Supporting Information for:

Biotransformation of Trace Organic Contaminants in Open-Water Unit Process Treatment Wetlands

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20 pages
10 figures
4 tables

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Details of microcosm sampling

Triplicate microcosms containing unbuffered wetland water ([DOC]≈8 mg C L^{-1}; $[NO_3^-]$≈10 mg N L^{-1}) were amended with all six test compounds (5 μg L^{-1}) and sampled at regular intervals throughout the experiment. 1 mL aqueous samples were filtered through 1 μm glass-fiber Acrodisc syringe filters (Pall Corporation), amended with about 2.5 ng of each isotopically-labeled internal standard, and refrigerated until analysis (within 1 week). Test compounds were extracted from dewatered (centrifuged at 5,000 RPM, 10 minutes), wet biomat samples (about 0.1 g dry weight). Samples were agitated for approximately 8 hours on a rotisserie in 15 mL of methanol amended with about 2.5 ng of each isotopically-labeled internal standard. Methanol extracts were filtered (1 μm glass-fiber; Millipore, Bellerica, MA) and diluted to 1 L with deionized water prior to clean-up and concentration via solid phase extraction (SPE). The SPE media consisted of 50 mg Waters Oasis hydrophilic-lipophilic balance (HLB) in cartridges pretreated with 10 mL of methanol, followed by 10 mL of Milli-Q water. Cartridges were eluted with 12 mL of methanol, dried under a gentle nitrogen stream, and re-suspended in 1 mL of Milli-Q water prior to analysis via HPLC-MS-MS.

Details of pilot-scale wetland sampling

Aqueous samples were collected in 1 L, baked amber glass bottles, filtered through 1 μm glass fiber filters and refrigerated prior to analysis. 400 mL samples were amended with approximately 10 ng of isotopically-labeled internal standards and extracted via SPE, as described above, prior to analysis. In some cases, 1 mL samples were amended with 2.5 ng of isotopically-labeled internal standards, and analyzed by HPLC-MS/MS with direct injection of either 100 μL or 800 μL sample aliquots. Samples were analyzed within 3 days of collection.

Test compounds were extracted from biomat samples collected throughout the pilot-scale wetland using methanol, as described above.

Details of DNA Extraction, Sequencing, and Clone Library Creation

Approximately 1 g of biomass was sampled from dark microcosms and microcosms illuminated with visible light (635 nm) at the beginning, middle, and end of biotransformation experiments. Samples were shipped overnight on dry ice and stored at -80° C prior to extraction. DNA was extracted from 0.25g of sample using the Mo Bio PowerBiofilm DNA Isolation Kit per manufacturer's protocol. Extracted DNA was amplified in triplicate 25 µl reactions without Illumina adaptors or primers pads on a Roche LightCycler 480II. A portion of the 16S rRNA gene was amplified using Phusion Master Mix (New England BioLabs, Inc), 3% final volume DMSO, 0.4x final concentration SYBR Green, 200nM 515F (5' GTGYCAGCMGCCGCGGTAA 3'),[1] and 12bp Golay barcoded 806R (5'XXXXXXXXXXXXCCGGACTACHVGGGTWTCTAAT 3').[2] The amplification program was: 94°C 3 min; 94°C 45 sec , 50°C 10 sec, 72°C 90 sec. The program was stopped after all samples had amplified. Triplicates were pooled and purified using Agencourt AMPure XP and quantified using a Life Sciences Qubit 2.0 Flurometer. Normalized amplicons were sequenced on the Illumina MiSeq platform using NEBNext Ultra DNA Library Prep Kit and a MiSeq Reagent Kits v2 2x250 500 cycle kit.

The sets of 250 bp sequences were stitched together using ea-utils[3] fastq-join with a minimum base pair overlap of 100. Stitched sequences were reverse complimented with the fastx toolkit[4] in order to account for sequences that were sequenced in the reverse direction. The resulting sequences were processed in QIIME 1.7 dev[5] starting with sl_prep_fastq.py to create a

barcode .fastq file. The resulting sequence and barcode file were demultiplexed using split_libraries_fastq.py with default parameters, except for "--barcode 12", to negate error correcting of barcodes, as any sequences with errors would have been filtered out by sl_prep_fastq.py. Otus were piked *de novo* using Usearch 6.1[6] and chimeras were filtered out using the Greengenes gold database[7]. Representative sequences were aligned using PyNAST[8] and greengenes 13_5 aligned reference database. Taxonomy was assigned using the RDP classifier and greengenes 13_5 97 otu taxonomy database and the otu table was then rarified to 5050 sequences before further analysis. All phyla with less than 1% relative abundance were filtered out.

DNA for the 23S rRNA gene algal clone library was extracted in the same way as the microcosm samples and amplified with p23SrV_f1 and p23SrV_r1, GGA CAG AAA GAC CCT ATG AA, and TCA GCC TGT TAT CCC TAG AG, respectively, with the published amplification protocol.[9] Amplicon was purified via gel electrophoresis using the E. Z. N. A. gel extraction kit (Omega). Purified amplicon was then transformed into electro-competent *E. coli* cells using the TOPO TA Cloning kit (Invitrogen) per manufacture's instruction. Individual clones were Sanger sequenced by Wyzer Biosciences, Inc (Cambridge, MA). Sequences were analyzed in Geneious v6.0 and trimmed using default quality settings. After trimming any sequences with <90% high quality base scores were excluded from alignment. High quality sequences (16 out of 23) were aligned using the multiple alignment tool with default settings.

