

Supporting Information

Variation of Bacterial Communities with Water Quality in an Urban Tropical Catchment

Jean Pierre Nshimyimana^{1, 2, 4, 5}, Adam Joshua Ehrich Freedman^{2, 4}, Peter Shanahan^{2, 4}, Lloyd C. H. Chua³, and Janelle R. Thompson^{2, 4, *}

1. School of Civil and Environmental Engineering, Nanyang Technological University (NTU), 50 Nanyang Avenue, Singapore 639798, Singapore
2. Department of Civil and Environmental Engineering, Massachusetts Institute of Technology (MIT), 77 Massachusetts Avenue, Cambridge, MA 02139, USA
3. School of Engineering, Deakin University, Waurn Ponds, Geelong, Victoria 3216, Australia
4. Centre for Environmental Sensing and Modeling (CENSAM), Singapore-MIT Alliance for Research and Technology (SMART), 1 Create Way, Singapore 138602, Singapore
5. Singapore Center on Environmental Life Sciences Engineering (SCELSE), NTU, 60 Nanyang Drive, Singapore 637551

***Corresponding author:** Janelle Thompson: jthompson@mit.edu

Content Summary of Supporting Information

Page Number	Figures	Tables
S3	Figure S1	-
S4	Figure S2	-
S5	Figure S3	-
S6	Figure S4	-
S7	Figure S5	-
S8	-	Table S1
S9	-	Table S2
S10	-	Table S3
S11	-	Table S4
S15	-	Table S5

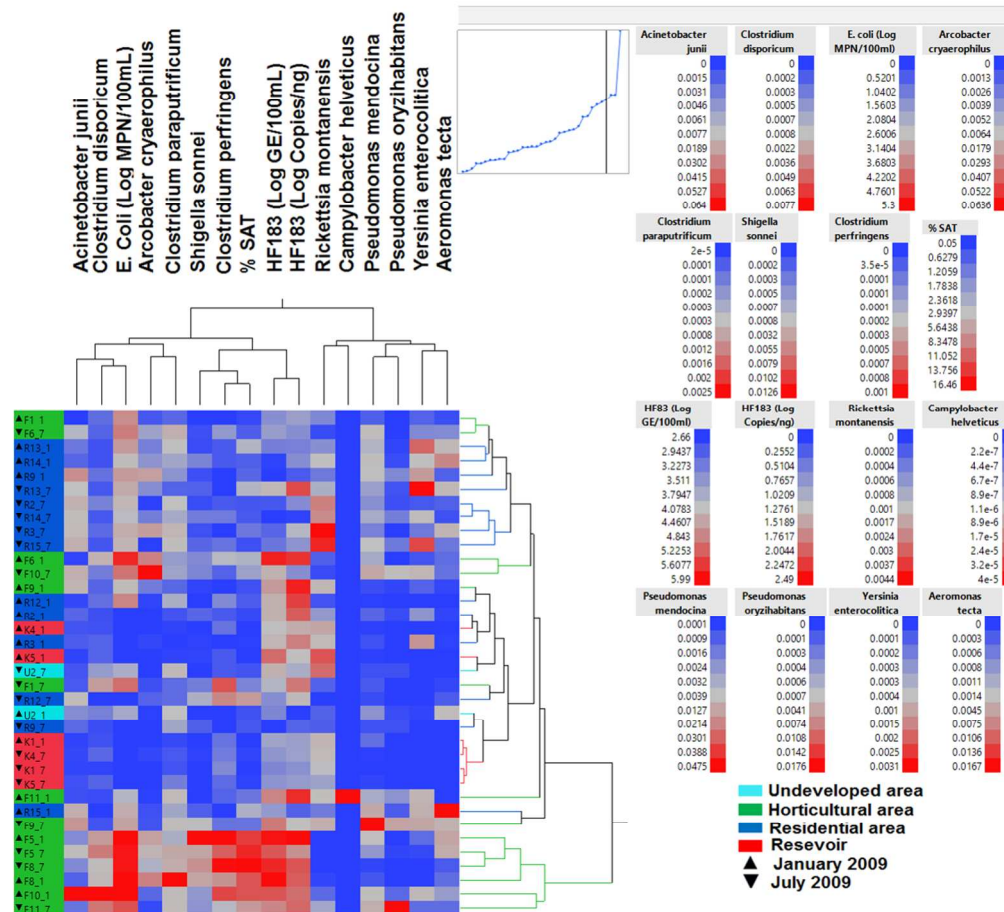


Figure S1: Hierarchical clustering of the distribution of selected pathogen-like sequences identified to species level (PLSs) including the ten most highly-represented and the five most closely related to gastro-intestinal pathogens. Comparison includes the proportion of % SAT, *E. coli*, and HF183. Heat map indicate sequence abundance as a fraction of the total sequences per sample and are individually scaled. *E. coli* and HF183 concentration are log10 transformed.

