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Aquatic Priming Effects in the York River Estuary and Implications for Dissolved Organic Carbon Mineralization

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AQUATIC PRIMING EFFECTS IN THE YORK RIVER ESTUARY AND IMPLICATIONS FOR DISSOLVED ORGANIC CARBON MINERALIZATION

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

ANOVA: Analysis of Variance

BGE: Bacterial Growth Efficiency

BOD: Biological Oxygen Demand

BP: Bacterial Production

BR: Bacterial Respiration

C: Carbon

Chl a: Chlorophyll a

CI: Confidence Interval

DIC: Dissolved Inorganic Carbon

DO: Dissolved Oxygen

DOC: Dissolved Organic Carbon

DOM: Dissolved Organic Matter

IRMS: Isotope Ratio Mass Spectrometer

OC: Organic Carbon

OM: Organic Matter

PE: Priming Effect

POC: Particulate Organic Carbon

POM: Particulate Organic Matter

RQ: Respiratory Quotient

TOC: Total Organic Carbon

Abstract

AQUATIC PRIMING EFFECTS IN THE YORK RIVER ESTUARY AND IMPLICATIONS FOR DISSOLVED ORGANIC CARBON MINERALIZATION

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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The priming effect (PE), characterized as the enhanced microbial processing of bio-recalcitrant organic matter with the addition of labile substrates, has been hypothesized to moderate carbon (C) cycling in aquatic systems. In this study, aquatic PEs were evaluated through bacterial respiration and dissolved organic C consumption in incubations of water collected from three locations along the York River estuary. Incubations from White's

Landing on the Pamunkey River, a tidal freshwater tributary of the York, and from Croaker Landing in the middle of the estuary, displayed positive PEs when amended with labile C. In contrast, amended incubations from Gloucester Point, near the mouth of the estuary, displayed negative PEs, or reduced relative C metabolism, based on our calculations, This study provides empirical evidence for the occurrence of aquatic PEs and serves to elucidate how they may enhance or retard the processing and mineralization of organic C during transport to the ocean.

Introduction

Significance of Carbon Cycling in Inland Waterways

The flux of carbon (C) amongst its principal reservoirs, the land, ocean, and atmosphere, has been a prominent area of research for decades. Recently there has been an enhanced focus on global-scale changes in C fluxes resulting from human influence. Models of the global C cycle seek to detail dynamic biogeochemical processes in an effort to gain a fundamental understanding of the positive and negative feedbacks associated with circulation of C. In doing so, anthropogenic forcings to the C cycle can be more accurately interpreted and predicted. As of the last half-decade, modern C cycle models have sought to incorporate inland waterways along with their terrestrial, oceanic and atmospheric counterparts, as they have been recognized as significant transformers and transporters of C. However, in order to best predict the effects of future human impacts and climate change, a comprehensive understanding of the biogeochemical processes mediating the flux of C from inland waterways is needed.

Due to their small areal coverage relative to the open ocean, inland waters have historically been excluded from global C models (Cole et al. 2007). Current estimates of the C load entering inland waterways suggest as much as three times more terrestrially derived-C enters these systems than is exported to the ocean. Much of this C (~75%) is

hypothesized to leave through riverine outgassing (Cole et al. 2007; Tranvik et al. 2009; Aufdenkampe et al. 2011). Raymond et al. (2013) estimated C evasion from inland waterways is 2.1 Pg C yr^{-1}, suggesting that, relative to surface area, the C evasion from these systems is comparable to the exchange between the ocean and atmosphere. Consequently, it is important to develop our understanding of the biogeochemical and microbial factors responsible for regulating the C flux to the atmosphere relative to downstream transport and ultimately export to the coastal sea.

Organic Carbon Bioavailability in Inland Water Systems

Current estimates indicate that 1.9 - 2.7 Pg C yr^{-1} enter inland waterways from the terrestrial environment; as such, terrigenous organic carbon (OC) comprises a significant portion of both the particulate and dissolved organic matter (POM, DOM, respectively) for these systems (Cole et al. 2007; Battin et al. 2009; Aufdenkampe et al. 2011; Regnier et al. 2013). Allochthonous organic matter (OM) may, in turn, provide a significant energy subsidy for aquatic microbial communities (McCallister and Bauer 2004; Raymond and Saiers 2010; Guillemette and del Giorgio 2011; Bianchi 2011). In general, allochthonous OM is considered to be of both lower quality and bioavailability than OM of autochthonous origin, due to high C:Nitrogen and C:Phosphorus stoichiometry, lignin and cellulose concentration, aromaticity, and other characteristics (Bianchi et al. 2004; Duan et al. 2007; Lane et al. 2012; Ylla et al. 2012). Despite the relative recalcitrance of terrestrial OM, system net heterotrophy, often documented in inland waterways, is often associated with the respiration of allochthonous OM subsidies (Cole et al. 2000; Raymond et al. 2000;