Details of HPLC-MS-MS Analysis

Trace organic compounds were separated by an Agilent 1200 HPLC using a 2.1 mm x 30 mm Zorbax SB-C18 3.5 μm column, eluted with 0.5 mL min^{-1} acetonitrile and 0.1%

111 acetic acid in water with the following gradient: 0 minutes, 5% acetonitrile; 5.5 minutes 55%
112 acetonitrile; 6 minutes, 100% acetonitrile; 9 minutes, 100% acetonitrile; 10 minutes, 5%
113 acetonitrile. Compounds were detected with an Agilent 6460 MS-MS using electrospray
114 ionization (ESI) with a gas temperature of 350°C, a sheath gas temperature of 400°C, a gas flow
115 rate of 11 L/min at 50 psi, and a capillary voltage of 3600 V. Compound-specific parameters are
116 given in Table SI 1.
117

118 **Details of Corrections for Evaporation**

119 The effect of evaporation in the open-water cell can be accounted for by including an
120 extra term in the calculation of the first-order rate constant, k, according to:

$$\ln\left(F\frac{C}{C_o}\right) = -kt$$

121 where C and C_o are the concentration at time t and the initial concentration, respectively, and F is
122 the fraction of water remaining after evaporation (F<1). This equation may be rearranged to give:

$$k = -\frac{\ln(F)}{t} - \frac{\ln\left(\frac{C}{C_o}\right)}{t} = -\frac{\ln(F)}{t} + k_{obs}$$

123 where k_{obs} is the pseudo first-order removal rate of a compound observed in the wetland. Thus,

$$k_{obs} = k + \frac{\ln(F)}{t}$$

124 where $\frac{\ln(F)}{t}$<0 and corrects for evaporation. Approximately 10% evaporation (i.e., F=0.9),
125 quantified using increases in chloride concentrations, was typical during summer between the
126 cell inlet and outlet (Figure SI 9). Based on a residence time of 1.5 days, $\frac{\ln(F)}{t}$=-0.07 d^{-1}.
127

Table SI 1. Compound-Specific Mass Spectrometry Parameters[a]

compound	precursor ion (amu)	fragmentor voltage (V)	product ions (amu)	collision energy (V)	cell accelerator (V)	ionization mode
1-naphthoxy acetic acid	201	80	143 115	15 45	0 5	negative[b]
4-OH-propranolol	276	115	173 116	15 15	5 5	positive[c]
Atenolol	267	130	145 190	24 16	7	positive
Atenolol-d_7	274	130	145	24	7	positive
Carbamazepine	237	120	179 194	35 15	7	positive
Carbamazepine-d_{10}	247	120	204	20	7	positive
Metoprolol	268	130	159 116	17 14	7	positive
Metoprolol-d_7	275	130	123 159	14 17	7	positive
Metoprolol-α-OH	284	130	116 74	15 15	7	positive
Metoprolol-α-OH-d_5	289	135	121	15	5	positive
Metoprolol acid	268	130	191 145	17 25	7	positive
Metoprolol acid-d_5	273	130	196	17	7	positive
Nor propranolol	218	100	155 127	15 45	5	positive
Nor propranolol-d_7	255	100	189	12	5	positive
Propranolol	260	98	116 183	13 12	7	positive
Propranolol-d_7	267	98	116	13	7	positive
Sulfamethoxazole	254	110	92 156	25 10	7	positive
Sulfamethoxazole-d_4	258	110	96	25	7	positive
Trimethoprim	291	140	123 261	20 17	7	positive
Trimethoprim-d3	294	140	123	20	7	positive

[a]All compounds were analyzed using a drying gas temperature of 350° C, a gas flow of 12 L min^{-1}, a nebulizer pressure of 60 psi, a sheath gas temperature of 400 ° C, a sheath gas flow of 12 L min^{-1}, and a nozzle voltage of 300 V. [b]Compounds analyzed by positive ionization used a capillary voltage of 3600 V. [c]Compounds analyzed by negative ionization used a capillary voltage of 4500 V.

Table SI 2. Average Aqueous Test Compound Concentrations of Trace Organic Compounds in the Pilot-Scale Open-Water Cell

Compound	**Concentration (ng L^{-1})[a]**				
	Inlet	***Point 1***	***Middle***	***Point 3***	***Outlet***
Atenolol	100±10	31±4	19±3	7.7±1.6	3.3±0.9
Metoprolol	58±3	38±3	30±3	20±3	8.6±0.9
Propranolol	41±2	23±3	16±2	10±1	4.5±0.6
Trimethoprim	8.4±0.9	7.8±0.8	6.3±0.3	5.1±0.7	4.7±0.7
Sulfamethoxazole	810±20	750±20	730±10	750±10	710±20
Carbamazepine	110±10	110±10	120±10	110±10	110±10

[a]Average ± standard error of the mean of 10 samples measured at each sample location in the wetland during the week of August, 20th 2012. See Figure SI 8 for plot of data.

Table SI 3. Wetland Temperatures[a]

Month	Average Temperature (˚C)
January	8.6±0.4
February	9.8±0.3
March	11.9±0.3
April	14.4±1.0
May	16.8±0.7
June	20.0±0.3
July	22.0±0.2
August	22.3±0.4
September	21.7±0.5
October	17.5±0.3
November	12.4±0.5
December	9.3±0.3

[a] Average monthly temperatures reported in Livermore, CA from 2007-2012 ± standard error of the mean.[10]

Table SI 4. Sorbed Test Compound Concentrations in Open-Water Wetland[a]

Compound	Concentration sorbed (µg kg^{-1})			Concentration sorbed (µg (kg organic carbon)$^{-1}$)[b]			Predicted aqueous concentration (ng L^{-1})[c]		
	Inlet	*Middle*	*Outlet*	*Inlet*	*Middle*	*Outlet*	*Inlet*	*Middle*	*Outlet*
Atenolol	0.2±0.1	0.6±0.1	0.5±0.0	2.9±1.0	1.9±0.3	1.6±0.1	22±8	12±3	12±1
Metoprolol	1.9±0.4	11.3±0.9	6.6±0.7	34±16	37±3	24±3	85±40	83±11	59±10
Propranolol	14±6	69±13	38±12	210±40	230±50	140±50	57±12	52±13	37±14
Trimethoprim	0.2±0.2	1.4±1.3	0.5±0.1	5.1±3.5	4.7±4.4	1.8±0.3	24±17	20±21	8.5±1.5
Sulfamethoxazole	1.8±0.9	2.2±0.8	5.7±1.2	25±3	7.4±2.5	20±5	1220±190	620±140	980±250
Carbamazepine	0.7±0.3	3.0±1.0	2.3±0.1	10±1	10±3	8.1±0.7	130±20	130±40	100±10

[a] Average ± one standard deviation. [b]Normalized based on percent organic carbon contents: inlet 7.2±3.7% organic carbon; inlet 30.2±0.2% organic carbon; inlet 28.3±1.3% organic carbon. [c]Aqueous concentration predicted based on organic carbon-normalized concentrations and log K_{oc} values measured in autoclaved test tubes containing wetland biomass (see Supporting Information Figure SI 2) according to: $C_{aq}=C_{sorb}/K_{oc}$.

