

## **Supplemental Materials for Staley, Z. et al.**

### **Additional Methods**

#### *Microcosm Establishment*

For both experiments, microcosms were established in a greenhouse at the University of South Florida Botanical Gardens (Tampa, FL). In the first, dark experiment, microcosms consisted of 11.3 L Rubbermaid plastic trash cans with opaque sides (29.97 x 22.86 x 33.65cm) containing 1 L of sediment disinfected by baking at 176.67°C and 2 L of autoclaved deionized water. These microcosms were covered with aluminum foil to prevent light penetration. In the second, light experiment, microcosms consisted of 2L glass beakers containing 0.5 L of sediment disinfected as above and 1.5 L of autoclaved de-ionized water. In both experiments, target bacteria were inoculated at densities of approximately  $10^7$  CFU/100 ml and target viruses at densities of approximately  $10^3$  copy numbers/ml.

#### *Bacterial Enumeration*

Culture-based methods of bacterial enumeration utilized selective differential media for each bacterial target, *E. coli* was enumerated on mTEC agar following incubation for 2 hours at 35°C and then at 44.5°C for 22 h (9), *Enterococcus faecalis* was enumerated on mEI agar after 24 h incubation at 41°C (8), *E. coli* O157:H7 was enumerated on Sorbitol MacConkey agar following 24 h incubation at 37°C (6), and *S. enterica* was enumerated on XLT-4 agar following incubation for 24 h at 37°C (2). Quantification via qPCR (performed only for the dark microcosms) utilized the primers and probes found in Table S1.

### *Growth Curves*

Growth curves were conducted by inoculating an isolated colony of *E. coli* ATCC 9637, *Ent. faecalis* ATCC 19433, *S. enterica* serovar Typhimurium, or *E. coli* O157:H7 EDL 933 into a centrifuge tube containing 20ml of M9 Minimal Media (supplemented with 12g/L of yeast extract for *Ent. faecalis* growth) and incubated for 24h at 37°C. The optical density at 600 nm (OD<sub>600</sub>) was measured for each overnight culture using a NanoDrop 2000 Spectrophotometer (Thermo Scientific) and then diluted 1:20 into individual tubes containing M9 Minimal Media. The OD<sub>600</sub> was measured for each of the tubes to get a baseline prior to agrochemical addition. Three replicates cultures of M9 amended with each singular agrochemical treatment (water and solvent controls, inorganic fertilizer, atrazine, malathion, or chlorothalonil) were established at 1x expected environmental concentration (EEC). The OD<sub>600</sub> was measured after 1 h and then after every subsequent 30 min for ~5 h. This procedure was repeated until growth curves had been conducted for all target bacteria at 1x and 2x EEC. Growth curves were also conducted as above for each of the four target bacteria as well as *E. coli* WW6 (isolated from a wastewater treatment plant in Tampa, FL) using agrochemical treatments including a water control, atrazine, atrazine-2-hydroxy, cyanuric acid, and atrazine exposed to 365 nm UV lamps for ~18h.

**Table S1.** Primers and probes used for qPCR analysis.

Target	Orientation	Primer or Probe	Sequence (5'-3')	Reference
HPyV	Forward	SM2	AGT CTT TAG GGT CTT CTA CCT TT	(5)
	Reverse	P6	GGT GCC AAC CTA TGG AAC AG	
	Probe	KGJ3	(FAM)-TCA TCA CTG GCA AAC AT-(MGBNFQ)	
Adenovirus	Forward	JTVXF	GGA CGC CTC GGA GTA CCT GAG	(1)
	Reverse	JTVXR	ACI GTG GGG TTT CTG AAC TTG TT	
	Probe	JTVXP	(FAM)-CTG GTG CAG TTC GCC CGT GCC A-(MGBNFQ)	
<i>E. coli</i> O157:H7	Forward	EcoOH-F	TCG AGC GGA CCA TGA TCA	(3, 7)
	Reverse	EcoOH-R	GGC GGC GTC TGA GAT AAC A	
	Probe	EcoOH-PR	(FAM)-AGA ACT TCA AAT CCA TCA TT-(MGBNFQ)	
<i>Salmonella</i> <i>enterica</i>	Forward	Sal-F	CGT TTC CTG CGG TAC TGT TAA TT	(3, 7)
	Reverse	Sal-R	AGA CGG CTG GTA CTG ATC GAT AA	
	Probe	Sal-probe	(FAM)-CCA CGC TCT TTC GTC T-(MGBNFQ)	
<i>E. coli</i>	Forward	Eco-F	GTC CAA AGC GGC GAT TTG	(3, 7)
	Reverse	Eco-R	CAG GCC AGA AGT TCT TTT TCC A	
	Probe	Eco-PR	(FAM)-ACG GCA GAG AAG GTA-(MGBNFQ)	
Enterococcus	Forward	EnteroF1A	GAG AAA TTC CAA ACG AAC TTG	(4)
	Reverse	EnteroR1	CAG TGC TCT ACC TCC ATC ATT	
	Probe	GPL813TQ	(FAM)-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-(TAMRA)	

**Table S2.** Correlations of bacterial concentrations obtained via culture-dependent methods vs. qPCR at T24 and T168. Pearson  $r$  correlation coefficient is shown.

