Supporting Information for:

Differential Resistance of Drinking Water Bacterial Populations to Monochloramine

Disinfection.

Tzu-Hsin Chiao,[†,‖,§] Tara M. Clancy,[†,§] Ameet Pinto,[†,⊥] Chuanwu Xi,[‡] and Lutgarde Raskin[*,†]

[†] Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States
[‡] Department of Environmental Health Sciences, University of Michigan, Ann Arbor, Michigan 48109, United States

Contents:

Table S1. Primers used for amplification of 16S rRNA gene sequences

Primer	Sequence (5' − 3')	Base Position (*E. coli* numbering)	Reference
180 bp (qPCR)			
338F	ACTCCTACGGGAGGCAGCAG	338-357	[1]
518R	ATTACCGCGGCTGCTGG	502-518	[1]
389 bp (pyrosequencing)			
563F	AYTGGGYDTAAAGNG	563-577	http://pyro.cme.msu.edu
909R	CCGTCAATTYHTTTRAGT	892-909	[2]
1379 bp (pyrosequencing)			
8F	AGRGTTTGATCMTGGCTCAG	8-27	[2]
1387R	GGGCGGWGTGTACAAGRC	1369-1387	[2]

Table S2. Pyrosequencing data summary

	Number of samples	Raw reads	Quality sequences	Sequences identified as chimeras	Sub-sampling (reads/sample)
Laboratory-scale inactivation samples	36	81980	60484	10142	763 (2123 when duplicate samples were combined)
Full-scale samples	6	1287	1175	17	106
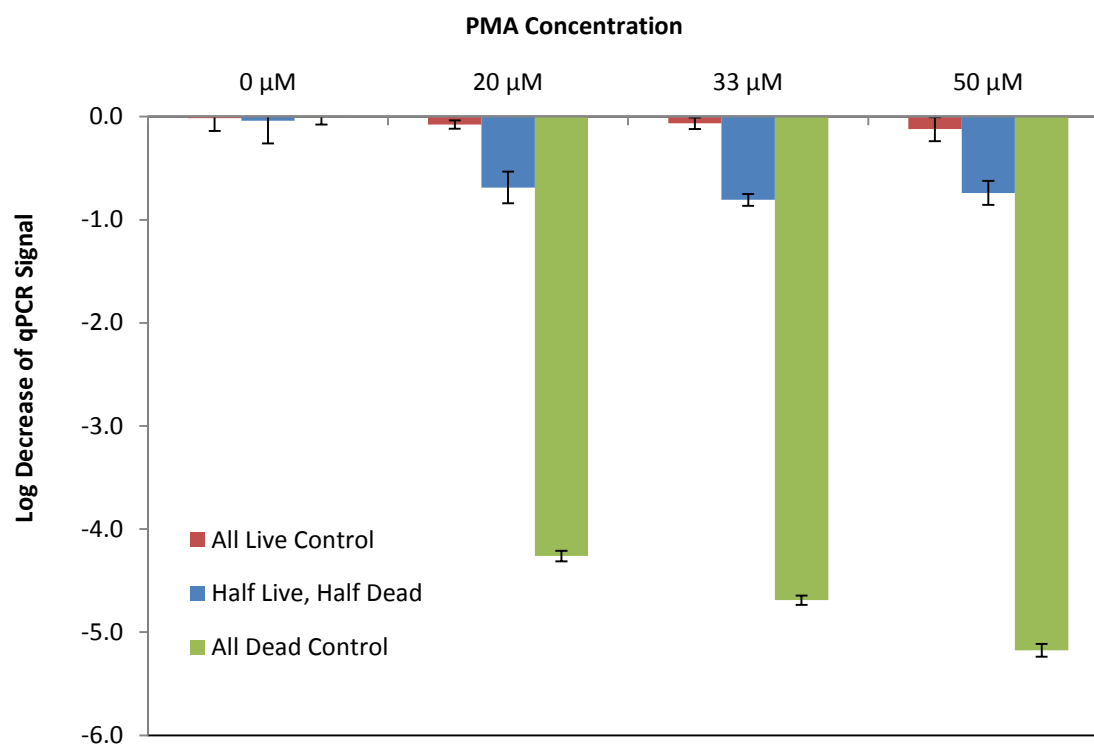
Figure S1. Results for qPCR with PMA treatment at various PMA concentrations for untreated (all live control), isopropanol treated (all dead control), and equal cell count live/dead mixture *E. coli* cells (half live, half dead). The 16S rRNA gene was quantified using the 338F and 518R primer pair. Average cell concentrations were approximately 10^6 to 10^7 cells/mL