Supporting Information

Periphyton biofilms influence net methylmercury production in an industrially contaminated system

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Quality assurance/quality control

Reference materials were analyzed to verify method performance. For ERM-CC580 methylmercury in estuarine sediment we measured 72.88 ng-MMHg/g versus the certified value of 75 ± 4 ng-MMHg/g and for IAEA-407 Fish Homogenate (methylmercury) we measured 0.192 µg-MMHg/g versus the certified 95% confidence interval of 0.188 – 0.212 ng-MMHg/g. Continuing calibration verification samples were ran at least every 30 samples (n = 11) with mean recovery 98 ±2%.

Sand used to disturb periphyton structure

Iota Standard quartz sand (Unimin Corp., New Canaan, CT), with a grain size range of 150-250 µm, was used; the sand had essentially no contamination from other primary or secondary minerals. The sand was soaked for at least 24 hours in 1 N HNO_3, then rinsed with reagent grade water until the rinse water had the same conductivity as reagent grade water. It was then baked at 550 °C in a muffle furnace for 16 hr.

Additional Site Description Details

Over the 12-month period beginning April 2014 the average stream discharge increased from 0.149 $m^3\ s^{-1}$ at the upstream sampling location to 1.35 $m^3\ s^{-1}$ at the downstream sampling location. Streamflow is higher in the winter and early spring and decreases in late-spring through mid-autumn due to increased evapotranspiration. Approximately 8 km upstream of our downstream sampling location the city of Oak Ridge wastewater treatment plant discharges treated effluent adding dissolved carbon, nitrate and phosphorous to EFPC. Nevertheless, the creek is meso- to eutrophic with respect to nitrogen and phosphorous along its entire length. The upper reach of EFPC is located in an urban environment. The downstream reach flows through residential, open-land, and forested areas. A comparison of the water chemistries between the two sites can be found in Table S2.

Periphyton MMHg flux assumptions and calculations

The methylation (k_m) and demethylation (k_d) rate potentials (d^{-1}) determined in the stable isotope assays were used, along with the bioavailable ambient Hg concentrations, to calculate the net methylation rate of periphyton (ng g-dw^{-1} d^{-1}; Figure 1C of the paper). This rate can be scaled up to estimate the flux of MMHg (mg d^{-1}) in EFPC due to periphyton activity.

$$Total\ MMHg\ Flux = \left(k_m[Hg^{II}_{bioav}] - k_d[MMHg_{bioav}]\right)(Total\ periphyton\ mass) \qquad \text{(S1)}$$

Hg^{II}_{bioav} is the ambient Hg^{II} concentration available for methylation and $MMHg_{bioav}$ is the ambient MMHg concentration available for demethylation. The total periphyton mass was estimated using the area density (calculated by taking the dry mass of the periphyton sample and dividing by the area of the disc to be 1 kg-dw m^{-2}), the total creek bed area and the percent of the creek bed covered by periphyton. Over the 18 km reach under consideration for this study, the average channel width is 12.6 m (inter-quartile range 9.93 – 13.7 m), assuming a triangular channel cross section with depth of 1 m the bed area = 229,639 m^2. Lacking any direct measurement, for the purposes of these calculations we adopted 60% as the percent of the bed covered by active periphyton. Additionally there is not a direct measure for the bioavailable Hg species concentrations. To estimate these values we used a percentage of the total ambient Hg and total ambient MMHg of the periphyton at the time of sampling. Since the percent availability of the Hg species may vary we used a range from 50% to 80%, along with the total periphyton mass, to estimate the MMHg flux attributed to periphyton activity.