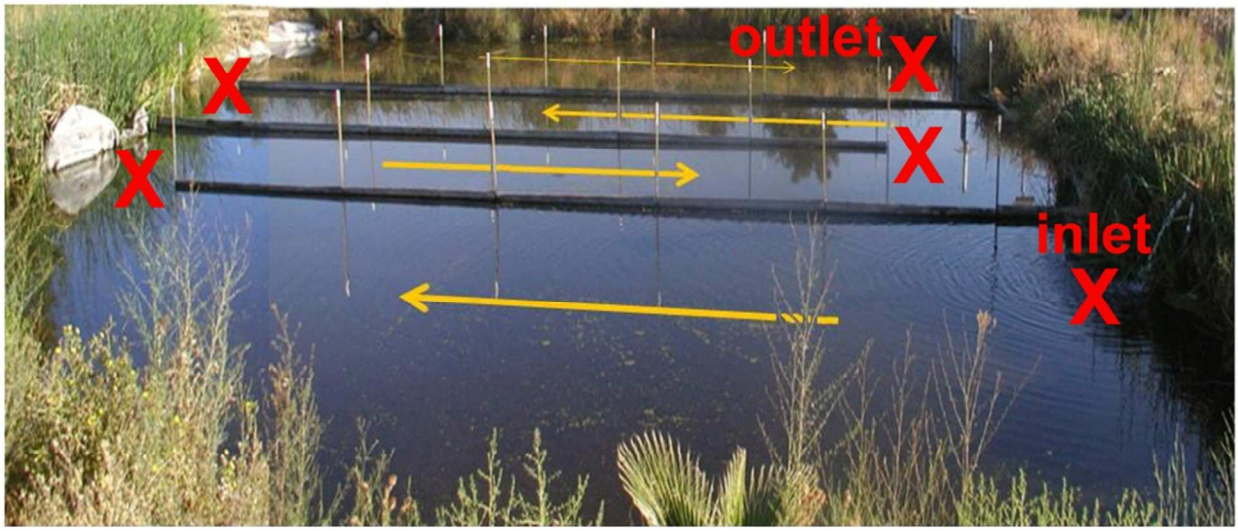

Figure SI 1. Photograph of the pilot-scale open-water unit process cell, located in Discovery Bay, CA (37.9˚N, 121.6˚W). Arrows indicate flow direct and X indicates sampling location.

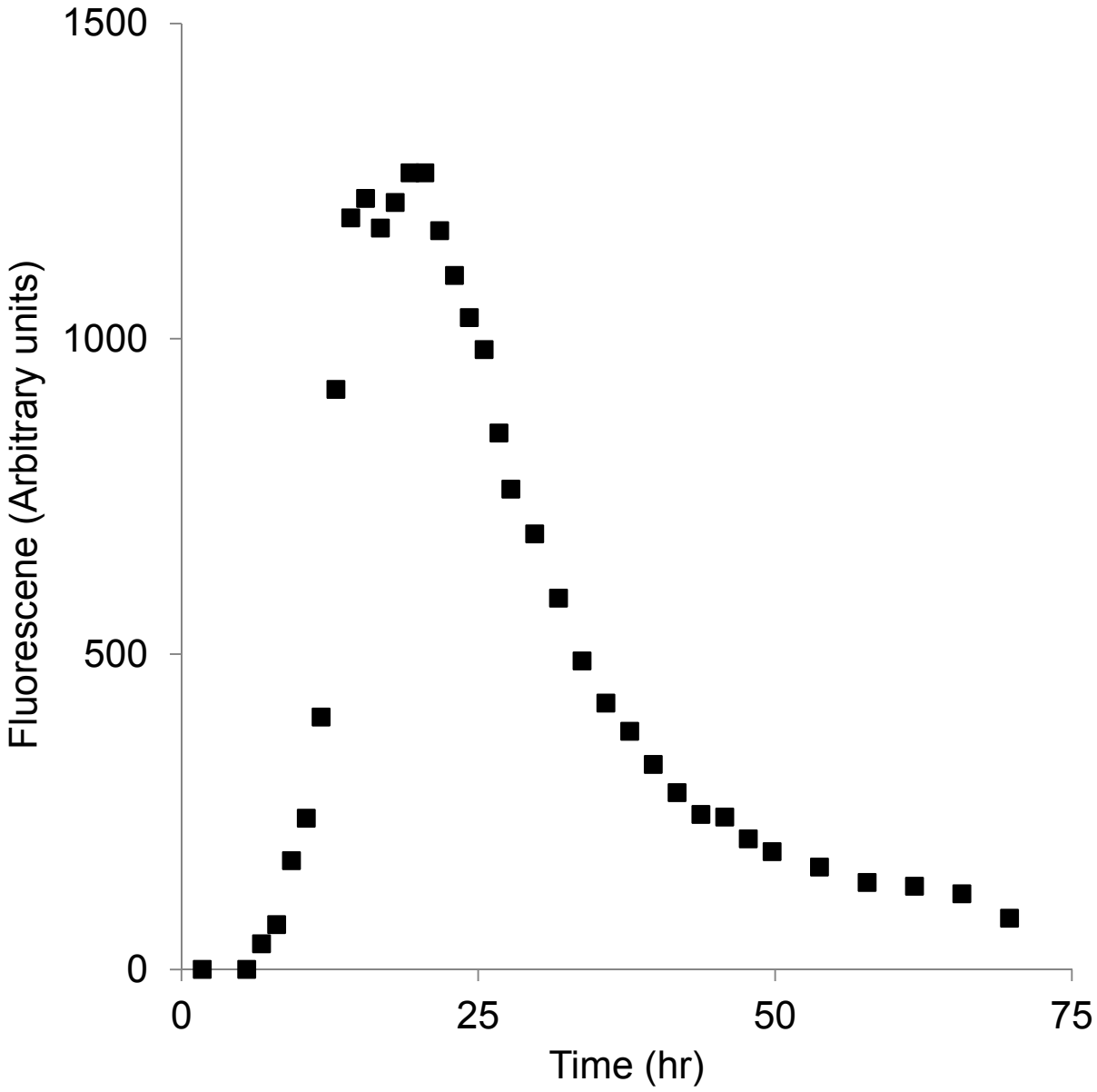

Figure SI 2. Breakthrough curve of rhodamine-WT in a tracer test in the pilot-scale open-water cell. Rhodamine-WT was injected at cell inlet at 0 hours and samples were collected at the cell outlet and analyzed by fluorometry (Turner TD 700). The center of mass was calculated to be 27 hours. The flow into the cell was 1.9×10^{-2} MGD (8.1×10^{-4} m^3 s^{-1}).