Cole and Caraco 2001; Karlsson et al. 2007; McCallister and del Giorgio 2012). In multiple systems, microbial respiration has been determined to be the primary driver of aquatic-atmospheric CO_2 fluxes (Richey et al. 2002; Karlsson et al. 2007; McCallister and del Giorgio 2008; Tranvik et al. 2009) with much of that C derived from terrestrial OM (Karlsson et al. 2007; McCallister and del Giorgio 2012).

Ongoing research has given scientists cause to reevaluate the broad classifications of OM bioavailability and recalcitrance in both soil (Ekschmitt et al. 2005; Guenet et al. 2012) and aquatic systems (Guenet et al. 2010a; Bianchi 2011; McCallister and del Giorgio 2012). Characteristics assumed to be linked with OM bioavailability, such as age (Hood et al. 2009; Guenet et al. 2012; McCallister and del Giorgio 2012) or relative persistence in an environment (Ekschmitt et al. 2005), have been shown to vary spatiotemporally with regard to actual bioavailability in natural systems. For allochthonous OM received by aquatic systems, environmental characteristics that promote stability or persistence in the terrestrial environment (e.g. physical occlusion in a substrate, structure of the soil complex) may have little or no influence on the relative recalcitrance of terrigenous OM in the aquatic context (Ekschmitt et al. 2005; Fontaine et al. 2007; McCallister and del Giorgio 2012; Guenet et al. 2013). Further, processes such as photo-mineralization and photosensitization may alter and moderate the bioavailability of OM in aquatic systems (Miller and Zepp 1995; Bertilsson and Tranvik 2000; Obernosterer and Benner 2004). In addition to these processes, other factors such as water table fluctuations in peatlands (Riedel et al. 2013), biochar products entering aquatic systems (Norwood et

al. 2013), and the aquatic priming effect (Guenet et al. 2010a; Bianchi 2011) may also play a role in regulating the relative bioavailability of OM in aquatic systems.

The Priming Effect in Aquatic Systems

The priming effect (PE) is speculated to be an underlying factor influencing OM utilization rates and relative bioavailability in inland waterways (Guenet et al. 2010a; Bianchi 2011; Regnier et al. 2013). In terrestrial systems, the PE is a well-documented process wherein the decomposition of recalcitrant OM is stimulated by the co-metabolism of labile OM (Kuzyakov et al., 2000 and sources therein). While the mechanism(s) responsible for PEs is not well understood, priming is hypothesized to result from: labile C providing energy that would otherwise limit microbial activity, microbial nutrient mining in an effort to meet stoichiometric demands resulting in increased recalcitrant OM turnover, enhanced bacterial production leading to greater OM turnover, or as a combination of some or all of these processes (Kuzyakov et al. 2000; Fontaine et al. 2003; Blagodatskaya and Kuzyakov 2008; Chen et al. 2014). As such, natural spatiotemporal variances or overall OM heterogeneity coupled with pulses of labile dissolved OC (DOC) may lead to the increased microbial processing of recalcitrant C sources (Ekschmitt et al. 2005; Thiessen et al. 2013; Bengtsson et al. 2014). Guenet et al. (2010a) and Bianchi (2011) both proposed that PEs might be an important pathway for the removal of recalcitrant C in aquatic systems. As an extension of their hypothesis, ecosystem-level characteristics, such as seasonality, may moderate PEs by influencing microbial

metabolism (del Giorgio and Cole 1998; Lane et al. 2012; Ylla et al. 2012) or labile OM availability in inland water systems (Bianchi et al. 2004; Duan et al. 2007).

Although PEs have been widely accepted as a factor influencing OM availability in terrestrial systems (Kuzyakov et al. 2000; Kuzyakov 2010), and hypothesized to influence OM processing in aquatic systems (Farjalla et al. 2009; Thouin et al. 2009; Bengtsson et al. 2012; Lane et al. 2012; Ylla et al. 2012; McCallister and del Giorgio 2012; d'Errico et al. 2012; Danger et al. 2013; Bianchi et al. 2013; Norwood et al. 2013; Guenet et al. 2013; Hansell and Carlson 2013; Fonte et al. 2013), to date there have been few empirical studies conducted of PE in the aquatic context (Danger et al. 2013; Franke et al. 2013; Guenet et al. 2013; Bengtsson et al. 2014). Support of the aquatic PEs hypothesis has led to the inclusion of PEs in aquatic C budget models for inland water systems (Regnier et al. 2013), although the ultimate significance of aquatic PE as a factor impacting C flux for these systems has not yet been empirically determined (Franke et al. 2013; Hotchkiss and Hall 2014; Bengtsson et al. 2014).