for all three types of samples. The results show that treatment with 50 μM PMA resulted in the greatest decrease in qPCR signal and thus the most complete removal of DNA from dead cells among the concentrations tested.
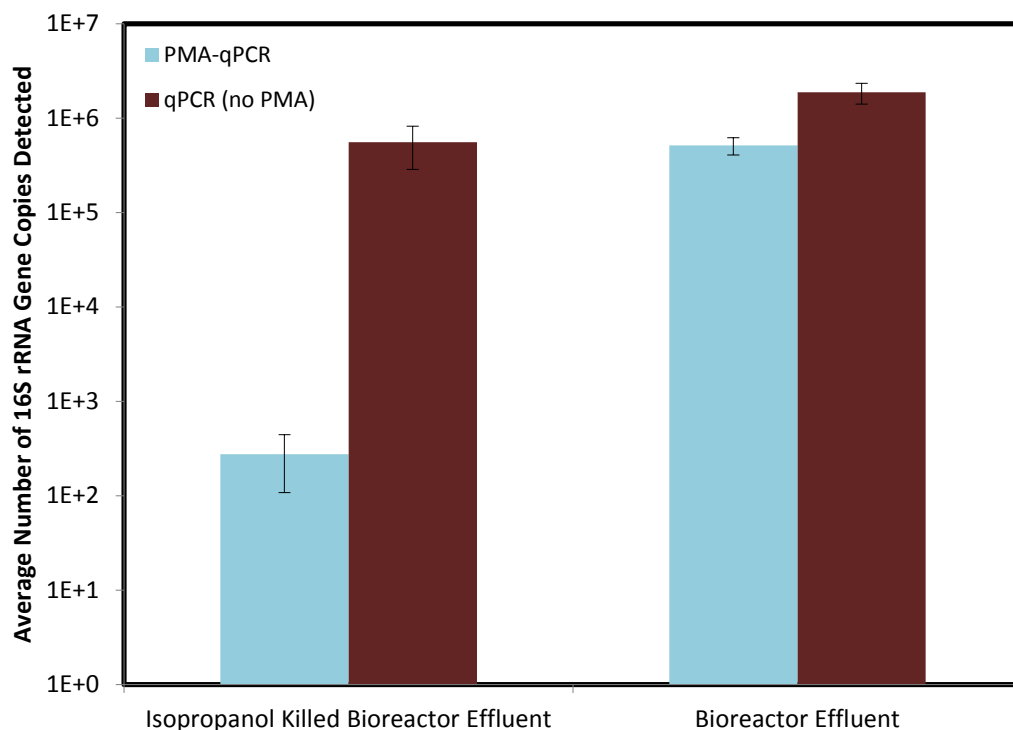
Figure S2. Results for qPCR with and without PMA treatment for isopropanol treated (killed) BAC filter effluent and untreated BAC filter effluent. The 16S rRNA gene was quantified using the 338F and 518R primer pair. The results show that PMA treatment helps to distinguish between live and dead cells. Without PMA treatment, there is little difference between the detection of live and dead cells. With PMA treatment, the number of cells measured in isopropanol treated controls were at least a 3-log units less than those in live controls.