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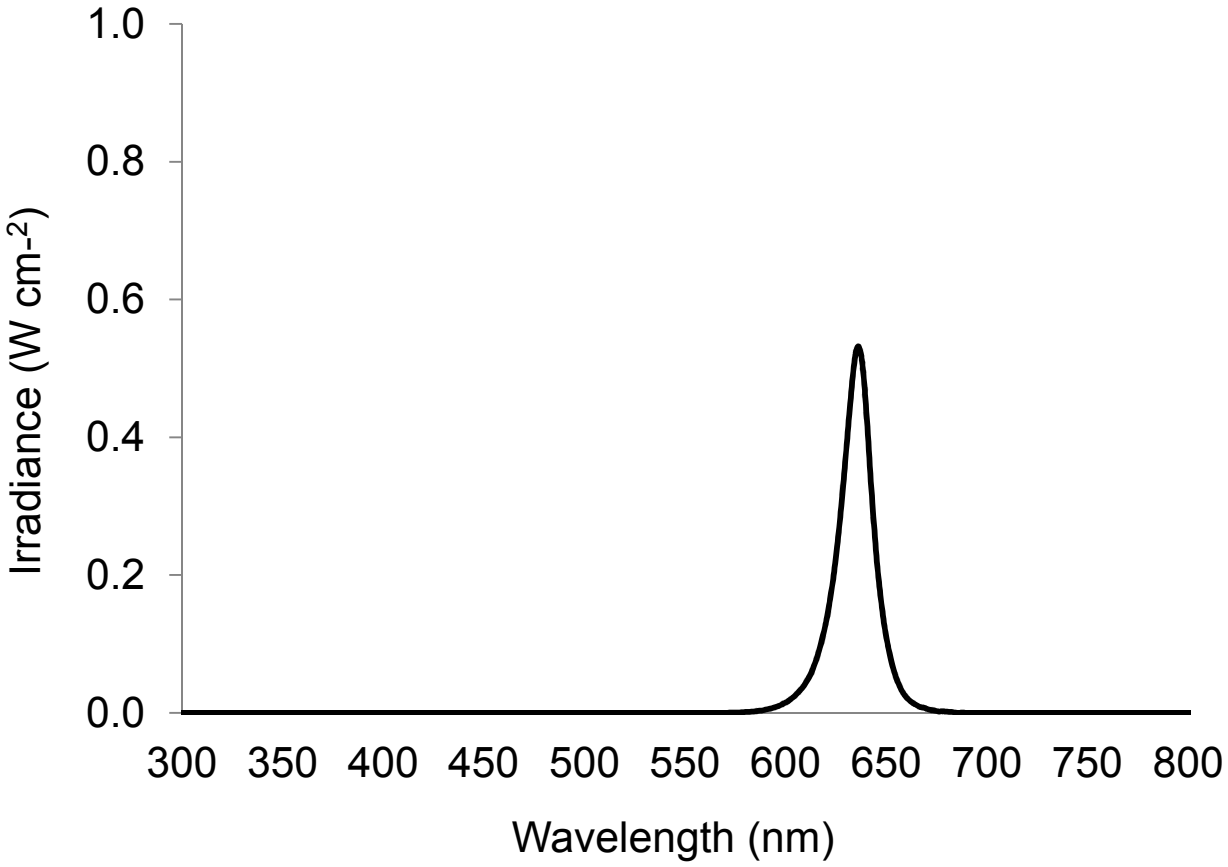

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159 **Figure SI 3**. Spectrum of the red lamp used in microcosm experiments measured with a

160 spectroradiometer (Stellarnet).

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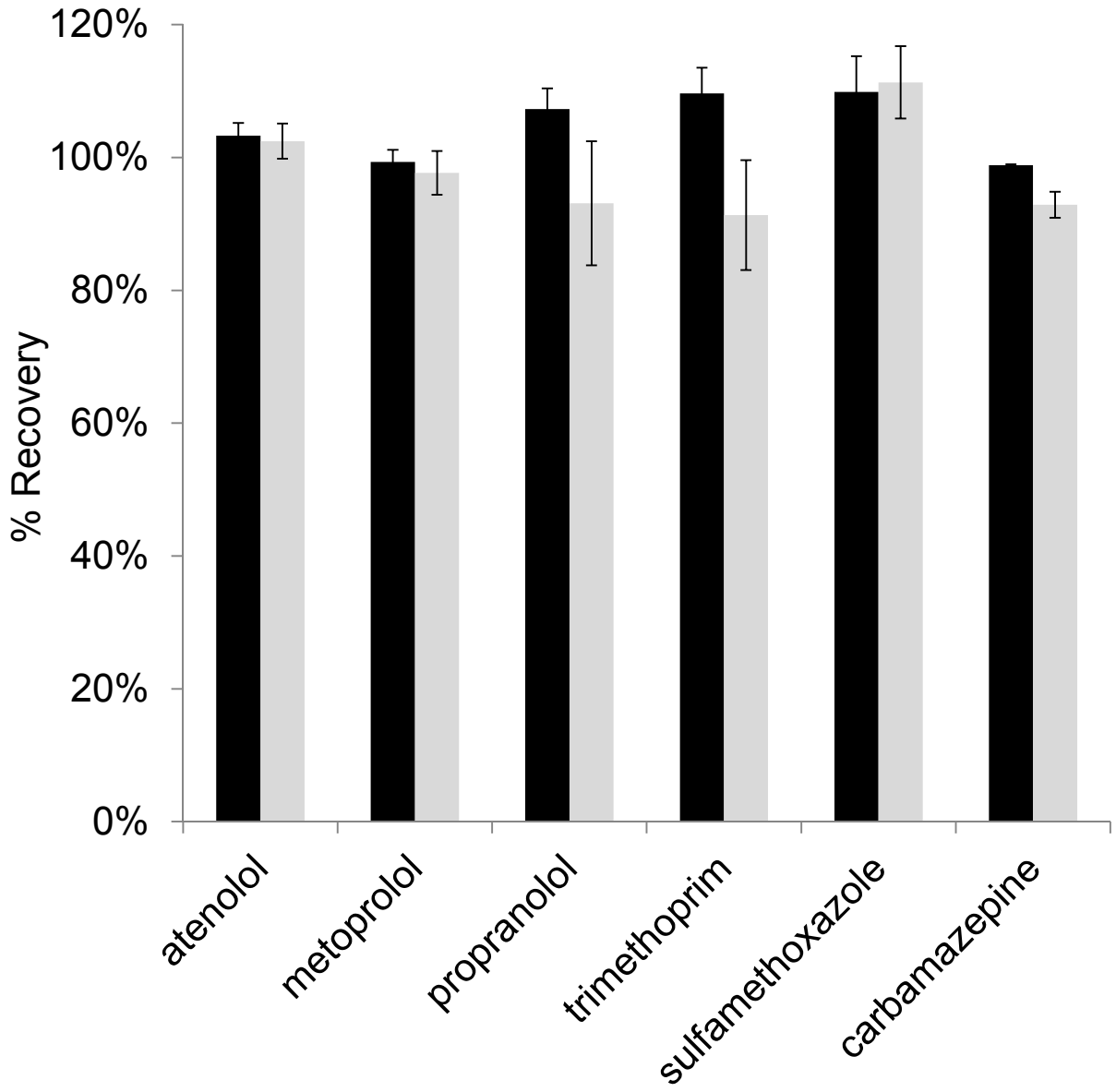