Study Objectives

The primary objective of this study was to establish an empirical method that could be used to assess the aquatic PE and secondarily, to apply that methodology to evaluate priming in aquatic samples collected along a model inland water system. The York River was chosen as the study system because: 1) it represents a natural salinity continuum, 2) it contains relatively distinct zones of terrestrial and marine OM sources (McCallister et al. 2006b) and 3) it is relatively pristine compared to the other prominent sub-estuaries of the

Chesapeake Bay (Schultz and Ducklow 2000; Schultz et al. 2003; McCallister et al. 2006b). This study focused on the metabolism of the DOC pool as bacterial consumption has been recognized as "one of the major sinks of DOC in the biosphere" (Guillemette and del Giorgio 2011), and PEs fundamentally stem from microbial metabolic processes (Kuzyakov et al. 2000). Unique to this study was the use of *Sorghum halepense* plant pollen leachate as the labile DOC source added to sample waters to evaluate potential PEs. Pollen leachate had advantages over simple compounds such as glucose since it was a heterogeneous mixture, provided a more complex DOC source, and likely provides a seasonal source of labile C to the York River.

Materials and Methods

The York River System and Sampling Locations

The York River Estuary is a tidal, partially mixed estuary that originates near West Point, VA, at the confluence of the Pamunkey and Mattaponi Rivers. It is considered pristine by comparison to neighboring sub-estuaries of the Chesapeake Bay, with the dominant land coverage of the watershed (~60%) consisting of forested land (Schultz et al. 2003). Of the two tributaries, the Pamunkey River contributes the majority (70-80%) of the total river flow to the estuary (Raymond and Bauer 2000; McCallister et al. 2006b). The Pamunkey River begins the transition from fresh to oligohaline water about ~45 river kilometers (rkm) before meeting the York River. Salinity in the estuary typically ranges from ~5 in surface waters near West Point to ~26 in bottom waters at the mouth off Gloucester Point (Friedrichs 2009).

Experimental Design

Aquatic PEs were assessed in York River water incubations, and differences in the bacterial metabolism of DOC, between groups amended with labile pollen leachate-C and control groups (water receiving no amendment), were used to identify Positive and Negative PEs. *Sorghum halepense* pollen leachate was chosen as the labile C source

received by amended groups as the leachate of the pollen is readily bioavailable and the plant grows along the York River (USDA; www.plants.usda.gov /core/profile?symbol=SOHA). As a C4 grass, *S. halepense* has a $\delta^{13}C$ isotopic signature distinct from that of terrestrial OM (approx. -27 ‰) and the bulk DOC signature of the estuary (Raymond and Bauer 2001; McCallister and Bauer 2004).

Sample Collection and Processing

Water samples were collected in March, 2014, from the York River Estuary, VA, at three locations: White's Landing on the Pamunkey River, off Croaker Landing on the north end of the York River State Park, and at the public pier off Gloucester Point beach near the termination point of the York into the Chesapeake Bay (Figure 1). Sampling locations are here after referred to as WL, CL and GP for White's Landing, Croaker Landing, and Gloucester Point, respectively. Samples of water from each site (36 L) were collected in acid-leached, sample-rinsed polycarbonate carboys and stored at room temperature prior to experimental setup (less than 24 hours). At the time of sampling, temperature, pH and salinity were measured at each collection site with a Mettler Toledo SG78 SevenGo Duo Pro pH/Ion/Conductivity meter calibrated prior to sampling. The chlorophyll a (Chl a) concentration in surface waters at each site was assessed at the time of sampling, by collection of particulate OM (POM) on GF/F filters (2 replicates per site). Filters were kept on ice while in the field and stored frozen (-80°C) in the lab until processing by pigment extraction in acetone (over 24 hours) prior to analysis on a TD-700 fluorometer.