The k_m and k_d from the February time series were used for these calculations. These rate potentials are from a full light assay from the downstream site. Since we measured differences in net methylation upstream versus downstream and light versus dark we wanted to try and account for these variables in our calculations. To do this we used three separate Eqn. S1's (downstream daytime; upstream daytime and nighttime) and summed them for the total periphyton MMHg flux. The upstream section is 7.2 km of the total 18 km and the downstream section is 10.8 km of the total 18 km; this division between upstream and downstream was based on watershed characteristics. The daytime was 10.5 h and the nighttime was 13.5 h. For the "downstream daytime" flux we took the MMHg flux (Eqn. S1) and multiplied it by 10.8 km/18 km to account for it being the downstream section and 10.5 h/24 h to account for it being during the daytime. Next, since we don't have a direct measure of the net methylation upstream for the February assay we used the ratio of the November upstream net methylation (0.223 ng g-dw^{-1} d^{-1}) divided by the November downstream net methylation (0.404 ng g-dw^{-1} d^{-1}) as a correction factor. We then multiplied the MMHg flux (Eqn. S1) by this factor as well as the daytime correction factor (10.5 h/24 h) and the upstream correction factor (7.2 km/18 km) to get the final "upstream daytime" flux. Finally for the "nighttime" flux we used the ratio of the dark May assay net methylation (0.035 ng g-dw^{-1} d^{-1}) divided by the light May assay net methylation (0.395 ng g-dw^{-1} d^{-1}) as a correction factor. We then multiplied the MMHg flux (Eqn. S1) by this factor as well as the nighttime correction factor (13.5 h/24 h) to get the final "nighttime" flux. The sum of the three parts (downstream daytime, upstream daytime and nighttime) gives the total flux. This calculation was done for a range of Hg species bioavailability (Figure S4). As a comparison the total MMHg flux in the water discharging from EFPC on the day we started the February assay was 14.8 mg d^{-1}.