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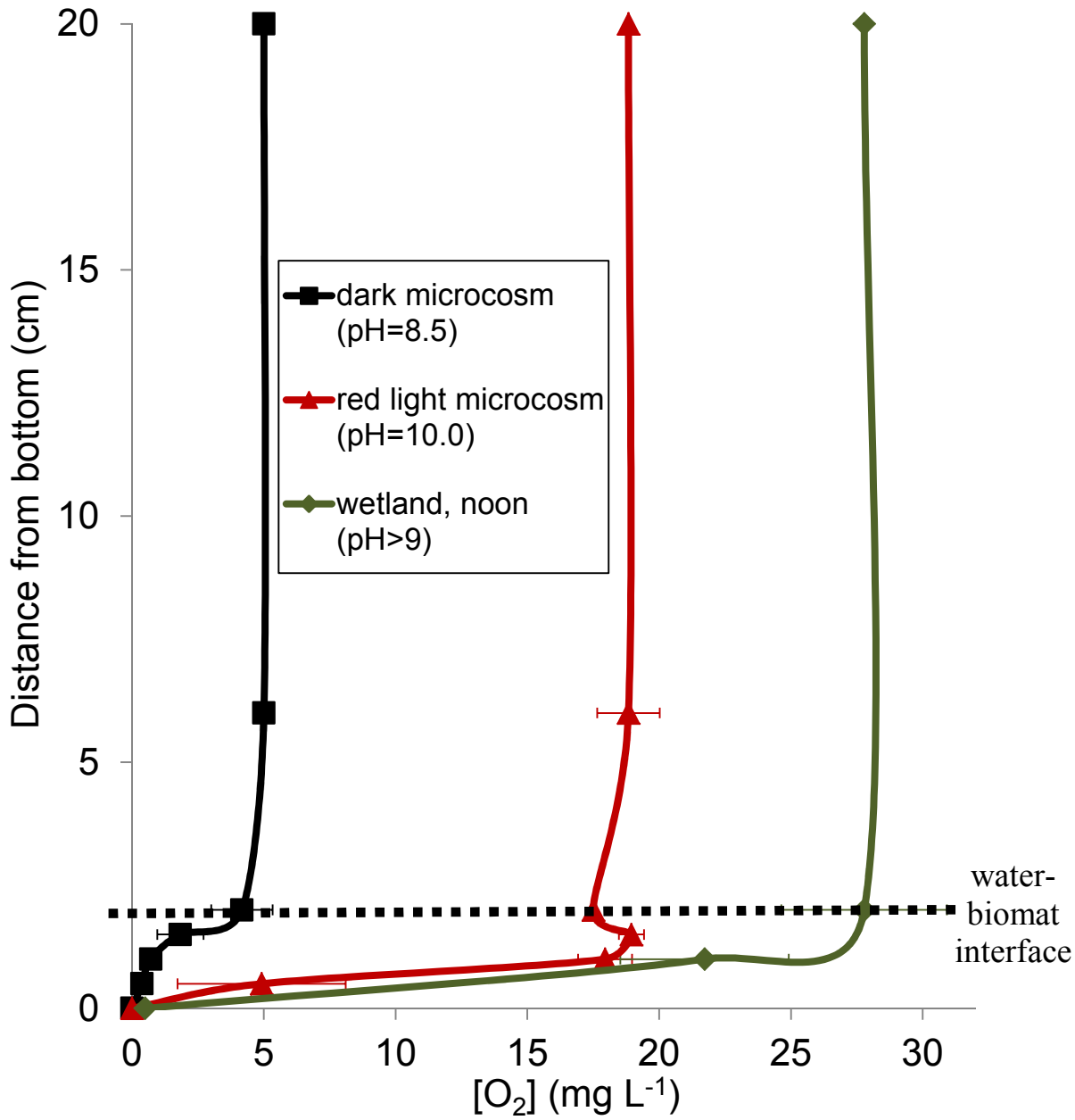
164 **Figure SI 4**. Percent mass recovery of test compounds amended to reactors containing

165 autoclaved wetland biomass (13 g L^{-1}) maintained at pH 8.7 (black) and pH 10.0 (gray) by

166 buffering with 20 mM borate. Reactors were gently mixed in triplicate on a rotisserie for 2 hours

167 at 4˚C in the dark prior to extraction. Error bars represent ± one standard deviation.

168



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170 **Figure SI 5**. Dissolved oxygen profiles in microcosms and at the pilot-scale open-water cell

171 (4-17-13) measured with a micro dissolved oxygen probe (Lazar Research Laboratories). Error

172 bars represent ± one standard deviation.