Sample waters were pumped (peristaltic) through a pre-combusted 0.7-µm GF/F pre-filter and a 0.2-µm Gelman capsule filter connected inline to remove POM, eukaryotes, and bacteria. Filtrate was distributed into glass carboys and inoculated with bacteria by the addition of 0.7-µm GF/F-filtered water (1% v/v). After inoculation, filtered sample waters were divided into two groups: a control group (sample waters receiving no OM addition) and an experimental treatment that received the labile DOM addition of pollen leachate at a target level of ~60 µM C. This concentration roughly represents a 10% increase in DOC for the system relative to the annual average (McCallister unpublished data). *S. halepense* pollen (Sigma-Aldrich) was leached (24 hours) the day prior to sampling, in 18-ohm deionized (DI) water at room temperature (20°C), then filtered (GF/F) to remove residual POM. After filtration, inoculation and division into treatment groups, waters were then added to 300-ml biological oxygen demand (BOD) bottles for dissolved oxygen (DO) measurement (three replicates per site) and δ^{13}C-DOC/DIC assessments (ten replicates per site), and to 1-L Nalgene incubation bottles for DOC concentration and bacterial production (BP) measurements (three replicates per site). All bottles were overflowed and incubated in the dark at room temperature (20°C) for 28 days. All BOD bottles were sealed with Thompson's silicone vacuum grease and housed submerged in DI water to prevent gas exchange with the atmosphere.

Dissolved Oxygen Detection for Bacterial Respiration Assessments

Incubation BOD bottles were outfitted with planar oxygen-sensitive foil ports for non-invasive DO analysis with a FIBOX 3 fiber optic oxygen meter. The concentration of

DO was assessed at the start of the incubation period, after 12 hours and at 1, 2, 3, 4, 7, 14, 21, and 28 days of incubation. At each sampling time point, DO was measured once per second for two minutes, and the data were averaged. The coefficient of variance for the two-minute assessments was less than 0.5% for all replicates. The FIBOX 3 was calibrated between each two-minute series to account for sensor spot variation and changes in atmospheric pressure. Resolution of the FIBOX, at 9.06 mg O_2 L^{-1} and 1013 hPa, is ± 50 µg O_2 L^{-1}. Resolution improves with decreasing O_2 concentration such that resolution becomes ± 10 µg O_2 L^{-1} at 2.72 mg O_2 L^{-1}. The FIBOX 3 was used in conjunction with a temperature probe that was placed in the insulated container housing all DO-BOD bottles, as measuring the temperature of individual bottles was not possible. Bacterial respiration (BR) was calculated as the change in DO, assuming a respiratory quotient (RQ) of 1:1 (mol CO_2 produced : mol O_2 consumed).

Dissolved Organic Carbon Concentration and Bioavailability Assessments

Subsamples for DOC concentration were collected from filled 1-L Nalgene bottles at the start of the incubation time course, at 12 hours and at 1, 2, 3, 4, 7, 14, 21, and 28 days of incubation. During each subsampling event, each Nalgene incubation bottle was subsampled once, yielding three replicate subsamples per treatment group per time point. Subsamples were dispensed into 40-ml amber glass vials, acidified with 100 µl of 12 M HCl, and sealed with acid-washed I-CHEM septum tops. The DOC concentration was determined by high temperature catalytic oxidation with a Shimadzu TOC-V_{CPH} analyzer containing high sensitivity platinum mesh catalyst and all subsamples with salinity greater

than 5 were automatically diluted with 18-ohm DI water in the TOC-VCPH prior to oxidation. The coefficient of variance amongst analytical replicates was less than 2%.

The bioavailability of bulk DOC in each group was calculated as the proportion of DOC removed relative to the total DOC at the start of the incubation. Bioavailability is expressed as a percentage.

Bacterial Production and Bacterial Growth Efficiency Estimates

Bacterial production (μg C L^{-1} hr^{-1}) was estimated by ^{3}H-leucine incorporation into bacterial protein according to the protocol outlined by Smith and Azam (1992). Subsampling for BP from 1-L Nalgene incubations (three technical replicates per bottle per time point) took place at the start of the incubation period, at 6 and 12 hours, and at 1, 1.5, and 2 days of the incubation time course. Aliquots (1.5 ml) of water, taken from 1-L Nalgene incubations, were added to 2 ml centrifuge vials and amended with 30 nM ^{3}H-leucine. Leucine-amended BP subsamples were kept in the dark, at room temperature for one hour, then terminated with the addition of 100 μl of 100% trichloroacetic acid. Blanks were generated by adding sample water, ^{3}H-leucine, and trichloroacetic acid to a centrifuge tube in concert. All vials were centrifuged after incubation and aspirated to isolate bacteria into a pellet, simultaneously removing any unincorporated ^{3}H-leucine. Pellets, adhered to the side of the centrifuge vials, were rinsed with a 5% trichloroacetic acid solution, vortexed, centrifuged, and aspirated to remove any residual isotope. The pellet was then rinsed in an 80% ethanol solution, vortexed, centrifuged, and aspirated, leaving only the rinsed bacterial pellet left in the centrifuge vial. Once vials were dry (~24 hours), vials

received 2 ml of scintillation cocktail and were run on a Beckman 6000IC scintillation counter with blanks interspersed every 15 vials. The coefficient of variance did not exceed 8% and average 4% for all BP technical replicates.