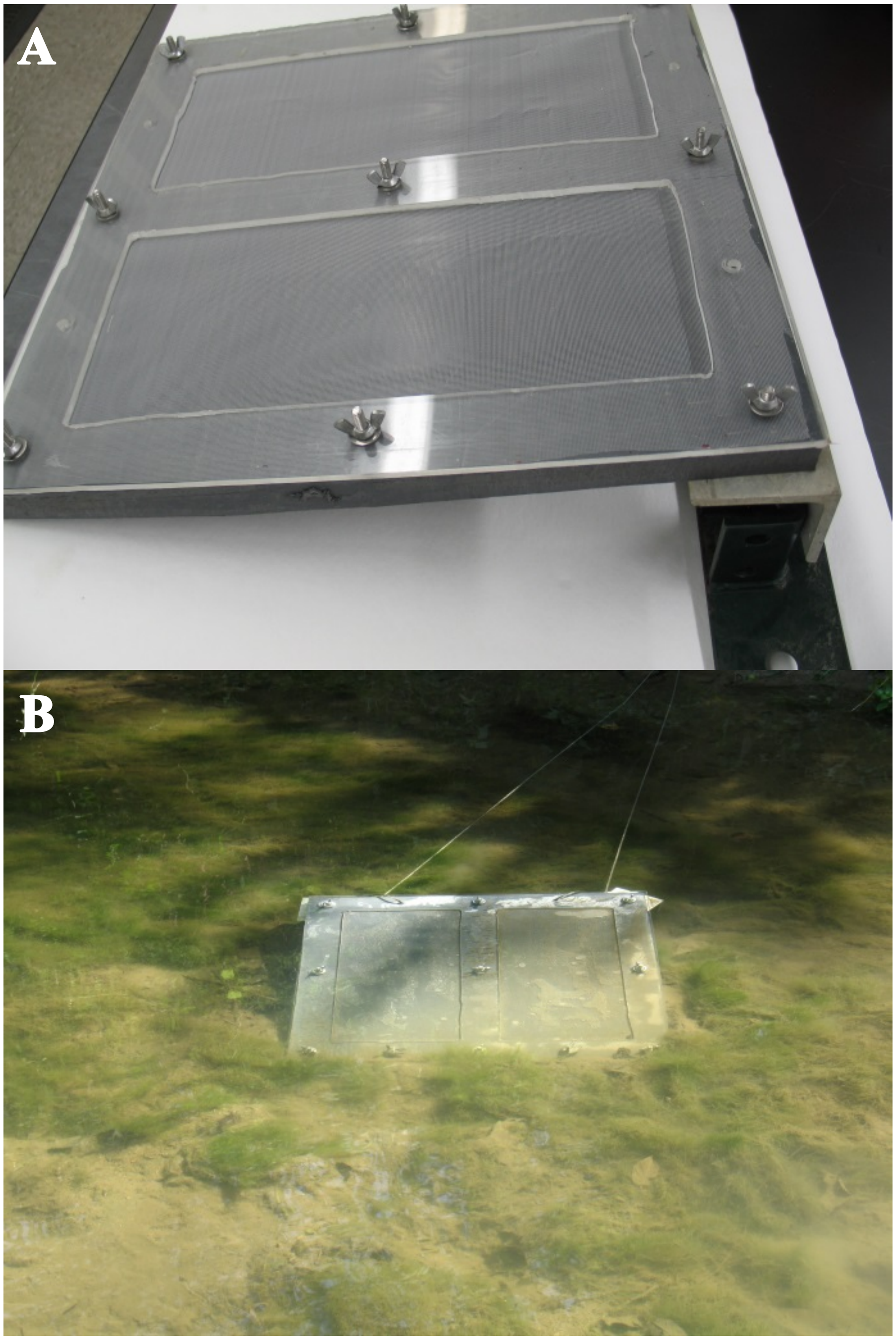

Figure S1. Periphyton growth structures before (A) and immediately after (B) stream deployment (9/19/2014) and illustrating the abundant periphyton growth on the creek bed. The structures consist of an 45.72 cm × 45.72 cm × 2.54 cm polypropylene base, an 45.72 cm × 45.72 cm × 0.635 cm frame with two 35.56 cm × 15.24 cm windows and an 45.72 cm × 45.72 cm polypropylene mesh sheet. The mesh sheet is sandwiched between the base and the frame so that two 35.56 cm × 15.24 cm sections of the mesh are exposed. The exposed sections are where the periphyton grew. The structure was secured in the creek with steel cables (B) and attached to rebar that was secured to the stream bank.

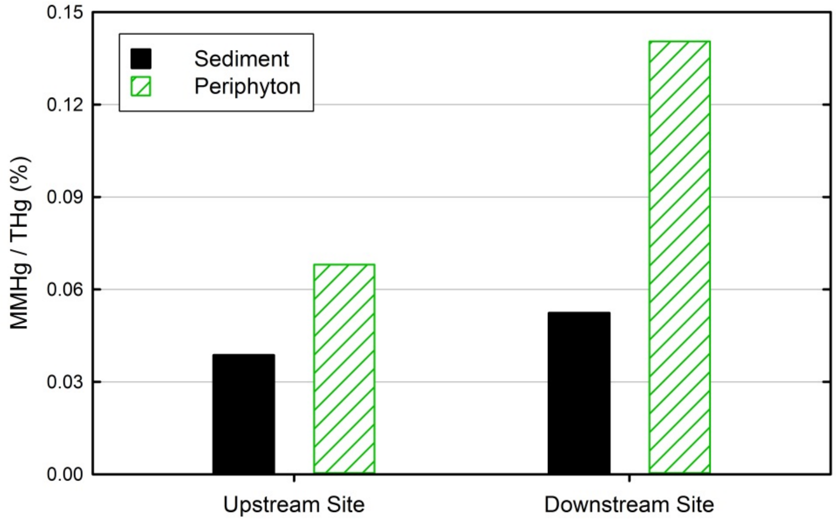

Figure S2. Longitudinal results from the upstream and downstream sites showing percent MMHg in sediment and periphyton. Each bar is the mean value of samples taken once a month from Sept. 2014 through Oct. 2015.

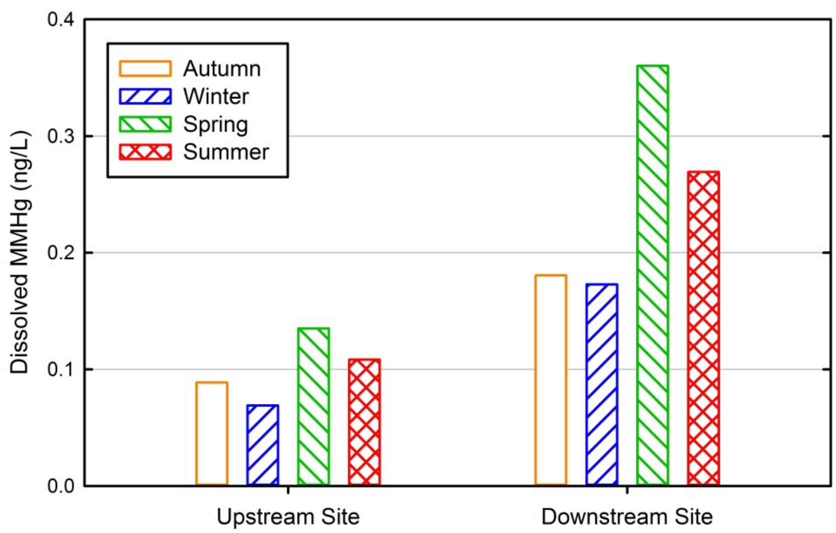

Figure S3. Dissolved MMHg concentrations in EFPC water column from the upstream and downstream sites. Each bar is the mean value of samples taken once a month from Sept. 2014 through Aug. 2015 separated by season.

