

Electronic Supplementary Material

**Biogeochemical and physical controls on the microbial degradation of dissolved organic
matter along a temperate microtidal estuary**

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Table S1 Resulting Variance Inflation Factors (VIFs) and corrected Akaike Information Criteria (AICc) with additional variables included in the DOC and DON multiple linear regression models

DOC					
Variable Added:	Correlation (r^2) with Δ DOC (%)	VIF	Colinear With:	AICc	Model adj. r^2
chl- <i>a</i>	0.00	1.08	-	248.8	0.50
salinity	0.01	4.35	Freshwater Age	249.5	0.49
DO	0.24	35.5	Temperature, Freshwater Age	246.1	0.52
pH	0.00	3.28	-	249.4	0.49
[DOC] _{initial}	0.27	2.62	-	249.3	0.49
[DIC] _{initial}	0.10	2.73	-	249.5	0.49
[DON] _{initial}	0.17	13.0	Temperature, Freshwater Age	177.3	0.16
DOC:DON	0.17	2.33	Temperature	176.5	0.19
[NH ₄ ⁺] _{initial}	0.20	1.60	-	248.4	0.51
[NO _x] _{initial}	0.09	1.79	-	223.3	0.46
<i>a</i> ₄₄₀ _{initial}	0.00	2.69	-	249.4	0.49
[CDOM _{protein-like}] _{initial}	0.22	1.97	-	249.5	0.49
[CDOM _{humic-like}] _{initial}	0.11	1.55	-	246.3	0.54
DON					
Variable Added:	Correlation (r^2) with Δ DON (%)	VIF	Colinear With:	AICc	Model adj. r^2
salinity	0.00	7.69	Flushing Time, pH	140.8	0.85
DO	0.01	6.36	Flushing Time	140.2	0.85
[DIC] _{initial}	0.03	3.74	Flushing Time, pH	140.7	0.85
[DON] _{initial}	0.17	9.08	Flushing Time	138.0	0.87
DOC:DON	0.10	4.64	Flushing Time	138.8	0.86
[NO _x] _{initial}	0.02	5.16	Flushing Time	140.7	0.85
<i>a</i> ₄₄₀ _{initial}	0.00	5.83	Flushing Time, pH	138.7	0.86
$\delta^{13}\text{C}_{\text{DOC}}$ (‰)	0.02	13.6	Flushing Time, Protein-like CDOM, Humic-like CDOM	140.7	0.85
Temperature (°C)	0.08	4.19	Flushing Time	140.2	0.85
Freshwater Age (d)	0.07	5.27	Flushing Time, pH	140.6	0.85

Table S2 Initial concentrations and values of biogeochemical variables included in regression modelling analyses. Numbers in parentheses are ± 1 standard error of the mean of triplicate measurements. n.d. = no data