Bacterial growth efficiency (BGE) was calculated for each 1-L Nalgene incubation replicate (three total per treatment) by integrating the six BP assessments and dividing by the absolute value of the change in DOC after 2 days of incubation. BGE is expressed as a percentage.

Isotopic Signature of DOC and Aqueous DIC

Waters from each treatment and site combination were allocated to 300-ml BOD bottles for incubation and collection of ^{13}C-DOC and aqueous ^{3}C-DIC samples. Bottles were overflowed with sample water, sealed with Thompson's silicone vacuum grease, and housed submerged in DI water. Bottles were sealed in this manner to ensure that respired CO_2 remained in the incubations as aqueous DIC. Bottles (two per time point) were randomly selected at the start of the incubation period, and after 2, 4, 7, and 28 days of incubation, for destructive sampling. Selected bottles were opened and waters immediately transferred to 40-ml amber glass vials. Vials were overflowed, amended with 40 µl of $ZnCl_2$ solution (50% w/v) to terminate bacterial activity, and sealed with acid-washed I-CHEM septum tops. Each randomly selected bottle yielded one sample for ^{13}C -DOC sample and one sample for aqueous ^{13}C-DIC analysis. Aqueous ^{13}C-DIC samples were acidified with phosphoric acid and the respired CO_2 was then analyzed on a GasBench II system interfaced to a Delta V Plus IRMS at the UC Davis Stable Isotope Facility. Fresh

water ^{13}C-DOC samples (salinity < 5) were analyzed using a TOC analyzer interfaced to an IRMS where in DOC is oxidized to CO_2 with the addition of sodium persulfate at the UC Davis Stable Isotope Facility. The variance of technical replicates of samples during analysis was less than or equal to 0.2‰. Results for stable isotope values are reported in standard per mil notation as:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 10^3 \qquad \textit{eq. 1}$$

where R is $^{13}C/^{12}C$.

Calculation of Priming Effects

Aquatic PEs were evaluated for each site as the amount of DOC consumption in amended treatments (those with pollen leachate-C additions) less the amount of added (primer C or pollen leachate-C) C metabolized in the amended treatments, in excess of the DOC consumed within the respective control incubations (eq. 2).

$$[\text{Primed C}] = |\Delta[\text{treatment C}]| - (A \times [B]) - |\Delta[\text{control C}]| \qquad \textit{eq. 2}$$

where A is the proportion of added pollen leachate-C metabolized in the amended treatment, and B is total pollen leachate-C added to the amended treatment. Amended incubations from each site were evaluated for the primed BR of C using the same method. Estimates of priming calculated without tracing the fate of the pollen leachate-C, are

conservative with regard to positive PEs, as 100% of the pollen-C was assumed to have been consumed. Alternatively, assuming 100% pollen-C consumption can lead to overestimation of negative PEs.

For the priming of DOC, the difference between the final concentrations of DOC in amended treatments and control treatments was used to create a confidence interval (CI) around the true mean difference; in this instance the mean difference, representing the amount of primed background C.

A more refined estimate of PEs was achieved by utilizing the $\delta^{13}C$ isotopic signature of the added pollen leachate-C to assess the amount of primer C metabolized in experimental treatments. A two-end-member mixing model (eq. 3) was used to estimate the amount of pollen leachate-C present in the DOC pool at selected times during the incubation, and a more accurate estimation of *B* was possible with equation 2.

$$\delta^{13}C_{mixture} = f_1\,\delta^{13}C_1 + f_2\,\delta^{13}C_2 \text{ where } f_1 + f_2 = 1 \qquad \textit{eq. 3}$$

where C_1 is the added pollen leachate-C and C_2 is the background C (the C from the York, present in both groups), while f_1 is the proportion of pollen leachate-C and f_2 is the proportion of background DOC in the amended treatment incubations.