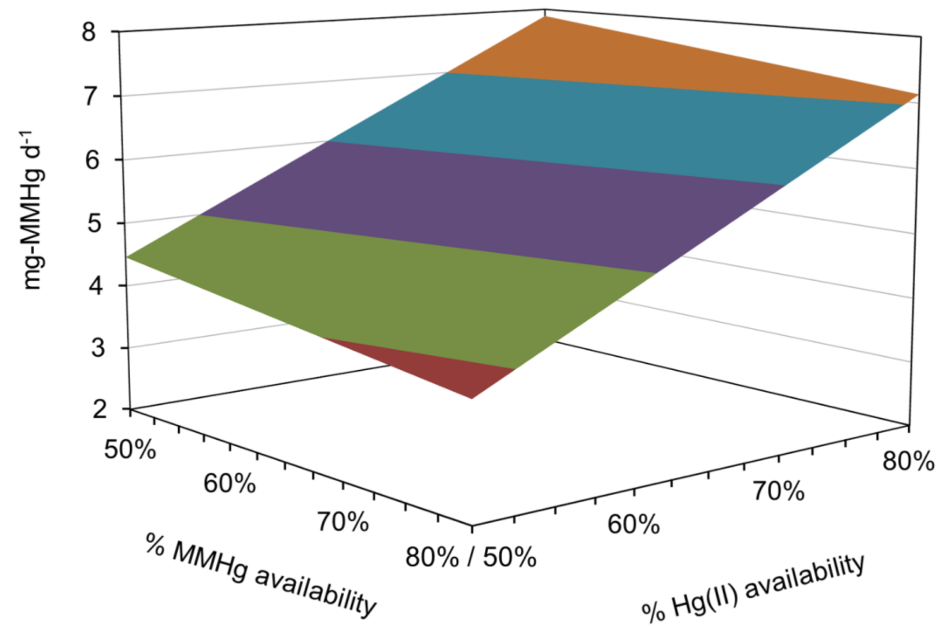

Figure S4. February 2015 time series showing the change in MMHg flux in EFPC that can be attributed to periphyton activity by varying the percent availability of the ambient MMHg and Hg^{II}. The space examined was held between 50% and 80% Hg species availability.