Month	Location along estuary	chl- <i>a</i> ($\mu\text{g L}^{-1}$)	DOC:DON	[DIC] (mM)	[NO _x] (μM)	[NH ₄ ⁺] (μM)	<i>a</i> ₄₄₀ (m ⁻¹)	<i>a</i> * ₄₄₀ (m ⁻² g ⁻¹)	$\delta^{13}\text{C}_{\text{DOC}}$ (‰)	Slope Ratio (S _R)
October	upper	20.4 (1.78)	n.d.	1.10 (0.09)	15.1 (0.27)	1.37 (0.20)	2.69 (0.23)	0.475 (0.063)	-27.3 (1.12)	1.09 (0.02)
	middle	27.4 (0.63)	n.d.	1.48 (0.02)	11.0 (0.44)	0.89 (0.19)	1.50 (0.05)	0.178 (0.005)	-25.3 (1.15)	1.16 (0.02)
	lower	10.5 (3.28)	n.d.	1.51 (0.02)	11.4	0.55 (0.08)	0.554 (0.103)	0.206 (0.069)	-24.7 (1.74)	1.18 (0.02)
February	upper	8.17 (0.21)	28.4 (0.7)	0.76 (0.00)	10.1(0.08)	0.49 (0.09)	0.711 (0.199)	0.168 (0.050)	-28.7 (0.04)	0.96 (0.06)
	middle	13.3 (0.44)	34.0 (5.1)	1.06 (0.00)	19.2 (0.01)	3.51 (0.06)	1.45 (0.12)	0.241 (0.002)	-29.0 (0.02)	1.12 (0.00)
	lower	23.9 (1.21)	37.0 (0.7)	1.25 (0.00)	34.5 (0.08)	7.93 (0.01)	1.20 (0.04)	0.315 (0.029)	-30.3 (0.02)	0.97 (0.03)
April	upper	4.59 (0.46)	28.7 (2.2)	0.86 (0.00)	17.0 (0.05)	10.6 (0.02)	1.18 (0.15)	0.265 (0.045)	-29.9 (0.30)	0.83 (0.02)
	middle	13.7 (0.57)	22.1 (0.8)	0.98 (0.00)	0.43 (0.09)	1.42 (0.51)	1.12 (0.03)	0.259 (0.016)	-28.2 (0.11)	0.81 (0.07)
	lower	59.8 (8.32)	25.4 (2.1)	1.17 (0.00)	3.70 (0.39)	2.12 (0.21)	0.599 (0.214)	0.128 (0.036)	-27.2 (0.68)	0.83 (0.04)
July	upper	39.3 (2.77)	18.4 (0.19)	1.22 (0.07)	0.44 (0.01)	3.94 (0.03)	2.06 (0.12)	0.411 (0.012)	-27.7 (0.61)	1.04 (0.01)
	middle	20.9 (3.12)	18.2 (0.34)	1.41 (0.07)	0.16 (0.03)	0.65 (0.08)	1.56 (0.20)	0.331 (0.043)	-25.9 (0.96)	1.10 (0.04)
	lower	9.58 (0.57)	22.7 (2.0)	1.43 (0.07)	0.18 (0.03)	0.79 (0.15)	0.377 (0.013)	0.077 (0.007)	-25.9 (1.16)	1.13 (0.03)

Table S3 Initial and final DOC and DON concentrations and CDOM fluorescence during 28d incubation experiments. Numbers in parentheses are ± 1 standard error of the mean of triplicate measurements. Bold values indicate significant differences ($p < 0.05$) between initial and final concentrations. n.d. = no data

