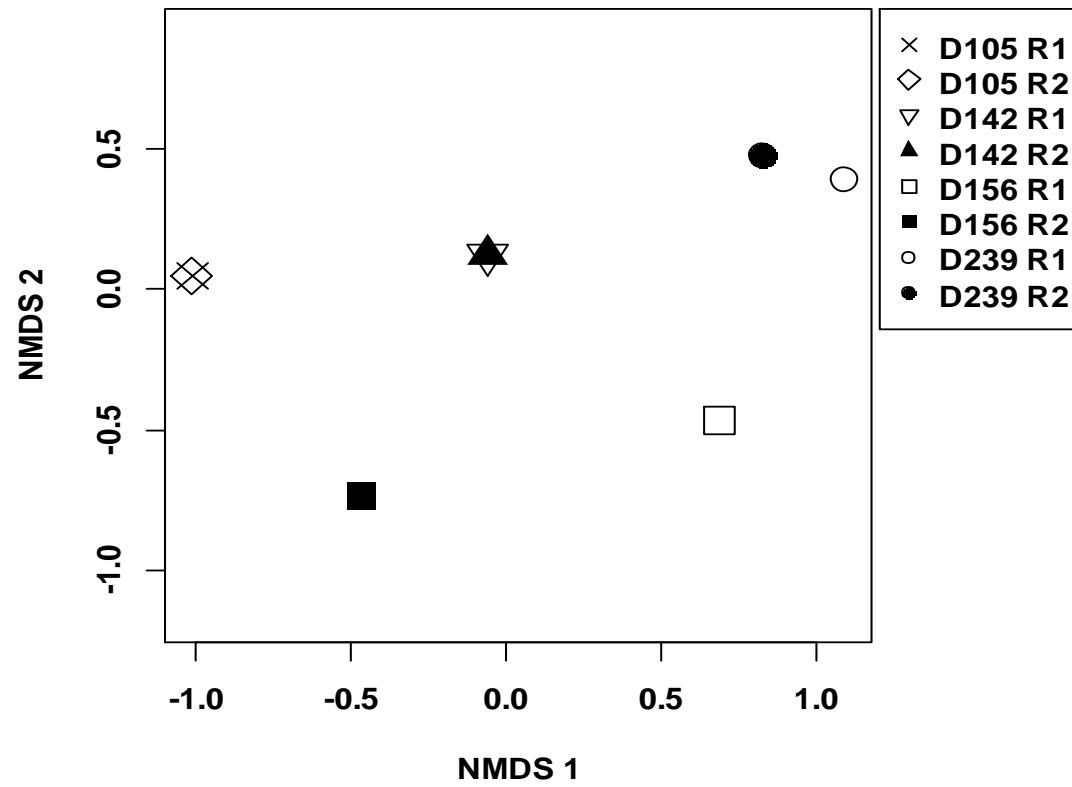
<i>Gp6</i>	-0.878	0.004
<i>Stenotrophomonas</i>	0.83	0.011
<i>Sorangium</i>	0.732	0.039
<i>Kaistia</i>	-0.878	0.004
<i>Ohtaekwangia</i>	0.976	<0.001
<i>Truepera</i>	-0.781	0.022
<i>Alkaliphilus</i>	0.781	0.022

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**Figure S1.** Rarefaction curves at 3% cutoff for sampling days 105, 142, 156, and 239. Error bars represent 95% confidence intervals.



**Figure S2.** Non-metric multidimensional scaling (NMDS) plot of replicate samples 1 and 2 (R1 and R2, respectively) from sampling days 105, 142, 156 and 239.



**Figure S3** Percentage of total bacterial sequences (mean of replicates) from the top 40 detected genera in the 4 sampling time points. Error bars represent the lower and upper values of actual replicates. Sequences labeled as Unclassified (Genera) could not be assigned to any particular genera using the Ribosomal Database Project Classifier at the selected bootstrap confidence estimate threshold of 50%. Subdivision 3 incertae sedis belongs to the phylum Verrucomicrobia.

### % Total Bacterial Sequences