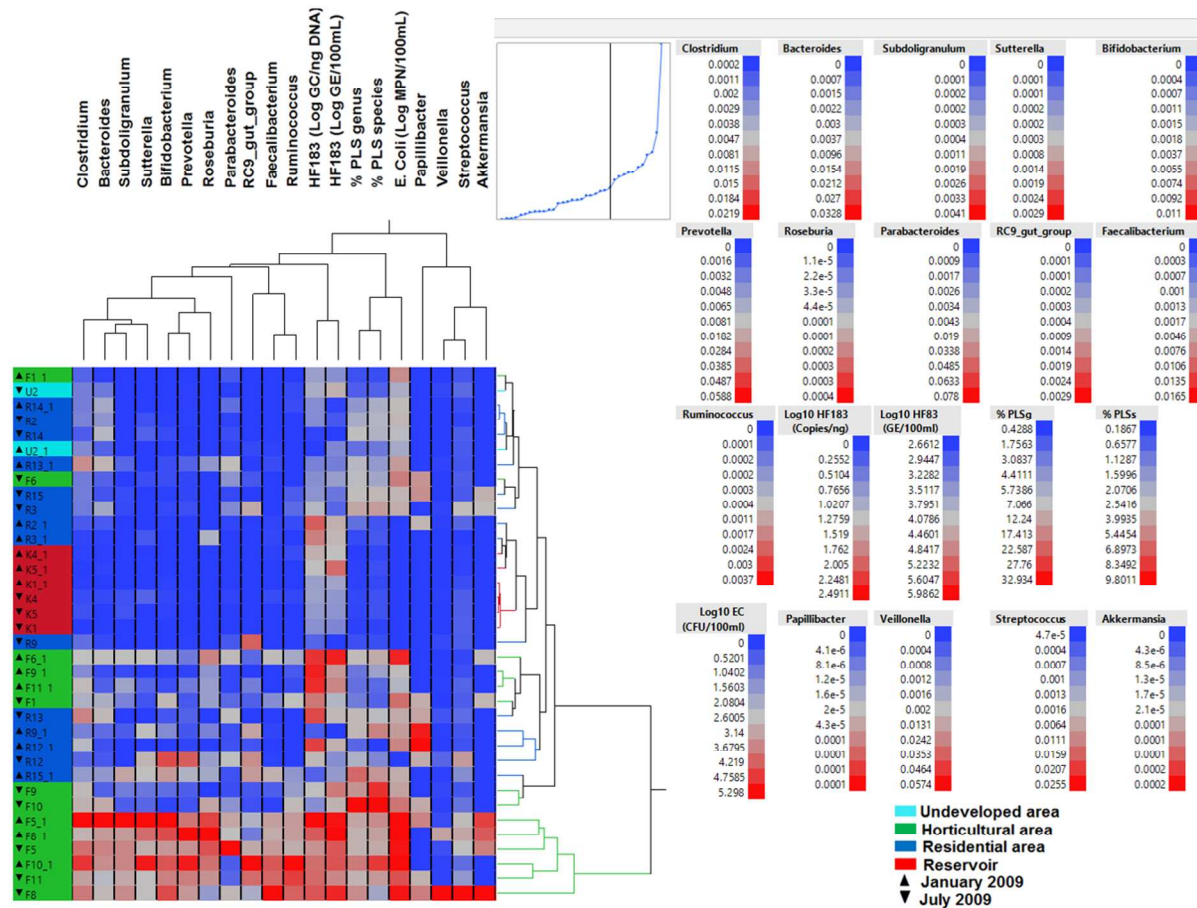