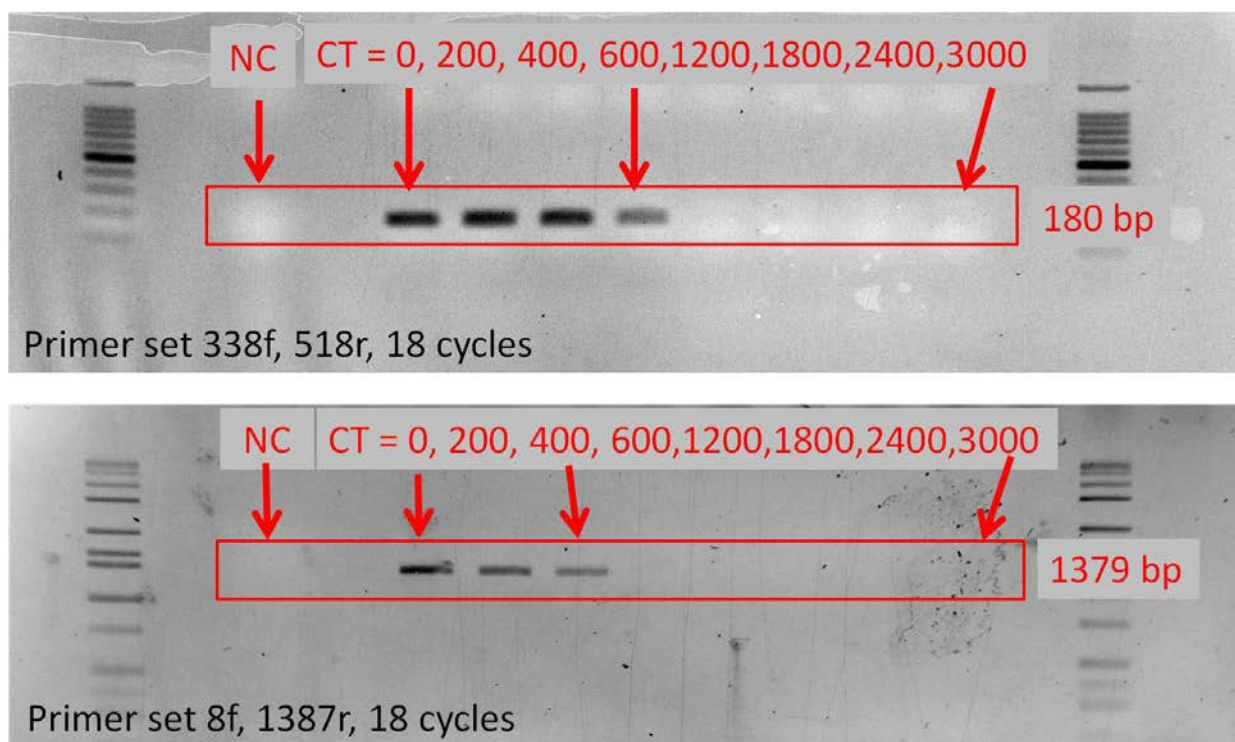
Figure S3. Gel image comparing PCR results with different amplicon lengths (top: 180 bp, bottom: 1379 bp) during inactivation of *E. coli* with 20 mg/L of monochloramine. Ct values in mg·min/L are provided above each band in the gel images and "NC" indicates negative control. Ladders are 100 bp and 1 kb, for top and bottom, respectively (Invitrogen, Life Technologies, Grand Island, NY and Takara[TM] Clontech Laboratories, Mountain View, CA).
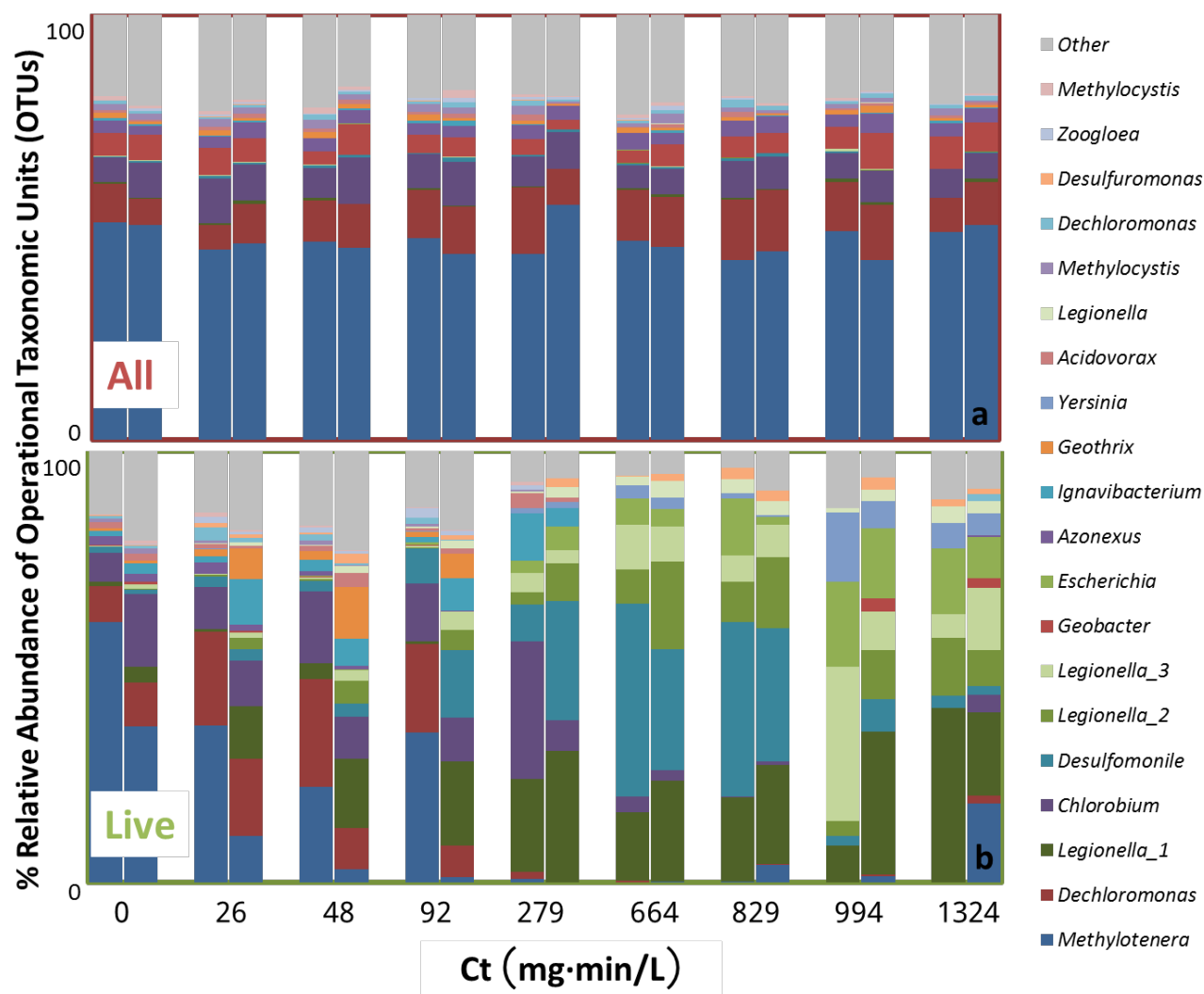
Figure S4. Relative abundance of OTUs represented by their taxonomic group for duplicate samples collected from laboratory-scale BAC filter inactivation experiment at different Ct values showing all bacteria (no PMA) (a) and the live fraction (PMA) (b).