Table S1: Methylation and demethylation rate potentials intercomparison

Study	Location	Water Body	Temp	Length	Light exposure	Intact	Tracer type	% Ambient Tracer used	k_m	k_d	Notes
			°C	h					d^{-1}	d^{-1}	
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	193%/65.8%	4.99E-05	0.085	EFPC: Downstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	50.6%/131.1%	4.43E-05	0.171	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	10	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	14.5%/22.5%	3.02E-05	0.068	EFPC: Downstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	35.3%/42.7%	5.23E-05	0.072	EFPC: Downstream
This work	Tennessee	East Fork Poplar Creek	20	48	Dark	Yes	$^{201}HgCl_2/MM^{202}Hg$	35.3%/42.7%	2.15E-05	0.071	EFPC: Downstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	99.8%/45.6%	4.12E-05	0.221	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	No	$^{201}HgCl_2/MM^{202}Hg$	99.8%/45.6%	2.12E-05	0.208	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	10	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	48.9%/182%	3.54E-05	0.099	EFPC: Downstream
This work	Tennessee	East Fork Poplar Creek	10	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	66.3%/355%	3.18E-05	0.152	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	38.2%/143%	4.93E-05	0.261	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	20	48	Dark	Yes	$^{201}HgCl_2/MM^{202}Hg$	38.2%/143%	2.32E-05	0.236	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	No	$^{201}HgCl_2/MM^{202}Hg$	38.2%/143%	2.98E-05	0.197	EFPC: Upstream
This work	Tennessee	East Fork Poplar Creek	20	48	Light	Yes	$^{201}HgCl_2/MM^{202}Hg$	38.2%/143%	4.83E-05	0.273	EFPC: Upstream (rocks)
Desrosiers 2006	Quebec, Canada	Lake Croche	25	12	12 h light/12 h dark	Yes	$^{203}HgCl_2/^{194}HgNO_3$	3.09%	5.90E-04		
Desrosiers 2006	Quebec, Canada	Lake Croche	20	12	12 h light/12 h dark	Yes	$^{203}HgCl_2/^{194}HgNO_3$	2.11%	4.40E-04		
Desrosiers 2006	Quebec, Canada	Lake Croche	15	48	12 h light/12 h dark	Yes	$^{203}HgCl_2/^{194}HgNO_3$	1.27%	1.60E-05		
Hamelin 2011	Quebec, Canada	St. Lawrence River	21-23	48	Incubated in field	Yes	$^{199}HgO/MM^{200}Hg$	46.2%/2.1%	1.70E-03	0.187	
Acha 2011	Bolivia	Lake La Granja	30	12	Dark	Yes	$^{200}HgCl_2/MM^{202}Hg$	- ND -*	2.32E-04	0.178	macrophyte: *E. crassipes*
Acha 2011	Bolivia	Lake La Granja	30	12	Dark	Yes	$^{200}HgCl_2/MM^{202}Hg$	- ND -	3.45E-04	0.232	macrophyte: *P. densiflorum*
Cleckner 1999	Florida	Everglades	Ambient	3	Ambient Light	Yes	^{203}Hg	50%-300%	2.26E-02		Site F1 12/95
Cleckner 1999	Florida	Everglades	Ambient	3	Dark	Yes	^{203}Hg	50%-300%	2.32E-01		Site F1 12/95
Cleckner 1999	Florida	Everglades	Ambient	3	Ambient Light	Yes	^{203}Hg	50%-300%	2.01E-04		Site U3 12/95
Cleckner 1999	Florida	Everglades	Ambient	3	Dark	Yes	^{203}Hg	50%-300%	5.15E-04		Site U3 12/95
Cleckner 1999	Florida	Everglades	Ambient	3	Ambient Light	Yes	^{203}Hg	50%-300%	1.93E-02		Site F1 03/96
Cleckner 1999	Florida	Everglades	Ambient	3	Dark	Yes	^{203}Hg	50%-300%	4.15E-02		Site F1 03/96
Cleckner 1999	Florida	Everglades	Ambient	3	Ambient Light	Yes	^{203}Hg	50%-300%	1.98E-03		Site U3 03/96
Cleckner 1999	Florida	Everglades	Ambient	3	Dark	Yes	^{203}Hg	50%-300%	5.18E-03		Site U3 03/96
Correia 2012	Bolivia	Lake La Granja	22-28	48	Dark	No	$^{203}HgCl_2$	- ND -	1.81E-01		macrophyte: *L. helminthorriza*
Correia 2012	Bolivia	Viejo River	22-28	48	Dark	No	$^{203}HgCl_2$	- ND -	2.15E-02		macrophyte: *H. donacifolia*
Correia 2012	Bolivia	Viejo River	22-28	48	Dark	No	$^{203}HgCl_2$	- ND -	1.90E-02		macrophyte: *H. rotundifolia*
Correia 2012	Bolivia	La Granja	22-28	48	Dark	No	$^{203}HgCl_2$	- ND -	1.38E-01		macrophyte: *P. densiflorum*
Correia 2012	Bolivia	Salinas	22-28	48	Dark	No	$^{203}HgCl_2$	- ND -	1.00E-03		macrophyte: *P. densiflorum*
Huguet 2010	French Guiana	Petit-Saut Reservoir	30	168	Dark	No	$^{199}HgCl_2$	2000%	5.00E-05		Reservoir Depth: 6.5 m
Huguet 2010	French Guiana	Petit-Saut Reservoir	30	168	Dark	No	$^{199}HgCl_2$	2200%	7.00E-05		Reservoir Depth: 15 m
Huguet 2010	French Guiana	Petit-Saut Reservoir	30	168	Dark	No	$^{199}HgCl_2$	1700%	2.60E-04		Reservoir Depth: 20 m
Huguet 2010	French Guiana	Sinnamary Estuary	30	168	Dark	No	$^{199}HgCl_2$	490%	1.50E-03		Passerelle Station
Huguet 2010	French Guiana	Sinnamary Estuary	30	168	Dark	No	$^{199}HgCl_2$	21000%	4.00E-04		Venus Station
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{203}HgCl_2$	- ND -	7.67E-02		macrophyte: *S. rotundifolia*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{203}HgCl_2$	- ND -	1.03E-01		macrophyte: *P. stratiotes*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{203}HgCl_2$	- ND -	1.62E-01		macrophyte: *E. crassipes*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{203}HgCl_2$	- ND -	1.71E-01		macrophyte: *C. demersum*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{200}HgCl_2/MM^{199}Hg$	- ND -	1.91E-02	0.068	macrophyte: *S. rotundifolia*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{200}HgCl_2/MM^{199}Hg$	- ND -	4.31E-02	0.092	macrophyte: *P. stratiotes*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{200}HgCl_2/MM^{199}Hg$	- ND -	6.00E-02	0.187	macrophyte: *E. crassipes*
Mauro 2002	Florida	Everglades	30	24	Dark	Yes	$^{200}HgCl_2/MM^{199}Hg$	- ND -	7.32E-02	0.224	macrophyte: *C. demersum*