Month	Location along estuary	DOC (μM)		DON (μM)		Total CDOM (RU)		Protein-like CDOM (RU)		Humic-like CDOM (RU)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
October	upper	684.7	444.1			0.593	0.493	0.108	0.057	0.486	0.436
		(50.3)	(11.5)	n.d.	n.d.	(0.026)	(0.075)	(0.012)	(0.013)	(0.037)	(0.088)
	middle	692.1	462.3			0.460	0.327	0.130	0.037	0.329	0.290
		(45.2)	(93.0)	n.d.	n.d.	(0.008)	(0.140)	(0.007)	(0.020)	(0.002)	(0.122)
February	upper	582.0	336.7			0.341	0.291	0.155	0.052	0.186	0.239
		(150.3)	(90.8)	n.d.	n.d.	(0.002)	(0.039)	(0.013)	(0.022)	(0.011)	(0.019)
	middle	319.7	299.2	12.5	11.3	0.451	0.221	0.055	0.023	0.396	0.198
		(44.1)	(37.4)	(0.6)	(0.4)	(0.228)	(0.003)	(0.017)	(0.002)	(0.211)	(0.001)
April	upper	435.6	447.7	14.9	12.4	0.360	0.375	0.025	0.088	0.335	0.288
		(112.8)	(114.0)	(0.9)	(0.2)	(0.067)	(0.036)	(0.024)	(0.029)	(0.091)	(0.007)
	middle	320.6	360.8	8.66	9.09	0.667	0.397	0.082	0.047	0.585	0.350
		(19.0)	(52.1)	(0.34)	(0.09)	(0.302)	(0.012)	(0.030)	(0.014)	(0.272)	(0.002)
July	upper	373.0	336.4	13.0	15.5	0.649	0.379	0.075	0.038	0.574	0.342
		(17.8)	(14.8)	(0.37)	(0.11)	(0.320)	(0.004)	(0.044)	(0.006)	(0.275)	(0.002)
	middle	359.9	311.7	16.3	15.6	0.565	0.355	0.083	0.089	0.482	0.266
		(12.0)	(4.38)	(0.04)	(0.93)	(0.314)	(0.047)	(0.050)	(0.044)	(0.263)	(0.003)
July	upper	380.9	318.6	15.0	18.9	0.232	0.247	0.065	0.056	0.167	0.191
		(31.4)	(6.81)	(0.02)	(0.95)	(0.005)	(0.008)	(0.002)	(0.008)	(0.004)	(0.00)
	middle	422.5	398.1	22.7	20.5	0.478	0.184	0.070	0.046	0.408	0.139
		(11.8)	(9.72)	(0.48)	(0.25)	(0.017)	(0.007)	(0.003)	(0.007)	(0.015)	(0.002)
July	upper	392.2	281.1	21.5	18.8	0.428	0.400	0.139	0.066	0.288	0.334
		(6.73)	(4.19)	(0.22)	(0.96)	(0.041)	(0.015)	(0.026)	(0.008)	(0.015)	(0.006)
	middle	418.0	347.1	18.1	16.1	0.190	0.563	0.084	0.046	0.106	0.517
		(54.2)	(47.9)	(0.70)	(0.54)	(0.007)	(0.009)	(0.007)	(0.005)	(0.00)	(0.004)

Table S4 ANOVA results for determining spatiotemporal differences in initial biogeochemical conditions and DOC, DON, and CDOM degradation along the YRE. Statistically significant results ($p < 0.05$) are bolded

Variable/Interactions	Date		Location		Date * Location	
df	3		2		6	
Variable	F	p	F	p	F	p
log(chl- <i>a</i>)	3.65	0.03	7.72	< 0.01	44.0	< 0.01
log([DOC])	15.0	< 0.01	0.64	0.54	0.70	0.65
[DON]	246	< 0.01	39.3	< 0.01	14.9	< 0.01
log(DOC:DON)	12.53	< 0.01	3.37	0.07	1.40	0.29
a_{440}	9.50	< 0.01	57.1	< 0.01	14.8	< 0.01
a^*_{440}	1.58	0.23	18.3	< 0.01	8.70	< 0.01
[DIC]	37.7	< 0.01	42.8	< 0.01	1.98	0.12
log([NO _x])	984	< 0.01	86.0	< 0.01	64.7	< 0.01
log([NH ₄ ⁺])	36.4	< 0.01	19.1	< 0.01	41.6	< 0.01
$\delta^{13}\text{C}_{\text{DOC}}$	6.94	< 0.01	2.58	0.10	0.78	0.60
[CDOM] _{protein-like}	7.67	< 0.01	1.55	0.24	1.86	0.14
log([CDOM] _{terrestrial humic-like})	2.22	0.12	11.0	< 0.01	2.25	0.09
log([CDOM] _M)	2.14	0.13	9.89	< 0.01	1.79	0.16
Slope Ratio	58.9	< 0.01	5.56	0.01	2.41	0.07
ΔDOC	9.95	< 0.01	0.44	0.65	1.00	0.45
ΔDON	42.6	< 0.01	15.6	< 0.01	5.88	0.01
$\Delta\text{CDOM}_{\text{total}}$	0.495	0.69	1.19	0.33	0.584	0.71
$\Delta\text{CDOM}_{\text{protein-like}}$	1.57	0.24	1.23	0.32	1.55	0.24
$\Delta\text{CDOM}_{\text{humic-like}}$	0.699	0.57	2.55	0.11	1.06	0.42