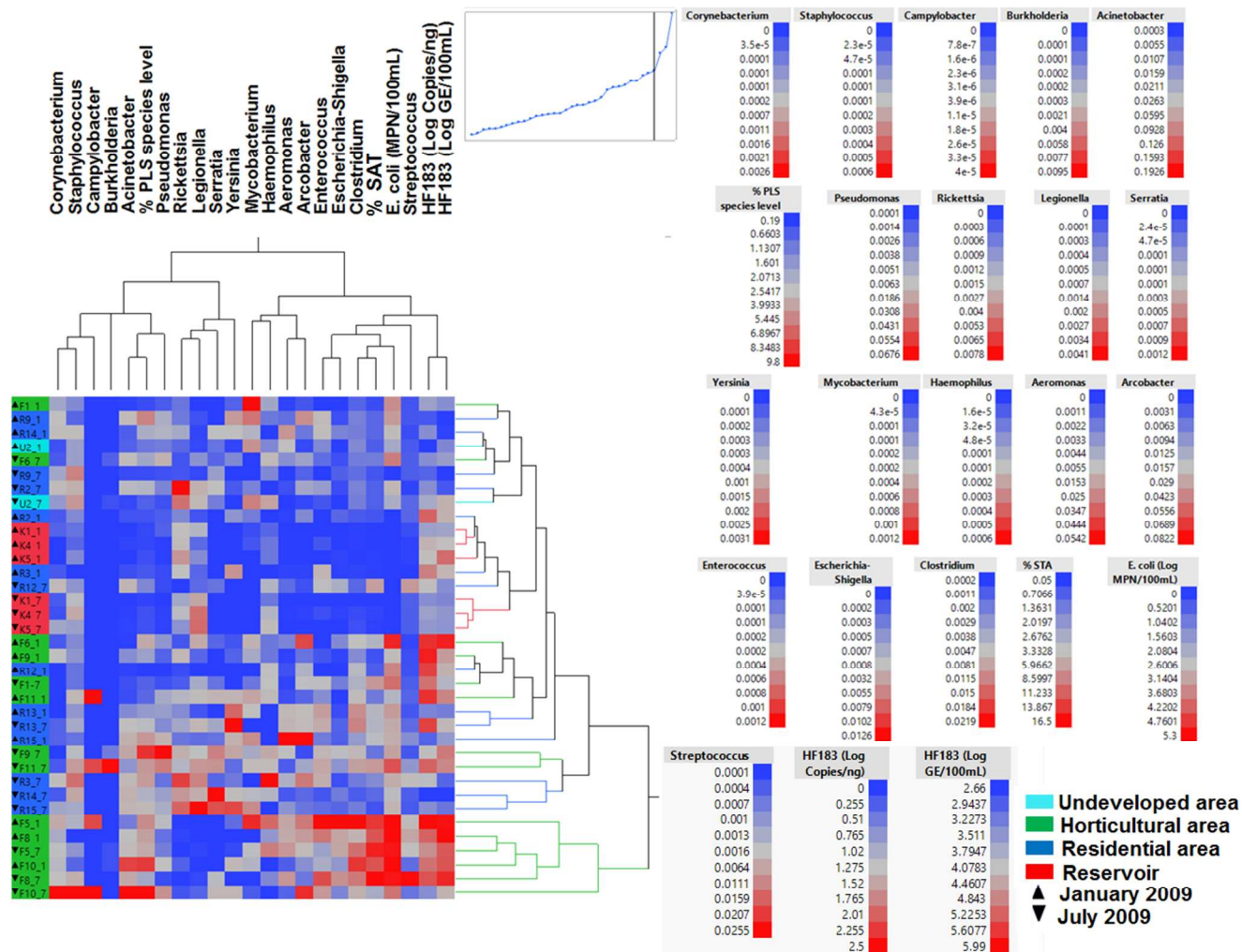