* ND : could not be determined with the information provided in the manuscript

Table S2: Ancillary water quality parameters measured at the upstream and downstream periphyton growth locations. Values represent the median (1st and 3rd quartiles) of 11 monthly samples collected between May 2014 and April 2015. The influence of the waste water treatment plant effluent is most evident in the elevated nitrate and soluble reactive phosphorous concentrations at the downstream location.

Parameter	**Units**	**Upstream Site**	**Downstream Site**
Chloride	mg L^{-1}	19.5 (18.6 - 25.1)	18.7 (12.9 - 23.9)
Dissolved Oxygen	mg L^{-1}	10.1 (8.74 - 10.9)	8.9 (7.3 - 11.1)
Dissolved Organic Carbon	mg L^{-1}	1.58 (1.34 - 2.34)	2.42 (1.66 - 2.80)
Ammonia	µg L^{-1}	18.8 (8.07 - 28.8)	20.8 (15.5 - 26.6)
Nitrate	mg L^{-1}	9.96 (9.76 - 10.7)	14.0 (11.9 - 16.9)
pH	SU	7.97 (7.48 - 8.10)	7.27 (7.02 - 7.66)
Specific Conductance	µS cm^{-1}	398 (354 - 407)	366 (299 - 422)
Soluble Reactive Phosphorous	µg L^{-1}	46.6 (24.7 - 64.3)	223 (47.0 - 398)
Sulfate	mg L^{-1}	33.5 (32.1 - 34.8)	21.9 (15.8 - 22.6)
Total Suspended Solids	mg L^{-1}	2.9 (2.3- 3.6)	5.6 (2.6 - 7.5)

Table S3: Matrix depicting treatment factors for which methylation and demethylation rate potentials were measured. Dates indicate when the samples were collected and assays initiated. Cell number in parentheses corresponds to the cell number listed Tables S5, S7, and S8.

	Location	**upstream**							
	Temperature	**20°C**				**10°C**			
	Exposure	**Light**		**Dark**		**Light**		**Dark**	
	Structure	**Intact**	**Disturbed**	**Intact**	**Disturbed**	**Intact**	**Disturbed**	**Intact**	**Disturbed**
Substrate	**PP Mesh**	(10) 11/24/14	(8) 9/15/15						
		(11) 9/15/15							
	Glass Frit					(6) 1/29/16			
	PP Sheet	(12) 6/9/16	(9) 6/9/16	(7) 6/9/16					
	Rock	(13) 6/9/16							

	Location	**downstream**							
	Temperature	**20°C**				**10°C**			
	Exposure	**Light**		**Dark**		**Light**		**Dark**	
	Structure	**Intact**	**Disturbed**	**Intact**	**Disturbed**	**Intact**	**Disturbed**	**Intact**	**Disturbed**
Substrate	**PP Mesh**	(4) 11/24/14		(3) 5/12/15		(1) 2/3/15			
		(5) 5/12/15							
	Glass Frit					(2) 1/29/16			
	PP Sheet								
	Rock								

PP = polypropylene

Table S4: Creek water temperatures for the 30-day period preceding assays.

Assay Date	**Median Temperature (°C) (1st and 3rd quartiles)**
November 2014	10.9 (8.8 – 12.5)
February 2015	7.9 (6.9 – 8.6)
May 2015	15.3 (14.6 – 16.1)
September 2015	23.1 (22.4 – 23.8)
January 2016	8.4 (6.5 – 9.7)
June 2016	19.6 (18.1 – 21.5)

Table S5: Treatment overview and results for net methylation assays. Cell number corresponds to cell number given in Tables S3, S7 and S8. For Location, US = upstream, DS = downstream.