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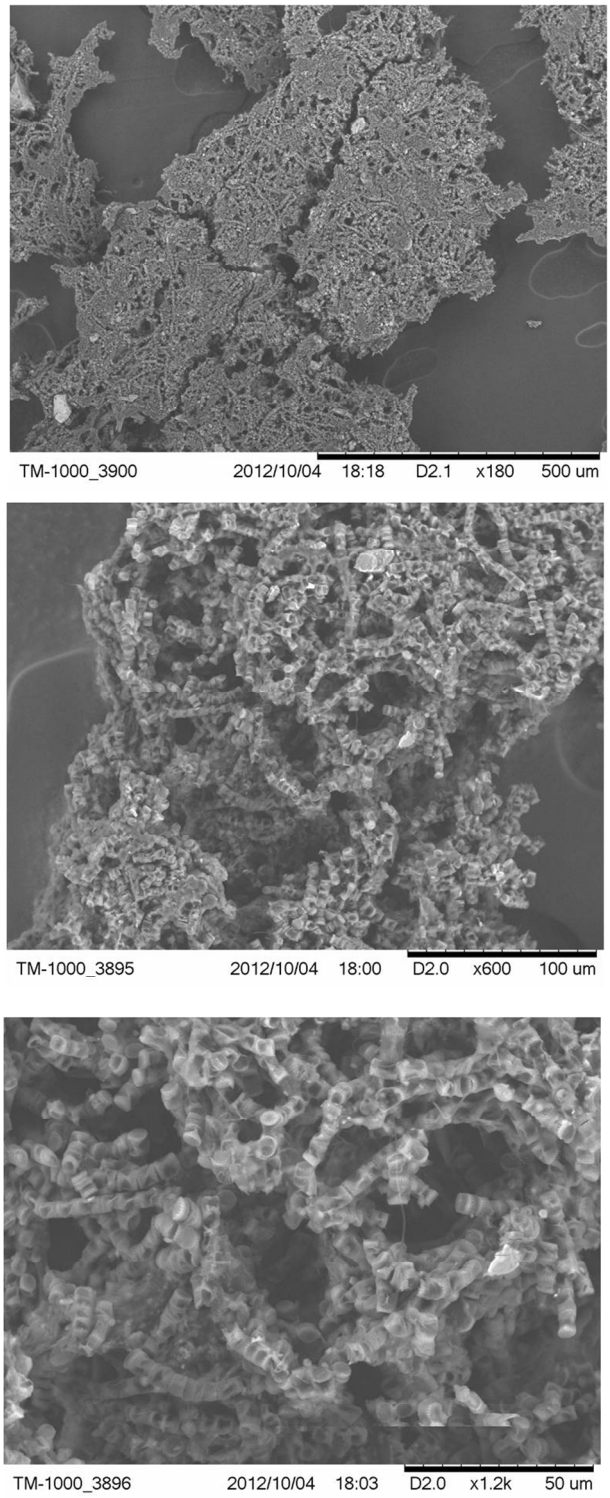

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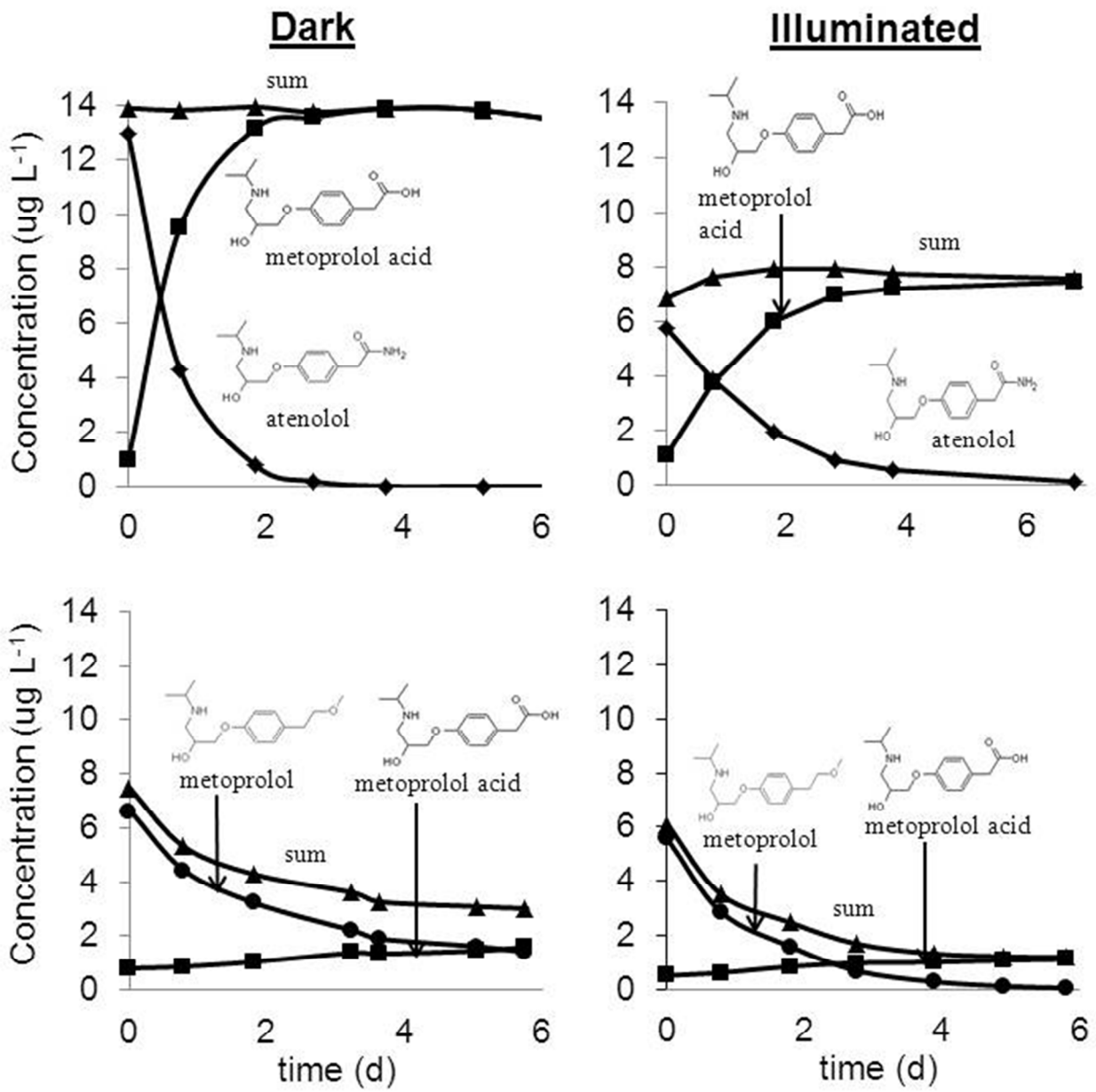
175 **Figure SI 6.** Scanning electron microscopy of wetland biomat at 180X (top), 600X (middle),

176 and 1200X (bottom) magnification.