Table S5 Selected results (instances when $p < 0.05$) from Tukey's Honest Significant Difference test for multiple comparisons following two-way ANOVAs conducted on measured variables. n.a. = not applicable

Variable	Date	p	Location	p	Date*Location	p
log(chl- <i>a</i>)	July - February	0.02	upper - middle	0.01	February, upper - February, lower	< 0.01
			upper - lower	< 0.01	April, upper - April, lower	< 0.01
					July, upper - July, lower	< 0.01
					October, middle - October, lower	< 0.01
log([DOC])	October - February	< 0.01				
	October - April	< 0.01	n.a.	n.a.	n.a.	n.a.
	October - July	< 0.01				
[DON]	February - July	< 0.01	upper - middle	0.049		
	April - February	< 0.01	upper - lower	< 0.01	n.a.	n.a.
	April - July	< 0.01	middle - lower	< 0.01		
log(DOC:DON)	February - July	< 0.01				
	April - July	0.03	n.a.	n.a.	n.a.	n.a.
a ₄₄₀	October - February	< 0.01	upper - middle	0.01		
	October - April	< 0.01	upper - lower	< 0.01	n.a.	n.a.
	April - July	0.04	middle - lower	< 0.01		
a* ₄₄₀			upper - middle	< 0.01		
	n.a.	n.a.	upper - lower	< 0.01	n.a.	n.a.
			middle - lower	0.04		
[DIC]	October - February	< 0.01	upper - middle	< 0.01		
	October - April	< 0.01	upper - lower	< 0.01		
	February - July	< 0.01			n.a.	n.a.
	April - July	< 0.01				
log([NO _x])	October - February	< 0.01	upper - middle	< 0.01		
	October - April	< 0.01	upper - lower	< 0.01		
	October - July	< 0.01	middle - lower	< 0.01		
	February - April	< 0.01				
	February - July	< 0.01				

	log([NH ₄ ⁺])	April - July	< 0.01				
		October - February	< 0.01	upper - middle	< 0.01		
		October - April	< 0.01	upper - lower	< 0.01		
		October - July	0.03				
		February - July	< 0.01				
		April - July	< 0.01				
	$\delta^{13}\text{C}_{\text{DOC}}$	October - April	0.03				
		October - February	< 0.01	n.a.	n.a.	n.a.	n.a.
		February - July	0.03				
	[CDOM] _{protein-like}	October - February	< 0.01	n.a.	n.a.	n.a.	n.a.
		October - April	0.01				
	log([CDOM] _{terrestrial humic-like})	n.a.	n.a.	upper - lower middle - lower	< 0.01 0.01	n.a.	n.a.
	Slope Ratio	October - February	< 0.01	upper - middle	0.02		
		October - April	< 0.01	upper - lower	0.046		
		February - April	< 0.01			n.a.	n.a.
		April - July	< 0.01				
	ΔDOC	October - February	< 0.01				
		October - July	0.04	n.a.	n.a.	n.a.	n.a.
	ΔDON	February - April	< 0.01	upper - middle	0.01		
		April - July	< 0.01	middle - lower	< 0.01	n.a.	n.a.
	$\Delta\text{CDOM}_{\text{total}}$	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	$\Delta\text{CDOM}_{\text{protein-like}}$	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	$\Delta\text{CDOM}_{\text{humic-like}}$	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table S6 Bulk and relative loss of DOC, DON, and CDOM during 28d incubation experiments. Numbers in parentheses are ± 1 standard error of the mean of triplicate measurements. Bold values indicate when changes in percent and absolute concentration (DOC and DON) and fluorescence (CDOM) were significantly different ($p < 0.05$) from zero. n.d. = no data