Cell	Date	Substrate	Location	Temp (°C)	Exposure	Structure	Ambient MMHg (ng g-dw^{-1})	Ambient THg (ng g-dw^{-1})	k_m (d^{-1})	k_d (d^{-1})	Net (ng g-dw^{-1} d^{-1})
4	11/24/2014	mesh	DS	20	Light	Intact	3.36	13800	4.48E-05	8.64E-02	3.28E-01
									4.57E-05	8.20E-02	3.55E-01
									5.92E-05	8.62E-02	5.27E-01
10	11/24/2014	mesh	US	20	Light	Intact	2.62	15100	3.58E-05	1.50E-01	1.46E-01
									4.76E-05	1.64E-01	2.89E-01
									4.96E-05	1.97E-01	2.33E-01
1	2/3/2015	mesh	DS	10	Light	Intact	2.5	14200	2.98E-05	5.55E-02	2.85E-01
									3.75E-05	7.11E-02	3.54E-01
									2.32E-05	7.70E-02	1.37E-01
5	5/12/2015	mesh	DS	20	Light	Intact	3.09	11800	9.56E-05	8.99E-02	8.51E-01
									3.25E-05	6.26E-02	1.90E-01
									2.89E-05	6.40E-02	1.43E-01
3	5/12/2015	mesh	DS	20	Dark	Intact	3.09	11800	2.33E-05	6.98E-02	5.90E-02
									2.03E-05	6.84E-02	2.84E-02
									2.08E-05	7.34E-02	1.86E-02
11	9/15/2015	mesh	US	20	Light	Intact	1.85	13700	4.31E-05	2.17E-01	1.90E-01
									4.38E-05	2.44E-01	1.49E-01
									3.67E-05	2.03E-01	1.28E-01
8	9/15/2015	mesh	US	20	Light	Disturbed	1.85	13700	2.26E-05	2.29E-01	-1.13E-01
									2.06E-05	1.89E-01	-6.79E-02
									2.03E-05	2.06E-01	-1.03E-01
2	1/29/2016	frit	DS	10	Light	Intact	2.04	13289	3.58E-05	1.02E-01	2.68E-01
									3.93E-05	9.17E-02	3.35E-01
									3.10E-05	1.05E-01	1.98E-01
6	1/29/2016	frit	US	10	Light	Intact	1.40	13056	4.11E-05	1.46E-01	3.32E-01
									2.89E-05	1.52E-01	1.65E-01
									2.56E-05	1.61E-01	1.09E-01
12	6/9/2016	sheet	US	20	Light	Intact	3.12	22192	4.80E-05	2.68E-01	2.28E-01
									5.24E-05	2.43E-01	4.02E-01
									4.76E-05	2.73E-01	2.04E-01
7	6/9/2016	sheet	US	20	Dark	Intact	3.12	22192	2.95E-05	2.84E-01	-2.33E-01
									2.08E-05	2.21E-01	-2.31E-01
									1.92E-05	2.03E-01	-2.09E-01
9	6/9/2016	sheet	US	20	Light	Disturbed	3.12	22192	2.64E-05	1.97E-01	-2.97E-02
									3.27E-05	1.71E-01	1.90E-01
									3.05E-05	2.25E-01	-2.60E-02
13	6/9/2016	rock	US	20	Light	Intact	3.12	22192	4.33E-05	2.51E-01	1.79E-01
									5.43E-05	2.94E-01	2.87E-01
									4.72E-05	2.76E-01	1.84E-01

Table S6: Analysis of variance results from cell means model for k_m and k_d.

Source	DF	Sum of Squares	Mean Square	F Value	Prob > F	R^2
k_m						
Model	12	4.68×10^{-9}	3.90×10^{-10}	2.88	0.012	0.571
Error	26	3.53×10^{-9}	1.36×10^{-10}			
Corrected Total	38	8.21×10^{-9}				
k_d						
Model	12	0.210	0.0175	44.47	<0.001	0.953
Error	26	0.010	0.0004			
Corrected Total	38	0.220				

Table S7: Probabilities of pairwise cell differences. Differences with probabilities ≤ 0.05 are indicated in boldface. Loc. = location, DS = downstream, US = upstream. Tmp. = temperature. Exp. = light exposure treatment, LT = incubated in light, DK = incubated in dark. Str. = structure, IN = intact, DS = disturbed. Sub. = substrate, M = polypropylene mesh, F = glass frit, S = polypropylene sheet, R = rock from stream. Cell number corresponds to cell number indicated in Tables S3, S5, and S8.