Figure S2. Hierarchical clustering of the distribution of SAT (genera). Comparison includes proportion of PLSg, PLSs, *E. coli*, and HF183. Heat map indicate sequence abundance as a fraction of the total sequences per sample and are individually scaled. *E. coli* and HF183 concentration are log10 transformed.



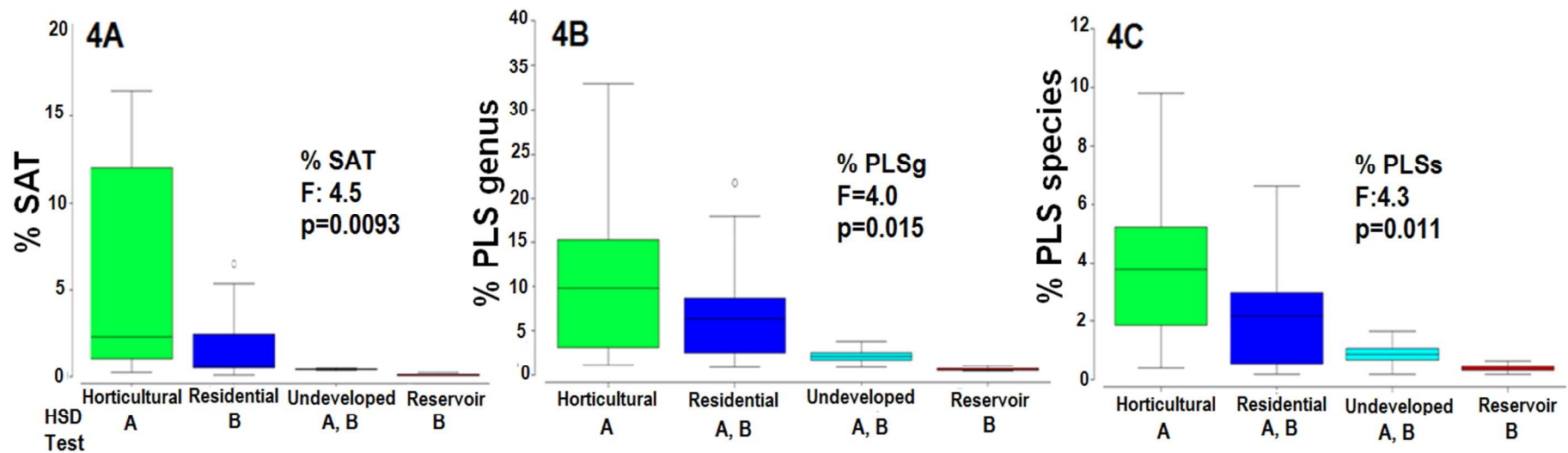


Figure S4. The distribution of sequences from (A) sewage-associated taxa (SAT) and (B, C) pathogen-like sequences identified to genus and species level (PLSg and PLSs, respectively) as a percentage of the total bacterial community composition for different land-use categories sampled in this study. Box and whiskers correspond to the median and quartile distribution. Tukey-Kramer Honest Significant Difference Test (HSD) and ANOVA analysis demonstrated variation of % SAT, % PLSg, and % PLSs across catchment land uses and reservoir. Letters of HSD test (A and B) indicate that catchment land use not represented by same letters have significantly different SAT, PLSg and PLSs.