Month	Location along estuary	Δ DOC (μ M)	Δ DOC (%)	Δ DON (μ M)	Δ DON (%)	Δ CDOM _{total} (RU)	Δ CDOM _{total} (%)	Δ CDOM _{protein-like} (RU)	Δ CDOM _{protein-like} (%)	Δ CDOM _{humic-like} (RU)	Δ CDOM _{humic-like} (%)
October	upper	241 (60)	-34.1 (6.82)	n.d.	n.d.	0.133 (0.084)	-15.8 (10.5)	0.051 (0.001)		0.050 (0.051)	-12.1 (12.2)
		280 (78)	-33.7 (12.1)	n.d.	n.d.	0.155 (0.215)	-22.8 (31.8)	0.093 (0.021)		0.039 (0.124)	-11.5 (37.8)
	lower	245 (90)	-41.2 (11.7)	n.d.	n.d.	0.013 (0.053)	-2.64 (11.4)	0.103 (0.009)		-0.053 (0.029)	30.2 (17.7)
February	upper	20.5 (6.6)	-6.24 (1.22)	-1.24 (0.22)	-9.87 (1.29)	0.396 (0.401)	-34.0 (35.5)	0.032 (0.015)		0.199 (0.211)	-30.5 (37.0)
		-12.1 (1.2)	2.90 (0.48)	-2.51 (0.70)	-16.6 (3.72)	0.054 (0.213)	0.78 (35.1)	-0.063 (0.005)		0.047 (0.097)	-6.81 (27.2)
	lower	-40.2 (71.1)	13.9 (23.0)	0.43 (0.25)	5.07 (3.13)	0.395 (0.460)	-23.7 (35.6)	0.035 (0.044)		0.235 (0.270)	-23.9 (35.1)
April	upper	36.6 (3.0)	-13.9 (0.20)	2.46 (0.47)	19.0 (4.17)	0.402 (0.475)	-23.0 (37.3)	0.038 (0.050)		0.232 (0.274)	-22.9 (36.8)
		48.3 (16.3)	-19.1 (5.65)	-0.70 (0.97)	-4.27 (5.95)	0.359 (0.541)	-9.43 (56.3)	-0.006 (0.094)		0.216 (0.266)	-21.0 (43.7)
	lower	62.3 (38.3)	-21.7 (11.6)	3.95 (0.93)	26.3 (6.17)	-0.033 (0.004)	9.67 (0.92)	0.009 (0.007)		-0.024 (0.004)	14.3 (2.54)
July	upper	24.4 (5.5)	-5.73 (1.19)	-2.22 (0.37)	-9.74 (1.50)	0.455 (0.031)	-62.1 (2.09)	0.025 (0.009)		0.269 (0.015)	-65.9 (1.44)
		111 (10)	-28.3 (2.06)	-2.71 (0.74)	-12.7 (3.54)	-0.002 (0.036)	1.13 (5.66)	0.073 (0.021)		-0.046 (0.009)	16.4 (3.9)
	lower	70.9 (16.7)	-17.0 (3.96)	-2.03 (0.31)	-11.1 (1.52)	-0.626 (0.010)	197 (10)	0.037 (0.006)		-0.410 (0.004)	386 (5)

Table S7 Initial and final concentrations of DOC and DON during 28d incubations of control treatments (0.2µm filtrate only). Numbers in parentheses are $\pm$ 1 standard error of the mean of triplicate measurements. n.d. = no data