Loc.	DS	DS	DS	DS	DS	US	US	US	US	US	US	US	US
Tmp.	10	10	20	20	20	10	20	20	20	20	20	20	20
Exp.	LT	LT	DK	LT	LT	LT	DK	LT	LT	LT	LT	LT	LT
Str.	IN	IN	IN	IN	IN	IN	IN	DS	DS	IN	IN	IN	IN
Sub.	M	F	M	M	M	F	S	M	S	M	M	S	R
Cell	1	2	3	4	5	6	7	8	9	10	11	12	13
k_m													
1		0.590	0.367	**0.048**	**0.028**	0.864	0.466	0.353	0.971	0.149	0.256	0.055	0.068
2			0.156	0.139	**0.086**	0.713	0.210	0.148	0.567	0.355	0.544	0.155	0.186
3				**0.006**	**0.003**	0.285	0.860	0.978	0.386	**0.024**	**0.048**	**0.007**	**0.009**
4					0.799	0.069	**0.009**	**0.006**	**0.045**	0.563	0.370	0.951	0.866
5						**0.040**	**0.005**	**0.003**	**0.026**	0.407	0.253	0.752	0.672
6							0.370	0.274	0.836	0.200	0.333	0.078	0.096
7								0.839	0.488	**0.035**	0.069	**0.011**	**0.014**
8									0.372	**0.022**	**0.045**	**0.007**	**0.009**
9										0.140	0.242	0.051	0.064
10											0.747	0.605	0.682
11												0.403	0.465
12													0.914
13													
k_d													
1		0.062	0.872	0.306	0.794	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
2			0.085	0.373	0.103	**0.003**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
3				0.385	0.920	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
4					0.442	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
5						**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
6							**<0.001**	**0.002**	**0.010**	0.287	**<0.001**	**<0.001**	**<0.001**
7								0.094	**0.025**	**<0.001**	0.360	0.133	0.029
8									0.526	**0.028**	0.428	**0.003**	**<0.001**
9										0.105	**0.160**	**<0.001**	**<0.001**
10											**0.004**	**<0.001**	**<0.001**
11												**0.020**	**0.003**
12													0.457
13													

Table S8: Results of the cell means analysis for methylation rate potential (k_m) and demethylation rate potential (k_d). The group identifier denotes those cells whose means are statistically indistinguishable at $p \leq 0.05$.

Cell	Mean	Group	Location[a]	Temperature	Exposure	Structure	Substrate[b]
k_m							
8	0.0000212	A	US	20	Light	Disturbed	mesh
3	0.0000215	A	DS	20	Dark	Intact	mesh
7	0.0000232	AB	US	20	Dark	Intact	sheet
9	0.0000298	ABC	US	20	Light	Disturbed	sheet
1	0.0000302	ABC	DS	10	Light	Intact	mesh
6	0.0000318	ABC	US	10	Light	Intact	frit
2	0.0000354	ABCD	DS	10	Light	Intact	frit
11	0.0000412	BCD	US	20	Light	Intact	mesh
10	0.0000443	CD	US	20	Light	Intact	mesh
13	0.0000483	CD	US	20	Light	Intact	rock
12	0.0000493	CD	US	20	Light	Intact	sheet
4	0.0000499	D	DS	20	Light	Intact	mesh
5	0.0000524	D	DS	20	Light	Intact	mesh
k_d							
1	0.0679	A	DS	10	Light	Intact	mesh
3	0.0705	A	DS	20	Dark	Intact	mesh
5	0.0722	A	DS	20	Light	Intact	mesh
4	0.0848	A	DS	20	Light	Intact	mesh
2	0.0995	A	DS	10	Light	Intact	frit
6	0.153	B	US	10	Light	Intact	frit
10	0.171	BC	US	20	Light	Intact	mesh
9	0.198	CD	US	20	Light	Disturbed	sheet
8	0.208	D	US	20	Light	Disturbed	mesh
11	0.221	DE	US	20	Light	Intact	mesh
7	0.236	EF	US	20	Dark	Intact	sheet
12	0.261	FG	US	20	Light	Intact	sheet
13	0.274	G	US	20	Light	Intact	rock

[a] US = upstream; DS = downstream
[b] mesh = polypropylene mesh; frit = glass frit; sheet = perforated polypropylene sheet; rock = rocks from the stream