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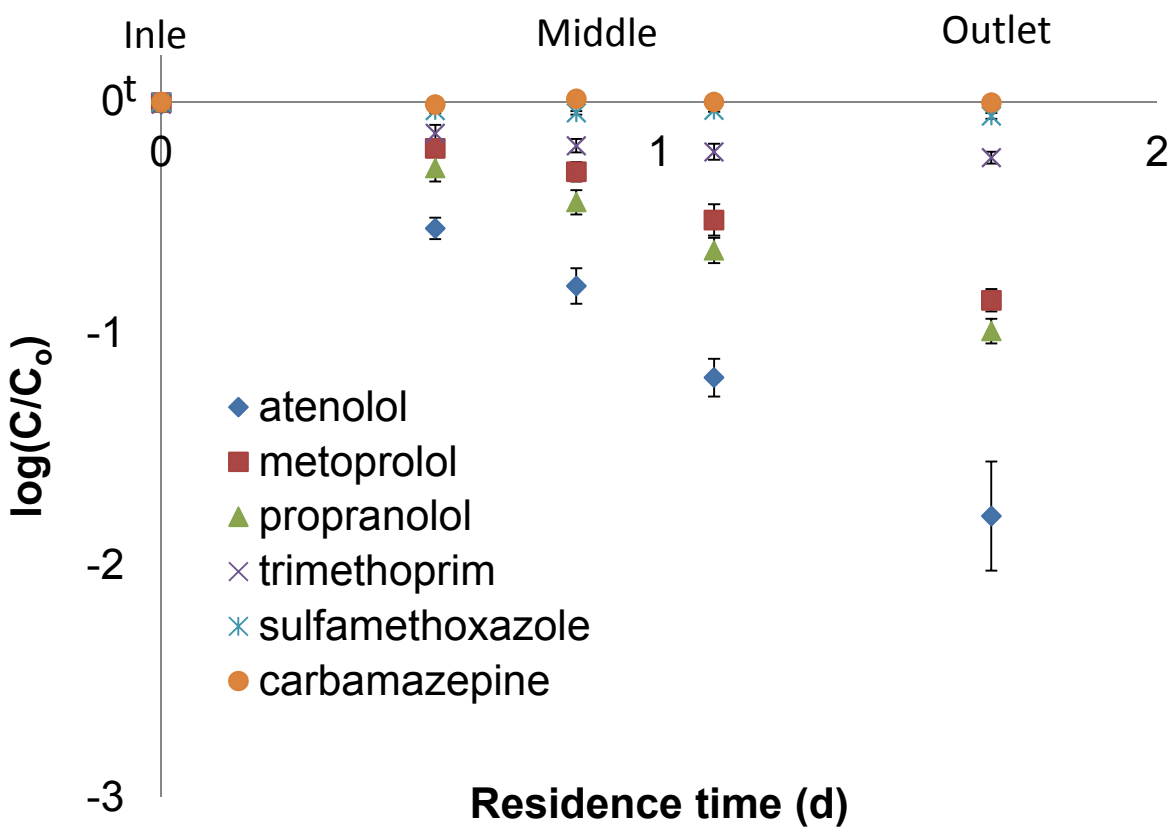
178 **Figure SI 7**. Formation of the biotransformation product metoprolol acid (■) in dark (left) and
179 illuminated (635 nm visible light; right) microcosms amended with either atenolol (top; ◆) or
180 metoprolol (bottom; ●). The sum of the concentrations of the parent products and the
181 transformation products are also shown (▲). Sorption accounted for loss of less than 1% of the
182 initial mass of atenolol and from 5% (illuminated microcosm) to 20% (dark microcosm) of the
183 initial concentration of metoprolol.



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185 **Figure SI 8**. Removal of test compounds in pilot-scale open-water wetland during August 2012.

186 Error bars represent ± standard error of the mean (n=10).

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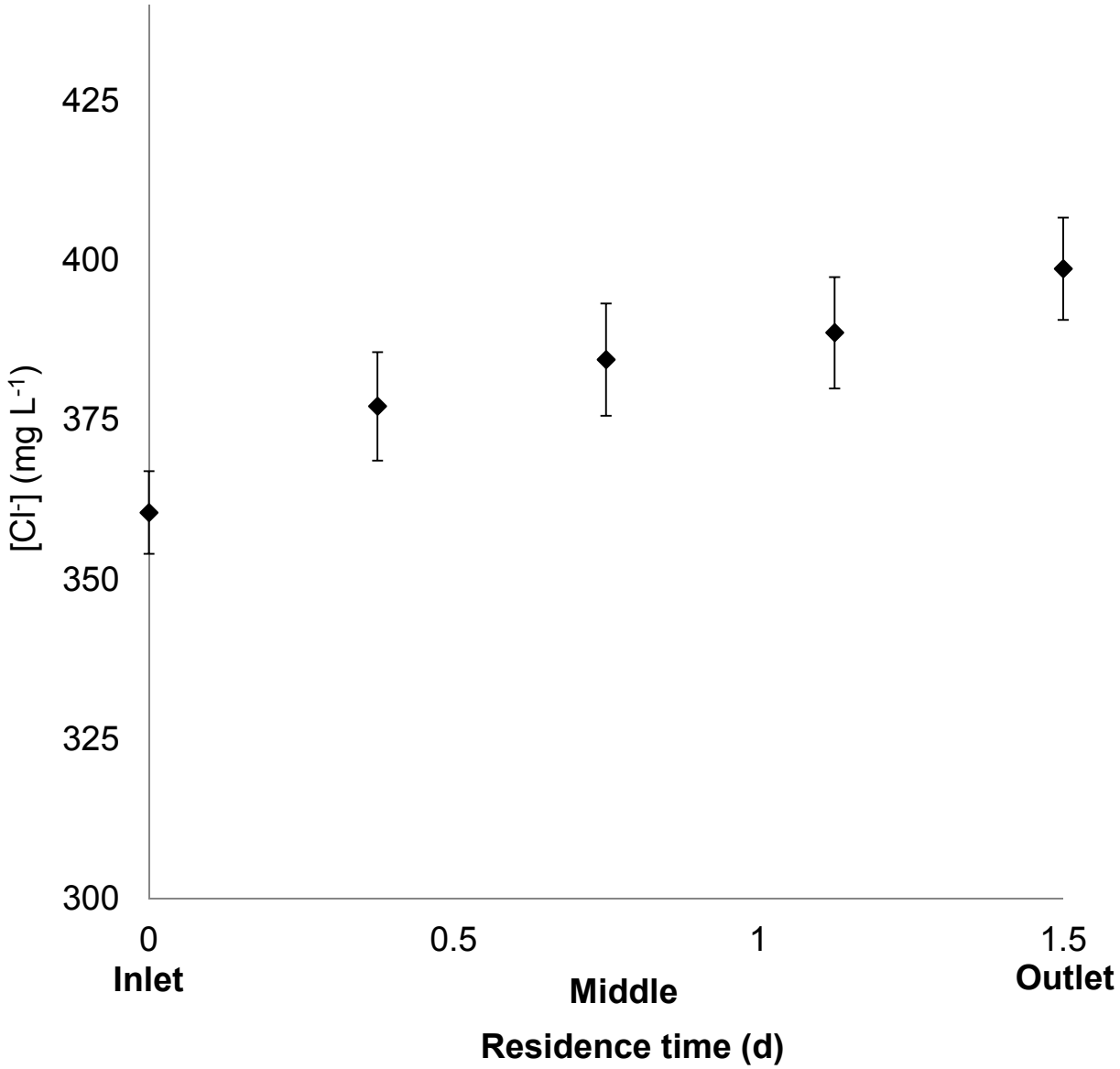