Month	Location along estuary	DOC (µM)		DON (µM)	
		<u>Initial</u>	<u>Final</u>	<u>Initial</u>	<u>Final</u>
October	upper	479.1 (28.7)	475.2 (59.3)	n.d.	n.d.
	middle	536.1 (14.9)	553.0 (48.3)	n.d.	n.d.
	lower	324.3 (77.5)	368.5 (91.2)	n.d.	n.d.
February	upper	299.7 (67.8)	296.9 (56.7)	13.2 (1.8)	12.2 (1.3)
	middle	430.2 (56.8)	428.4 (92.8)	15.7 (1.6)	14.7 (2.7)
	lower	361.8 (52.7)	360.4 (55.1)	10.8 (2.3)	12.1 (1.1)
April	upper	391.3 (34.4)	388.4 (40.6)	16.3 (0.4)	20.3 (3.4)
	middle	368.1 (29.6)	357.3 (46.3)	17.5 (0.5)	15.8 (0.4)
	lower	435.7 (43.5)	445.4 (65.8)	21.5 (0.4)	17.2 (1.0)
July	upper	372.3 (30.3)	377.5 (26.7)	20.2 (0.3)	21.5 (1.5)
	middle	315.9 (20.8)	320.9 (33.6)	22.1 (1.1)	21.6 (0.7)
	lower	379.8 (42.1)	374.4. (17.8)	13.7 (2.1)	14.4 (1.9)

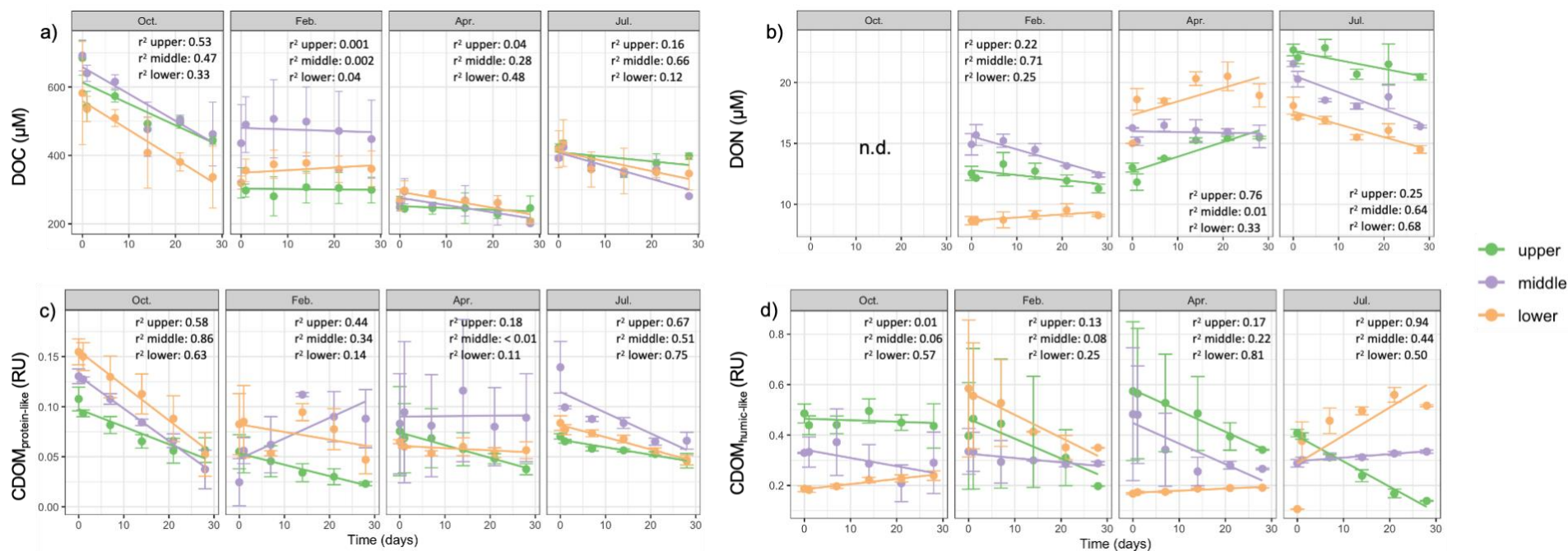


Fig. S1 Changes in a) DOC and b) DON concentrations and c) protein-like CDOM and d) humic-like CDOM fluorescence over 28-day dark incubations of surface water collected from the upper, middle, and lower of the YRE during October 2018 and February, April, and July 2019. Concentrations were measured at the onset of each incubation (0 days) and again after 1, 7, 14, 21, and 28 days. Error bars are standard errors of the mean of triplicate samples. n.d. = no data