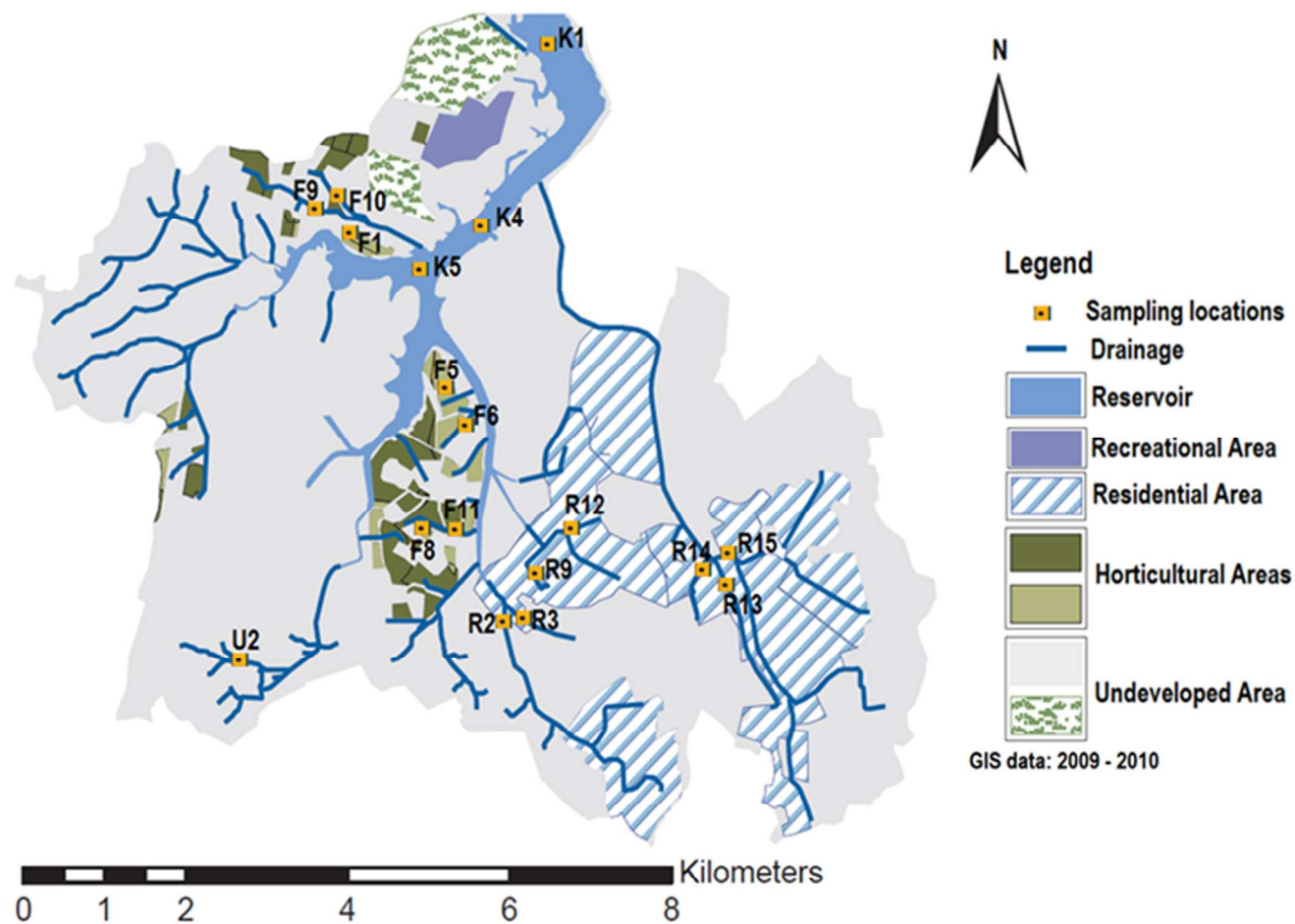


Figure S5. Land use and sampling sites in a tropical urban watershed in Singapore. Adapted from Nshimiyimana, J. P.; Ekklesia, E.; Shanahan, P.; Chua, L. H. C.; Thompson, J. R., Distribution and abundance of human-specific *Bacteroides* and relation to traditional indicators in an urban tropical catchment. *Appl. Microbial.* 2014, 116, (5), 1369-1383. Copyright © 1999 – 2017 John Wiley & Sons, Inc. All Rights Reserved. Open source publication under Creative Commons License .

Table S1: Summary of PERMANOVA results for evaluation of land-use and sample month as factors that structure the bacterial community in the catchment.

Source	df	SS	MS	Pseudo-F	P (Value)	Number of Permutations
Bacterial community structure (OTU)						
Land use	2	8,788	4,394	2.0	0.009	998
Sampling Month	1	2,725	2,725	1.2	0.16	999
Interaction of land use and month	2	2,224	1,112	0.5	1	996
Sewage-associated taxa (SAT) at genus level						
Land use	2	9,242	4,621	3	0.004	999
Sampling Month	1	1,323	1,323	1	0.5	999
Interaction of land use and month	2	1,514	757	0.4	1	999
Pathogen-like sequences at genus level (PLSg)						
Land use	2	3,614	1,807	2	0.031	998
Sampling Month	1	2,505	2,505	2	0.008	999
Interaction of land use and month	2	1,543	771	0.7	1	999
Pathogen-like sequences at species level (PLSs)						
Land use	2	2,226	1,113	2	0.031	999
Sampling Month	1	1,161	1,161	2	0.057	998
Interaction of land use and month	2	632	316	0.5	1	997

Table S2. Sewage associated taxa (Genus level) from this study

Family	Genus	Taxa associated with human gut or sewage sample*	Taxa previously identified as sewage-associated ¹
<i>Bifidobacteriaceae</i>	<i>Bifidobacterium</i>	√	√
<i>Bacteroidaceae</i>	<i>Bacteroides</i>	√	√
<i>Porphyromonadaceae</i>	<i>Parabacteroides</i>	√	√
<i>Ruminococcaceae</i>	<i>Faecalibacterium</i>	√	√
<i>Lachnospiraceae</i>	<i>Roseburia</i>	√	√
<i>Ruminococcaceae</i>	<i>Ruminococcus</i>	√	√
<i>Alcaligenaceae</i>	<i>Sutterella</i>	√	√
<i>Akkermansiaceae</i>	<i>Akkermansia</i>	√	√
<i>Bacteroidaceae</i>	<i>Papillibacter</i>	√	√
<i>Bacteroidaceae</i>	<i>Subdoligranulum</i>	√	√
<i>Prevotellaceae</i>	<i>Prevotella</i>	√	-
<i>Rikenellaceae</i>	<i>RC9_gut_group</i>	√	-
<i>Clostridiaceae</i>	<i>Clostridium</i>	√	-
<i>Veillonellaceae</i>	<i>Veillonella</i>	√	-
<i>Streptococcaceae</i>	<i>Streptococcus</i>	√	-

* based on sequence annotation or literature search

“√” Taxa classified by indicated criteria

“-” Taxa not classified by indicated criteria

Table S3. Pathogen-like bacterial 16S rRNA gene sequences recovered in this study.