The amount of C attributable to the pollen leachate C and to the bulk background C present at each site in the DOC pool can then be found through the following equation:

$$f_1 = [(\delta^{13}C_{mixture} - \delta^{13}C_2)/(\delta^{13}C_1 - \delta^{13}C_2)] \text{ and } f_2 = 1 - f_1 \qquad \textit{eq. 4}$$

For PEs, the amount of pollen-C consumed was then calculated as the difference between the initial amount of pollen-C received by the incubation group and the amount remaining as calculated by equation 4. The resultant proportion was substituted as "*A*" in eq. 2 and the amount of background C removal in experimental treatments at 2, 4, 7, and 28 days of incubation was calculated.

Statistics

The statistical significance of the differences between control groups and amended treatments for each of the three sites, for BP, BGE, total BR, total DOC consumption, initial DOC, final DOC, and DOC bioavailability were determined via two tailed *t* tests. Significant differences between control groups across sites for integrated BP, BGE, total BR, and DOC (initial, final, total consumption, and bioavailability) were determined by analysis of variance (ANOVA) and Tukey's HSD post-hoc tests. Simple linear regressions were used to evaluate correlations between salinity, BGE, and DOC bioavailability. ANOVAs, *t* tests, and linear regressions were performed at the $\alpha = 0.05$ level of significance. All assessments were carried out in R statistical computing software (http://R-project.org).

Results

Study System

Riverine discharge in the Pamunkey at the time of sampling was at the minimum for the month, at ~22 m^3 sec^{-1} compared to the daily average of ~33 m^3 sec^{-1} for March. The combined flow of the Mattaponi and the Pamunkey Rivers, the two primary freshwater tributaries of the estuary, was also at the monthly minimum with a flow of ~39 m^3 sec^{-1} relative the March daily average of ~53 m^3 sec^{-1} (preliminary 2014 data, USGS).

Surface waters at WL (93 rkm), on the Pamunkey River, had a salinity of ~0.5 and a pH of 8.05. The Chl a concentration (3.1 ± 0.2 μg L^{-1}) in surface waters off WL was the lowest of the three sampled locations. Surface water at CL on the northern reach of the York River State Park (~42 rkm) had a salinity of ~10 and pH of 8.47. The concentration of Chl a in CL surface waters, 6.3 ± 0.4 μg L^{-1}, was two times greater than the concentration from White's Landing. The third site at GP (~13 rkm from the Chesapeake Bay) had a salinity of ~21 with a pH of 8.67 and a Chl a concentration of 5.3 ± 0.9 μg L^{-1} (Table 1).

DOC Characteristics for Control and Amended York River Water Samples

The concentration of DOC in the York River at CL (336±12 μM C) was higher than GP at (245±12 μM C) and at WL on the Pamukey River (267±3 μM C). The initial concentrations of DOC in amended treatment groups from each site were significantly different from that of controls (all p values < 0.01) at 312 ± 3 μM C, 390 ± 7 μM C, and 308 ± 4 μM C for WL, CL, and GP respectively.

Removal rates of DOC peaked within the first two days for all incubation groups. Consumption of DOC with the first four days represented more than half of the total consumption over the entire 28-day incubation period for most groups. The one exception was the WL control group wherein consumption was 36% after 4 days, relative to the total consumption by the end of incubation. The CL pollen leachate-C amended group had the largest removal of DOC (122 ± 4 μM C) while the WL control group had the smallest removal at 25 ± 4 μM C (Table 2 and Figure 5). The final concentrations of DOC present in amended groups for WL (233 ± 2 μM C) and CL (268 ± 7 μM C) were significantly different from their corresponding control groups at 242 ± 3 μM C and 295 ± 3 μM C, respectively) for WL (p-value = 0.017) and CL (p-value = 0.010). The GP pollen leachate-C amended group had a final concentration of DOC also significantly different than that of its corresponding control group (215 ± 6 μM C vs 185 ± 3 μM C; p-value = 0.007) at the end of the incubation period, although unlike for WL and CL, the amended treatment final concentration was higher than the control. Bulk DOC bioavailability after 28 days represented 9%, 12%, and 25% for control groups from WL, CL, and GP respectively (Table 2). For The pollen leachate-C amended incubations the bioavailability averaged

25% for WL, 31% for CL, and 30% for GP. The percentage of bulk DOC consumed during the time course was significantly different between amended groups and control groups for all sites (all p-values < 0.05).