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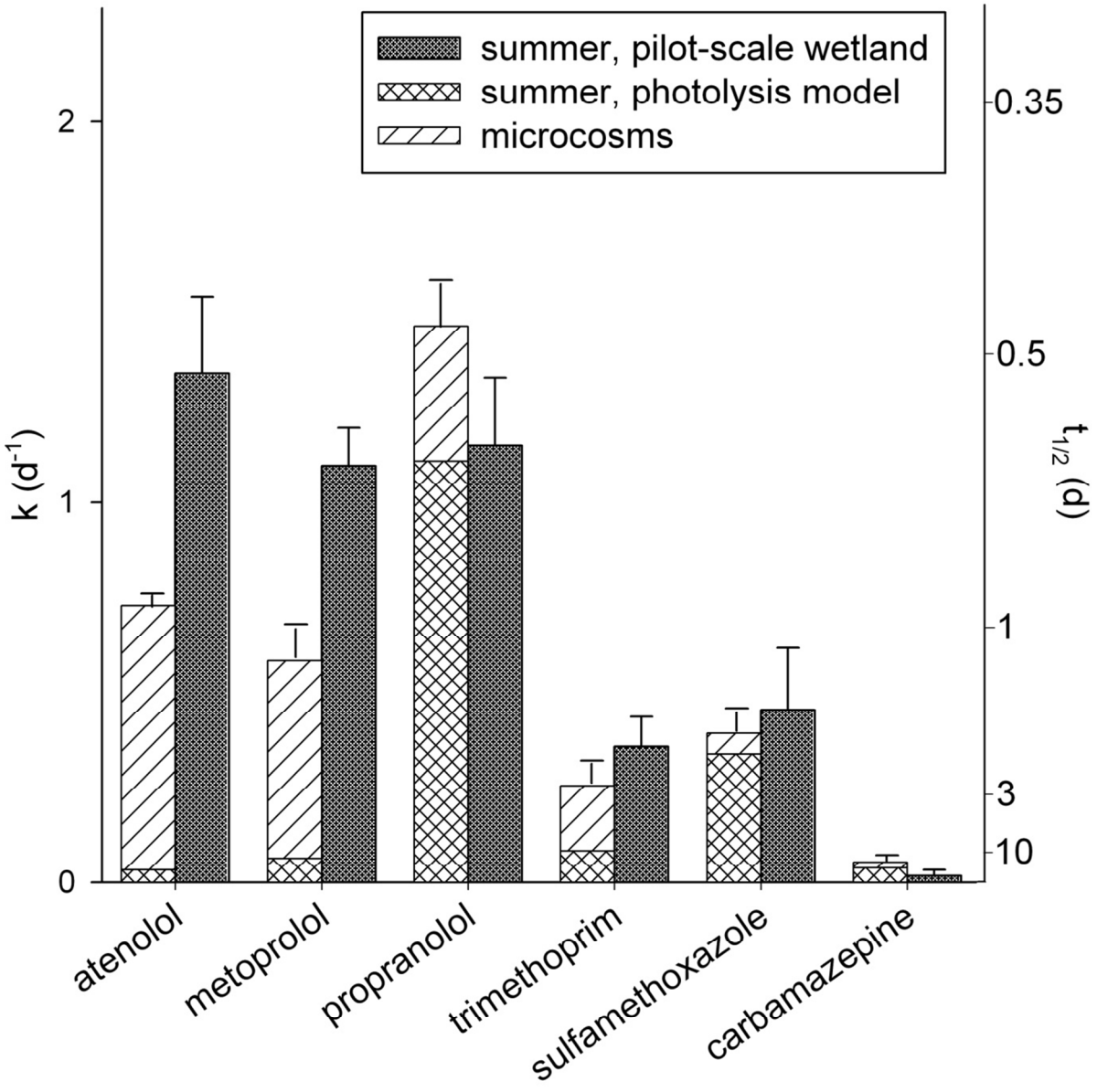
189

190 **Figure SI 9.** Average Cl^- concentrations throughout pilot-scale open-water cell during summer

191 2013. Error bars represent ± standard error of the mean.



192

193 **Figure SI 10.** Comparison of measured or predicted removal rates of test compounds to removal
194 rates observed in the Discovery Bay pilot-scale open-water wetland during summer, corrected
195 for evaporation. Photolysis rates were corrected assuming 25% of the hydraulic residence time
196 was spent in the biomat and that 7% of the water evaporated per day (see Figure SI 9). Error
197 bars represent ± one standard deviation for photolysis model and microcosms and ± standard
198 error of the mean for pilot-scale wetland.

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