OTUs from this study	Pathogen Genus*	Human pathogen species**	Accession Numbers	Diseases	Clinical References
OTU0005	<i>Acinetobacter</i>	<i>Acinetobacter junii</i>	Z93438.1	Septicemia	2
OTU0511	<i>Acinetobacter</i>	<i>Acinetobacter ursingii</i>	NR_025392.1	Bacteremia	3
OTU1889	<i>Acinetobacter</i>	<i>Acinetobacter soli</i>	NR_044454.1	Bloodstream infection	4
OTU0070 & OTU0706	<i>Clostridium</i>	<i>Clostridium paraputrificum</i>	NR_113021.1	Bloodborne pathogen	5
OTU0382	<i>Clostridium</i>	<i>Clostridium disporicum</i>	NR_026491.1	Opportunistic infection for immuno-compromised	6
OTU8629 & OTU0303	<i>Clostridium</i>	<i>Clostridium perfringens</i>	NR_121697.1	Diarrhea	7
OTU2955	<i>Clostridium</i>	<i>Clostridium intestinale</i>	AY781385.1	Bacteremia	8
OTU0965	<i>Rickettsia</i>	<i>Rickettsia honei</i>	NR_114449.1	Fever	9
OTU0131	<i>Rickettsia</i>	<i>Rickettsia montanensis</i>	CP003340.1	Fever	10
OTU0025	<i>Pseudomonas</i>	<i>Pseudomonas mendocina</i>	KF862906.1	Endocarditis in a patient with situs inversus	11
OTU0232	<i>Pseudomonas</i>	<i>Pseudomonas oryzihabitans</i>	NR_114041.1	Peritonitis, endophthalmitis, and bacteremia	12
OTU3872 & OTU7452	<i>Pseudomonas</i>	<i>Pseudomonas stutzeri</i>	AF094748	Opportunistic pathogen	13
OTU9011 & OTU3265	<i>Legionella</i>	<i>Legionella pneumophila</i>	NR_074231.1	Pneumonia / Opportunistic	14
OTU0963 & OTU1273	<i>Legionella</i>	<i>Legionella gresilensis</i>	NR_028744.1	Legionnaires' Disease	15
OTU0916	<i>Legionella</i>	<i>Legionella rowbothamii</i>	NR_036804.1	Legionnaires' Disease	16
OTU5429	<i>Staphylococcus</i>	<i>Staphylococcus sciuri</i>	AB212276.1	Endocarditis	17
OTU1038	<i>Staphylococcus</i>	<i>Staphylococcus epidermidis</i>	AB680360.1	Bacteremia	18
OTU0440	<i>Yersinia</i>	<i>Yersinia enterocolitica</i>	HE803717.1	Gastroenteritis	19
OTU0163	<i>Aeromonas</i>	<i>Aeromonas tecta</i>	NR_118043.1	Bacteremia	20
OTU0024	<i>Shigella</i>	<i>Shigella sonnei</i>	NR_074894.1	Shigellosis	21
OTU0622	<i>Streptococcus</i>	<i>Streptococcus oralis</i>	AY485602.1	Septicemia	22
OTU0758	<i>Enterococcus</i>	<i>Enterococcus casseliflavus</i>	NR_041704.1	Enterococcal meningitis	23
OTU1693	<i>Haemophilus</i>	<i>Haemophilus parainfluenzae</i>	NR_116168.1	Bronchiectasis	24
OTU1204	<i>Serratia</i>	<i>Serratia marcescens</i>	FJ584421	Sepsis (neonatal unit)	25
OTU0887	<i>Burkholderia</i>	<i>Burkholderia dolosa</i>	NR_104973.1	Lung disease	26
OTU0321	<i>Campylobacter</i>	<i>Campylobacter helveticus</i>	U03022.1	Gastroenteritis	27
OTU1837	<i>Mycobacterium</i>	<i>Mycobacterium setense</i>	EU371507.1	Lower Respiratory Tract Infection	28
OTU_1166	<i>Corynebacterium</i>	<i>Corynebacterium tuberculostearicum</i>	NR_028975.1	Isolated in patients with mastitis	29
OTU_16 & OTU_6394	<i>Arcobacter</i>	<i>Arcobacter cryaerophilus</i>	EU669899.1	Diarrhea	30

* Taxonomic affiliation with genus listed on US NIH etiological agents list

** Top BlastN match with >99% nucleotide identity

Table S4. SAT and PLS bacteria contributing to the dissimilarity between the catchment land uses and reservoir and the catchment

Criteria	Taxa	Catchment: Average Abundance	Reservoir: Average Abundance	Average Dissimilarity	Dissimilarity /Standard deviation	% Contribution	% Average Dissimilarity
Dissimilarity between Catchment and Reservoir Microbial Communities	OTUs level contributing to dissimilarity between Catchment and Reservoir						
	OTU_36 (<i>Flexibacter</i>)	0.7	5.3	0.1	1.7	0.2	
	OTU_31 (<i>Chitinophagaceae</i> <i>Spp.</i>)	1.1	5.5	0.1	1.7	0.2	
	OTU_26 (<i>Flexibacter</i>)	1.4	5.2	0.1	1.5	0.1	
	OTU_47 (<i>hgcI_clade</i>)	1.5	4.9	0.1	1.5	0.1	
	OTU_30 (<i>Microcystis</i>)	1.7	5.0	0.1	1.6	0.1	
	OTU_18 (<i>Alcaligenaceae</i> <i>Spp.</i>)	1.6	4.7	0.1	1.6	0.1	74.7
	OTU_87 (<i>NS9_marine_group</i> <i>Spp.</i>)	0.5	3.6	0.1	1.5	0.1	
	OUT_7 (<i>Arcobacter</i>)	3.2	0.6	0.1	1.7	0.1	
	OUT_6 (<i>I2up</i>)	3.1	2.9	0.1	1.3	0.1	
	OUT_32 (<i>Hydrogenophaga</i>)	3.5	2.0	0.1	1.5	0.1	
	SAT genus level contributing to dissimilarity between Catchment and Reservoir						
	<i>Prevotella</i>	2.8	0.2	8.0	2.3	14.7	
	<i>Bacteroides</i>	2.9	0.9	7.0	1.9	12.9	
	<i>Parabacteroides</i>	2.6	0.8	6.1	1.6	11.2	
	<i>Clostridium</i>	3.3	1.9	4.6	2.1	8.5	
	<i>Bifidobacterium</i>	1.8	0.6	4.4	1.5	8.2	
	<i>Faecalibacterium</i>	1.4	0.4	3.8	1.2	7.0	54
	<i>RC9_gut_group</i>	1.1	0.0	3.4	1.0	6.2	
	<i>Ruminococcus</i>	1.1	0.3	3.1	1.2	5.7	
	<i>Subdoligranulum</i>	1.1	0.4	3.0	1.2	5.4	
	<i>Sutterella</i>	1.0	0.2	2.9	1.2	5.4	