Bacterial Production and Bacterial Growth Efficiency Estimates

In control incubations for WL and GP, BP peaked after approximately one day (1.62 ± 0.45 and 1.05 ± 0.12 μg C L^{-1} hr^{-1}, respectively), while the control incubations for CL, (6.42 ± 0.04 μg C L^{-1} hr^{-1}) peaked after 12 hours. In pollen leachate-C amended groups, BP peaked after 6 hours for WL (7.91 ± 0.42 μg C L^{-1} hr^{-1}) and CL (9.03 ± 0.44 μg C L^{-1} hr^{-1}) while BP in the GP pollen leachate-C amended treatment group peaked after two days (13.52 ± 0.92 μg C L^{-1} hr^{-1}) and was over an order of magnitude higher than its corresponding control. Differences in integrated BP (μg C L^{-1}) at 48 hours were significant for all pollen leachate-C amended treatments relative to their respective controls (all p-values < 0.01) with amended groups at least three times greater than control groups (Figure 2).

Pollen leachate-C amended treatment groups from WL and GP had calculated BGEs significantly different (p values = 0.016 and 0.002, respectively) than corresponding control groups. The GP site had the lowest BGE of all groups (7%) after 48 hours, while WL and CL control groups were similar with 24% and 31% BGE, respectively. In the pollen leachate amended treatment groups from WL and CL the BGE exceeded 50% whereas BGE for GP was lower at 35%. For WL and CL, BGE was nearly two times greater for pollen leachate-C amended incubations relative to controls, while the BGE of

GP pollen leachate-C amended treatments was more than five times greater than its control (Figure 3).

Bacterial Respiration in Control and Amended Treatments

BR rates peaked for all groups within the first three days of incubation. Total respired C in the control groups ranged from 350 μg C L^{-1} to 772 μg C L^{-1} at the end of the incubation period, with similar concentrations of C respired at WL (406 ± 49 μg C L^{-1}) and GP (496 ± 30 μg C L^{-1}). In contrast, BR was ~50% greater in the CL control group relative to the other control groups (731 ± 48 μg C L^{-1} after 28 days). The pollen leachate-C amended incubations for the CL site had the highest total respiration of all of the groups by the end of the incubation (1550 ± 43 μg C L^{-1}) and those from WL and GP had substantially lower total respired C at 1092 ± 36 μg C L^{-1} and 1207 ± 40 μg C L^{-1}, respectively (Figure 4). There were significant differences between total BR in control groups relative to pollen leachate-C amended treatments for all sites at the end of the 28-day incubation period (all p values < 0.0001).

Primed Dissolved Organic Carbon Consumption and Bacterial Respiration

Priming effects were calculated according to eq. 2 as differences between the control groups and the pollen amended treatments at each site, with the assumption of 100% pollen leachate-C consumption when calculated without incorporating isotope mixing model results. PE assessments were made using both the total respired C and total DOC removal after the 28-day time course. Calculated PEs for DOC removal in WL and

CL pollen leachate-C amended incubations represented 9 and 27 μM C-background C consumed in excess of their respective control groups; those amounts correspond to a 37% (95% CI: 12% to 62%) increase in background DOC consumption for WL incubations and a 67% (95% CI: 31% to 103%) increase in background DOC consumption for CL incubations (Table 3 and Figure 5). There was a calculated negative PE, or decreased removal of background DOC (-30 μM C) relative to the corresponding controls, observed in the GP amended incubations; -50% (95% CI: -72% to -28%). The PE calculated for WL pollen leachate-C amended incubations using total BR assessments was +38%, or ~153 μg C L^{-1}. Amended incubations from CL also displayed positively primed BR equivalent to ~174 μg C L^{-1}, or a 24% increase relative to the total respiration in the control group. Primed respiration in the GP amended treatments was calculated to be -43 μg C L^{-1}, or -9% relative to total BR in the control group (Table 3 and Figure 4).

$DO^{13}C$ Analysis and Mixing Model Priming Calculations in the White's Landing Group

Isotopic analysis of the pollen leachate-C indicated it had a carbon signature reflective of C4 plants (-15.4 ± 0.2‰). The $\delta^{13}C$ signature of the bulk DOC at WL averaged -27.6 ± 0.1‰, while the $DO^{13}C$ signature in the pollen leachate-C amended treatments averaged -25.7 ± 0.2‰. At the end of the 28-day incubation period, the WL control group (-27.9 ± 0.1‰) and the pollen leachate-C amended treatment group (-27.9 ± 0.2‰) had similar $DO^{13}C$ signatures, indicating less of a presence of the ^{13}C enriched C source (pollen leachate-C) in the amended group (Figure 6B).