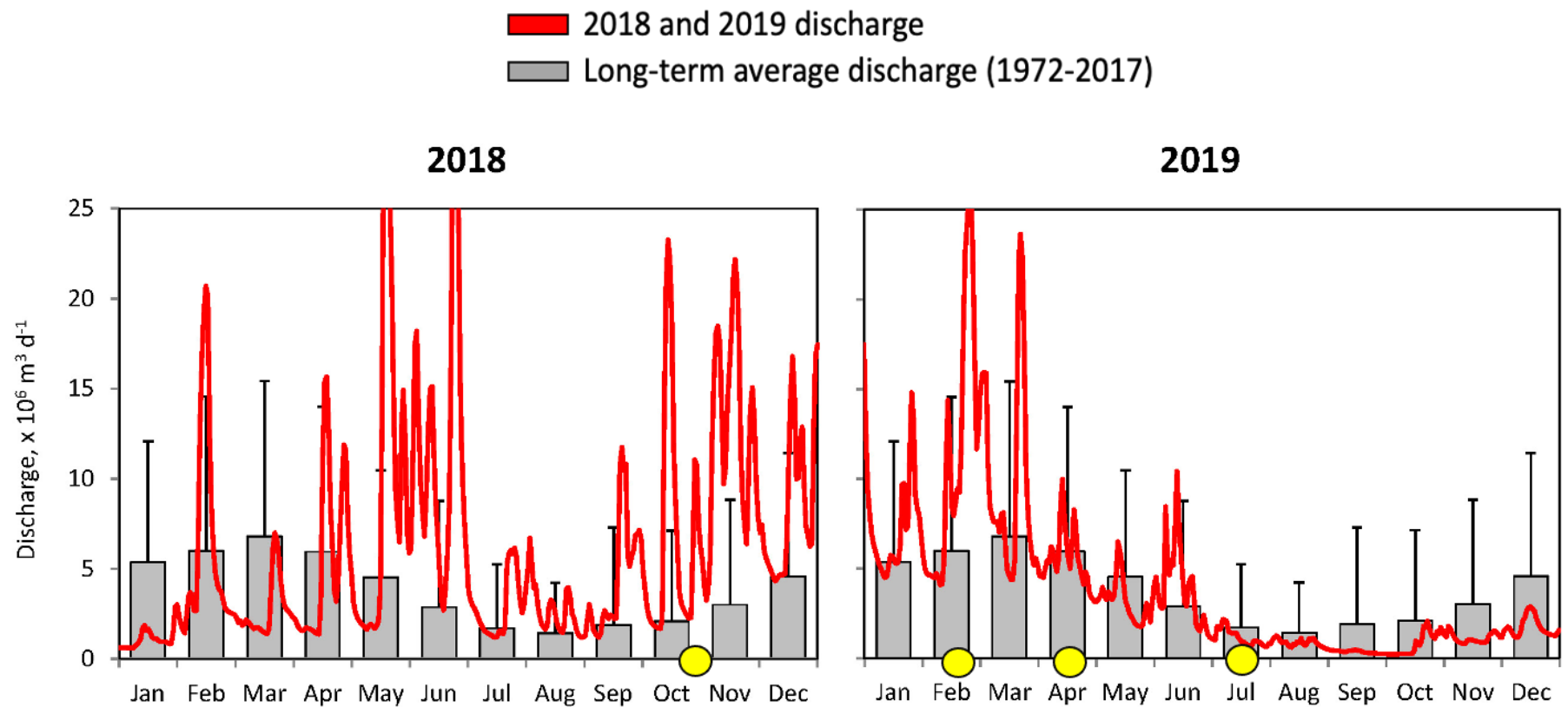


Fig. S2 Freshwater discharge to the YRE from the Pamunkey and Mattaponi Rivers. 2018-2019 daily discharge data is represented by the red line while the long-term monthly average discharges from 1972 to 2017 are represented by the gray bars (± 2 standard deviations). Sampling dates are indicated by the yellow dots along the x-axis. Discharge data were obtained from USGS gauging stations (details in text)