Table S4.

Criteria	Taxa	Catchment: Average Abundance	Reservoir: Average Abundance	Average Dissimilarity	Dissimilarity /Standard deviation	% Contribution	% Average Dissimilarity
Dissimilarity between Catchment and Reservoir Microbial Communities	PLS genus level contributing to dissimilarity between Catchment and Reservoir						
	<i>Arcobacter</i>	4.3	1.1	4.4	2.3	10.0	44.1
	<i>Aeromonas</i>	3.0	0.4	3.6	1.9	8.1	
	<i>Acinetobacter</i>	5.0	2.1	3.6	1.7	8.1	
	<i>Enterobacter</i>	2.5	0.6	2.6	1.7	6.0	
	<i>Erysipelothrix</i>	2.0	0.0	2.6	1.8	6.0	
	<i>Pseudomonas</i>	3.3	2.0	2.1	1.6	4.7	
	<i>Yersinia</i>	1.5	0.0	2.0	1.5	4.5	
	<i>Clostridium</i>	3.3	2.0	1.9	1.9	4.3	
	PLS species level contributing to dissimilarity between Catchment and Reservoir						
	<i>Arcobacter cryaerophilus</i>	3.0	1.0	4.1	1.4	8.6	47.1
	<i>Aeromonas tecta</i>	2.0	0.0	3.6	1.8	7.6	
	<i>Acinetobacter junii</i>	3.6	2.0	2.9	1.5	6.2	
	<i>Yersinia enterocolitica</i>	1.5	0.0	2.6	1.5	5.6	
	<i>Legionella gresilensis</i>	0.4	1.5	2.6	1.3	5.5	
	<i>Shigella sonnei</i>	1.6	0.3	2.5	1.4	5.4	
	<i>Pseudomonas mendocina</i>	3.0	2.0	2.4	1.6	5.1	
	<i>Rickettsia montanensis</i>	2.0	2.7	2.1	1.2	4.4	
	<i>Enterococcus casseliflavus</i>	1.3	0.3	2.1	1.6	4.4	
	<i>Mycobacterium setense</i>	1.1	0.0	2.0	1.4	4.3	

Table S4.

Criteria	Taxa	Horticultural: Average abundance	Reservoir: Average Abundance	Average Dissimilarity	Dissimilarity /Standard deviation	% Contribution	% Average Dissimilarity
Dissimilarity between Horticulture and Residential area microbial communities	OTUs level contributing to dissimilarity between horticulture and Residential Land Uses						
	OTU_6 (<i>I2up</i>)	2.4	4.4	0.1	1.1	0.1	69
	OTU_2 (<i>Cynobacteria Spp.</i>)	2.0	2.3	0.1	0.7	0.1	
	OTU_11 (<i>Novispirillum</i>)	1.6	3.7	0.1	1.0	0.1	
	OTU_7827 (<i>I2up</i>)	1.5	3.0	0.1	0.8	0.1	
	OTU_14 (<i>Tolumonas</i>)	3.4	2.4	0.1	0.8	0.1	
	OTU_35 (<i>Megasphaera</i>)	2.0	1.5	0.1	0.7	0.1	
	OTU_2638 (<i>Prevotella</i>)	2.7	0.7	0.1	1.3	0.1	
	OTU_112 (<i>Comamonadaceae Ssp.</i>)	1.6	2.9	0.1	0.9	0.1	
	OTU_32 (<i>Hydrogenophaga</i>)	3.6	4.0	0.1	1.1	0.1	
	OTU_5002 (<i>Aeromonadaceae Ssp.</i>)	2.1	1.9	0.1	0.8	0.1	
	SAT genus level contributing to dissimilarity between horticulture and Residential Land Uses						
	<i>Prevotella</i>	3.6	2.2	4.4	1.6	11.8	38
	<i>Faecalibacterium</i>	2.2	0.7	3.8	1.5	10.1	
	<i>Bifidobacterium</i>	2.4	1.3	3.7	1.5	9.7	
	<i>Ruminococcus</i>	1.8	0.5	3.1	1.7	8.1	
	<i>Subdoligranulum</i>	1.8	0.5	3.0	1.7	7.9	
	<i>Parabacteroides</i>	3.1	2.2	2.8	1.3	7.3	
	<i>RC9_gut_group</i>	1.4	0.9	2.7	1.4	7.2	
	<i>Bacteroides</i>	3.2	2.6	2.6	1.4	7.0	
	<i>Sutterella</i>	1.6	0.6	2.5	1.5	6.6	
	<i>Veillonella</i>	1.4	0.7	2.5	1.0	6.5	

Table S4.