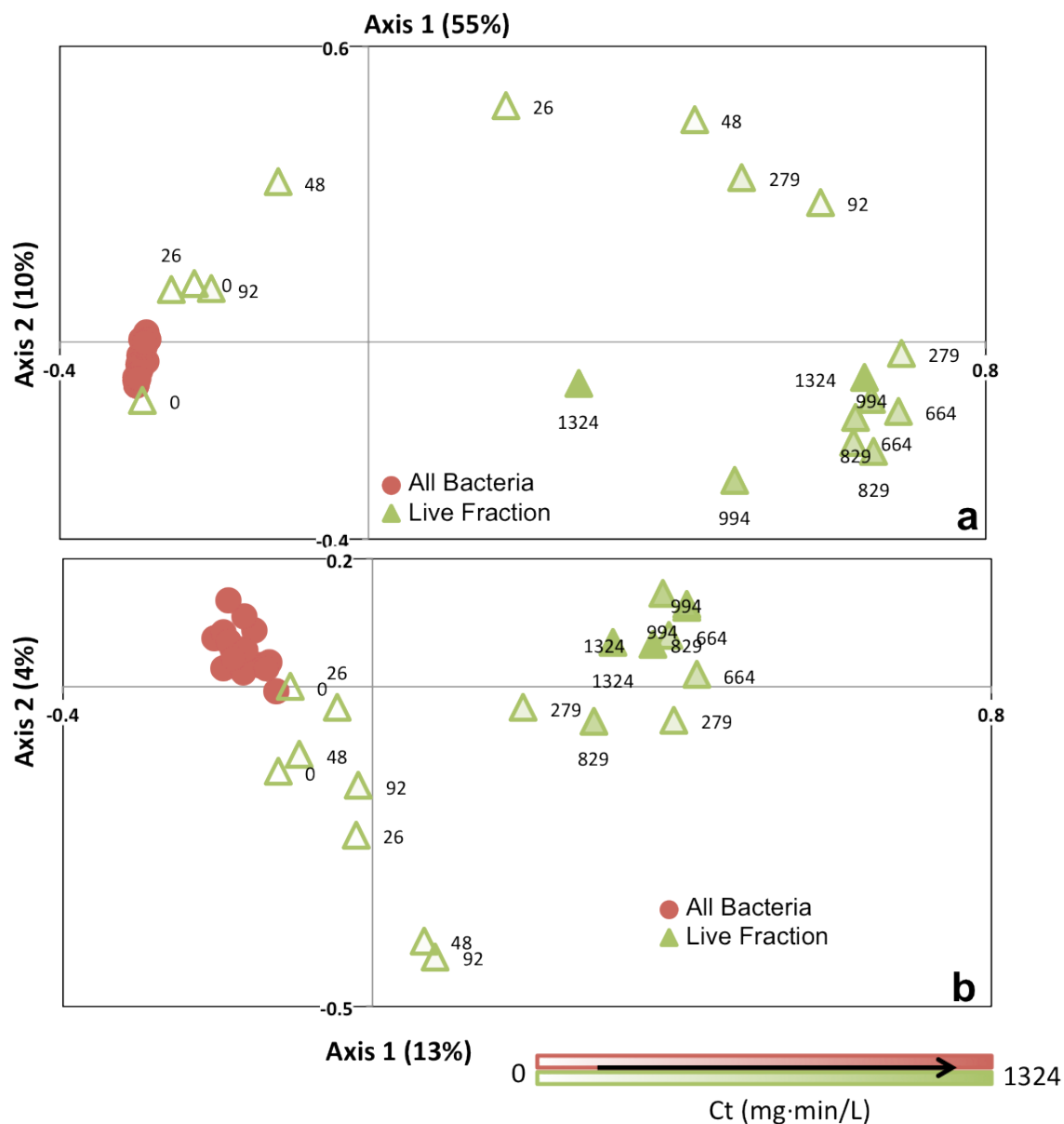
Figure S5. Principle coordinate plot showing the community structure (θ_{yc}) (a) and community membership (Jaccard Index) (b) for duplicate samples collected from the laboratory-scale BAC filter inactivation experiment for all bacteria (no PMA) and the live fraction (PMA treated). Numbers represent the Ct values in mg·min/L for the live fraction (PMA treated) samples.

References

1.   Einen, J.; Thorseth, I. H.; Ovreås, L. Enumeration of Archaea and Bacteria in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. *FEMS Microbiology Letters.* **2008,** *282* (2), 182-7.
2.   Pinto, A. J.; Raskin, L. PCR biases strongly distort bacterial and archaeal community structure in pyrosequencing. *PLoS One.* **2012,** *7* (8).