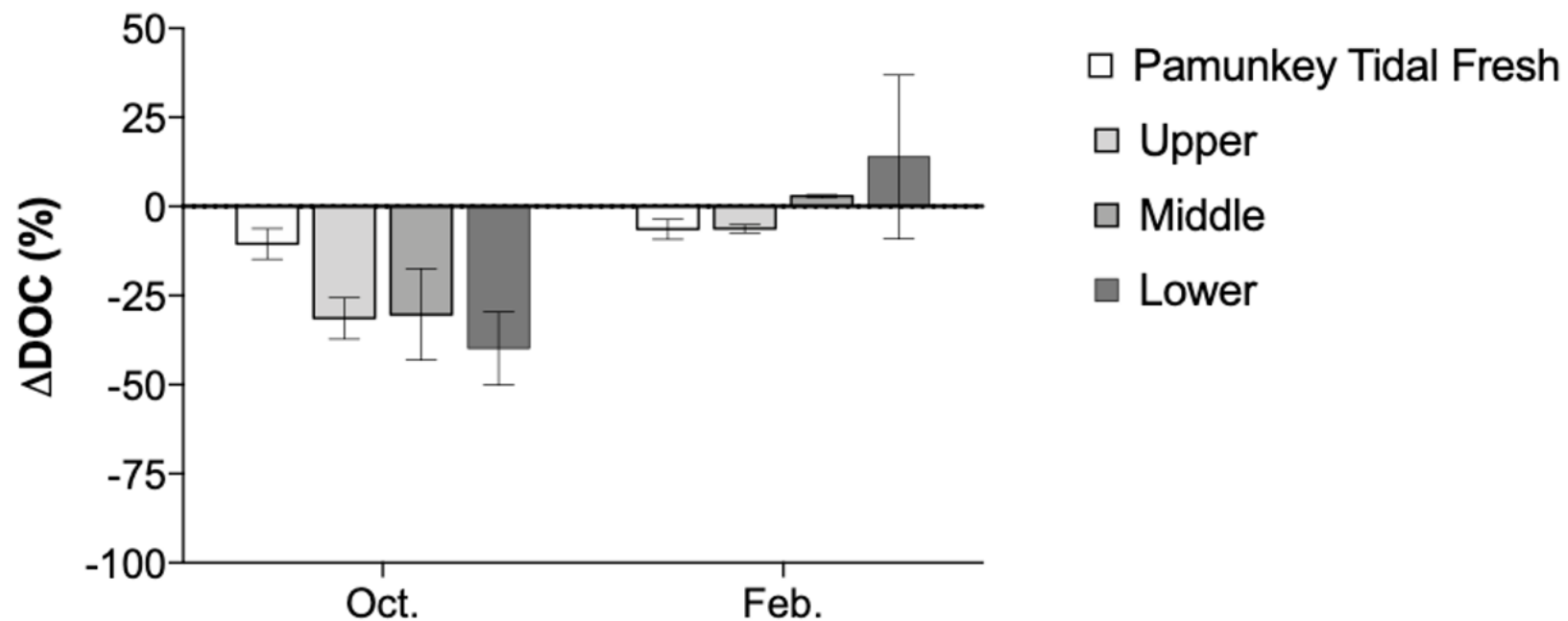


Fig. S3 Mean percent change in the concentration of DOC over 28-day incubation experiments. Samples were collected along the upper, middle, and lower YRE as well as in the tidal freshwater portion of the Pamunkey River, a tributary of the YRE, during October 2018 and February 2019. Error bars are ± 1 standard error of the mean of triplicate samples