Criteria	Taxa	Horticultural: Average abundance	Reservoir: Average Abundance	Average Dissimilarity	Dissimilarity /Standard deviation	% Contribution	% Average Dissimilarity
Dissimilarity between Horticulture and Residential area microbial communities	PLS genus level contributing to dissimilarity between horticulture and Residential Land Uses						
	<i>Acinetobacter</i>	5.0	5.0	2.1	1.3	7.4	29.0
	<i>Aeromonas</i>	3.0	3.0	1.6	1.3	5.6	
	<i>Arcobacter</i>	4.5	4.3	1.6	1.3	5.5	
	<i>Rickettsia</i>	1.5	2.5	1.4	1.4	5.0	
	<i>Pseudomonas</i>	3.6	3.0	1.4	1.2	5.0	
	<i>Enterobacter</i>	2.7	2.4	1.3	1.1	4.6	
	<i>Yersinia</i>	1.3	2.0	1.3	1.4	4.5	
	<i>Erysipelothrix</i>	1.8	2.0	1.3	1.2	4.5	
	<i>Legionella</i>	1.3	2.0	1.2	1.3	4.2	
	<i>Escherichia-Shigella</i>	2.0	1.4	1.2	1.2	4.2	
	PLS species contributing to dissimilarity between horticulture and Residential land uses						
	<i>Arcobacter cryaerophilus</i>	3.5	2.5	3.0	1.2	8.1	35.3
	<i>Acinetobacter junii</i>	3.5	4.0	2.1	1.3	6.0	
	<i>Aeromonas tecta</i>	2.0	2.1	2.0	1.2	5.4	
	<i>Yersinia enterocolitica</i>	1.3	2.0	2.0	1.3	5.2	
	<i>Rickettsia montanensis</i>	1.4	2.3	2.0	1.4	5.0	
	<i>Pseudomonas oryzihabitans</i>	1.1	1.0	1.7	1.2	5.0	
	<i>Clostridium disporicum</i>	2.3	1.3	1.7	1.2	5.0	
	<i>Shigella sonnei</i>	2.0	1.4	1.7	1.1	5.0	
	<i>Pseudomonas mendocina</i>	3.1	3.0	1.6	1.3	4.6	
	<i>Clostridium perfringens</i>	1.4	0.7	1.6	1.4	4.4	
	<i>Rickettsia honei</i>	0.5	1.0	1.4	1.2	4.0	

Table S5. List of barcodes used for sample identification during sequencing

Names of Sampling Sites	Sampling Months	Adaptors
F1	Jan-09	(AAGGTT)
F5	Jan-09	(AATCTG)
F6	Jan-09	(ACCATG)
F8	Jan-09	(ACCGGC)
F9	Jan-09	(AGTCCT)
F10	Jan-09	(ATATAG)
F11	Jan-09	(ATCAAT)
R2	Jan-09	(ATGACG)
R3	Jan-09	(CAACTT)
R9	Jan-09	(CAGGCG)
R12	Jan-09	(CCAGCC)
R13	Jan-09	(CCTATA)
R14	Jan-09	(CGACCA)
R15	Jan-09	(CGCTGG)
U2	Jan-09	(CGGATT)
K1	Jan-09	(CTAGTA)
K4	Jan-09	(CTCTAC)
K5	Jan-09	(CTTCGT)
F1	Jul-09	(GAATGC)
F5	Jul-09	(GACGCC)
F6	Jul-09	(GAGTTG)
F8	Jul-09	(GCCGAA)
F9	Jul-09	(GCGCGA)
F10	Jul-09	(GCTTAG)
F11	Jul-09	(GGATCT)
R2	Jul-09	(GGCAAG)
R3	Jul-09	(GTTCCA)
R9	Jul-09	(TACTTC)
R12	Jul-09	(TATGGC)
R13	Jul-09	(TCAGGA)
R14	Jul-09	(TCCGTT)
R15	Jul-09	(TGCAGT)
U2	Jul-09	(TGGCTG)
K1	Jul-09	(TGGTAC)
K4	Jul-09	(TTATCC)
K5	Jul-09	(TTCCGC)

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