<b>Target</b>	<b>Water</b>		<b>Sediment</b>	
	<b>T 24</b>	<b>T 168</b>	<b>T 24</b>	<b>T 168</b>
<i>E. coli</i>	0.86	0.70	0.83	0.65
<i>Ent. faecalis</i>	0.66	0.45	0.41	0.65
<i>E. coli</i> O157:H7	0.84	0.60	0.67	0.61
<i>S. enterica</i>	0.70	0.80	0.72	0.72

**Table S3.** Results of multivariate analysis of variance for the *Dark Microcosms* examining the effects of spatial block, agrochemical treatment, and sampling date on the density, quantified by qPCR, of *E. coli*, *Ent. faecalis*, *E. coli* O157:H7, *S. enterica*, HPyV, and adenovirus in the water column.

Effect	Wilk's <i>F</i>	<i>df</i> effect	<i>df</i> error	<i>P</i>
Intercept	1192.23	6	29	<0.001
Block	7.43	18	85.51	<0.001
Treatment	1.32	60	157	0.09
Sampling date	45.38	12	23	<0.001
Date*Block	16.55	36	68.68	<0.001
Date*Treatment	1.25	120	193.24	0.09

**Table S4.** Results of multivariate analysis of variance for the *Light Microcosms* examining the effects of spatial block, agrochemical treatment, and sampling date on the density of *E. coli*, *Ent. faecalis*, *E. coli* O157:H7, and *S. enterica* in the water column.

Effect	Wilk's <i>F</i>	<i>df</i> effect	<i>df</i> error	<i>P</i>
Intercept	32427.80	4	12	<0.001
Block	76.71	12	32.04	<0.001
Treatment	1.21	20	40.75	0.30
Sampling date	587.48	8	8	<0.001
Date*Block	263.18	24	23.80	<0.001
Date*Treatment	1.29	40	37.67	0.22

**Table S5.** Growth rates of bacteria cultures while exposed to agrochemicals. Error bars represent standard deviations.

Treatment	Concentration	<i>E. coli</i> 9637		<i>E. coli</i> WW6		<i>E. coli</i> O157:H7		<i>Ent. faecalis</i> 19433		<i>S. enterica</i>	
		Generation Time <sup>-1</sup>	Standard Deviation	Generation Time <sup>-1</sup>	Standard Deviation	Generation Time <sup>-1</sup>	Standard Deviation	Generation Time <sup>-1</sup>	Standard Deviation	Generation Time <sup>-1</sup>	Standard Deviation
Water	1x EEC	6.5E-01	1.03E-01			9.6E-01	8.17E-02	7.5E-01	8.16E-02	7.0E-01	9.76E-02
	2x EEC	8.5E-01	9.62E-02			6.1E-01	2.00E-02	7.7E-01	2.71E-02	9.3E-01	3.39E-02
	Intermediates	1.1E+00	5.83E-02	6.7E-01	2.62E-02	7.2E-01	7.18E-02	5.9E-01	3.47E-02	1.0E+00	1.50E-01
Solvent	1x EEC	6.4E-01	9.18E-02			1.0E+00	1.13E-01	3.9E-01	2.24E-01	7.5E-01	3.58E-02
	2x EEC	8.2E-01	3.81E-02			6.3E-01	4.66E-02	7.1E-01	3.11E-02	8.4E-01	2.83E-02
Fertilizer	1x EEC	6.2E-01	5.86E-02			9.5E-01	2.10E-01	6.0E-01	1.02E-01	6.8E-01	6.96E-02
	2x EEC	7.9E-01	3.59E-02			6.1E-01	4.66E-02	7.1E-01	3.58E-02	8.3E-01	3.70E-02
Malathion	1x EEC	6.3E-01	1.04E-01			1.0E+00	1.13E-01	5.4E-01	1.06E-01	7.1E-01	9.98E-02
	2x EEC	8.2E-01	5.69E-02			6.0E-01	2.92E-02	7.1E-01	3.12E-02	8.0E-01	2.21E-02
Chlorothalonil	1x EEC	6.8E-01	9.68E-02			9.0E-01	1.90E-02	5.5E-01	1.54E-01	7.6E-01	1.15E-01
	2x EEC	7.7E-01	3.10E-02			6.4E-01	7.60E-02	6.6E-01	6.53E-02	1.0E+00	1.26E-01
Atrazine	1x EEC	6.3E-01	7.73E-02			9.0E-01	7.31E-02	6.5E-01	2.10E-01	7.5E-01	1.36E-01
	2x EEC	8.3E-01	1.08E-01			5.3E-01	3.50E-02	7.2E-01	8.86E-02	7.9E-01	1.70E-02
	Intermediates	1.1E+00	4.89E-02	7.2E-01	2.88E-02	6.9E-01	1.56E-02	5.8E-01	1.78E-02	1.0E+00	9.04E-02
UV-atrazine	Intermediates	9.3E-01	4.13E-02	7.6E-01	3.22E-02	7.1E-01	4.47E-03	5.5E-01	4.14E-02	9.6E-01	4.57E-02
Atrazine-2-hydroxy	Intermediates	9.1E-01	5.79E-02	7.1E-01	5.85E-02	6.8E-01	4.88E-02	5.4E-01	5.37E-02	8.6E-01	2.22E-02
Cyanuric Acid	Intermediates	9.9E-01	3.01E-02	6.3E-01	2.18E-02	6.9E-01	3.70E-02	5.9E-01	2.02E-02	8.9E-01	2.44E-02

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