Combining the $\delta^{13}C$ signatures of the bulk DOC in the control incubations, the pollen leachate-C leachate amended treatments, and the pollen leachate-C itself, an estimate was made of the amount of pollen leachate-C removed in the WL amended group (Figure 6A) and the amount of background OC consumption at select time points (Figure 7). The background C removal in the WL pollen leachate-C amended treatment group (~19 μM C) after 2 days was 55% greater than the removal in the control group (12 ± 4 μM C) at that point in the incubation period. At 4 days into incubation, the background C consumption in the amended treatment (~25 μM C) was enhanced 176% relative to the control (9 ± 3 μM C; Figure 7). By the same calculations, background C consumption in the WL pollen leachate-C amended treatment at 7 days was ~33 μM C (84% increase) and at 28 days was ~34 μM C (38% increase). This evidence suggests that the majority (> 95%) of the background C removal in the WL pollen leachate-C amended treatment occurred within the first 7 days of the incubation of the 28-day incubation period.

Based upon the shift in isotopic signatures, ~99% of pollen-leachate C was consumed in the WL amended group after 28 days of incubation (Figure 6A), and as a result, the conservative assessment of primed DOC consumption (38%) was approximately equivalent to that calculated using the isotopic mixing model (38%) for the end of the incubation period.

Discussion

History of Priming in Aquatic Systems

While there has been much scientific focus on the PE in terrestrial environments, research on aquatic PE is still in its infancy. Although priming has frequently been invoked as a mechanism to metabolize "recalcitrant" OM (Freeman et al. 2004; McCallister and del Giorgio 2012; d'Errico et al. 2012; Danger et al. 2013; Regnier et al. 2013; Massicotte and Frenette 2013) there is little empirical support for this assertion. To date, there have been only two experimental studies looking specifically at priming in aquatic systems. Franke et al. (2013) and Bengtsson et al. (2014) examined the priming of C by biofilm communities in low order stream systems; the latter concluded that aquatic PE was not a significant factor on C metabolism in alpine streams, while the former stated that positive PEs were rare and only significant in two out of nineteen experiments in boreal catchments. Conversely, Hotchkiss and Hall (2014) used a bayesian process model to estimate PEs in western and Midwestern stream systems and suggested that primed metabolism of C potentially ranges from -130% to +370%.

The primary aim of this study was to evaluate the potential occurrence and nature of priming at three locations situated along the York River Estuary. This study provides the first empirical assessment of aquatic PE along an estuarine system. In contrast to priming

studies conducted in terrestrial systems (see Blagodatskaya and Kuzyakov 2008, for review) and bioavailability assessments in aquatic systems (Shimp and Pfaender 1985; Carlson et al. 2002; Thouin et al. 2009; Koehler et al. 2012; Franke et al. 2013; Guenet et al. 2013) which employed glucose as the labile C substrate, this study is unique in the use of *S. halepense* pollen. The pollen leachate-C added to experimental treatments represented a more heterogeneous and natural labile C source, comparable to a typical subsidy received by an inland water system.

Bioavailability of Dissolved Organic Carbon along the York River

The DOC in the York River (assessed through control incubations) displayed a linear trend of increased bioavailability with increasing salinity (linear regression, r = 0.83), mirroring results documented for the York River by Raymond and Bauer (2000). The concentrations of DOC (range = 230-350 μM) at WL, CL and GP were similar to values previously reported in the literature for the month of March (Raymond and Bauer 2000; McCallister et al. 2006a). The bioavailability of DOC (Table 2) was also similar to March values for the regions of the york near WL and CL (Raymond and Bauer 2000). Control incubations from GP, however, displayed a bioavailability (25%; Table 2) that is high compared to March literature estimates at the estuarine mouth (3.9% to 7.8%; McCallister et al. 2006b; McCallister unpublished data). The bioavailability of DOC in the more saline stretches of the York River estuary (16.6 ppt – 20.5 ppt) has been documented to range from 1.6% to 7.0% over the course of a year (Raymond and Bauer 2000). Raymond and Bauer (2000) found that bioavailability for York DOM, collected in March,

increased by a factor of four when incubated at 25°C relative to *in situ* temperatures. This suggests that the high bioavailability of DOC in GP control incubations may have been related to the temperature of incubation (room temperature vs. *in situ*).

Bacterial Metabolism in York River Incubations With and Without C Additions

Integrated BP in the control groups over the first 48 hours of incubation mirrored results published by McCallister et al. (2005), where the greatest production occurred in sample waters from the middle section of the estuary (CL), while BP was significantly lower at the WL site on the Pamunkey and the GP site near the mouth of the York River (ANOVA, p value < 0.001). Integrated BP was significantly higher (*t* tests, p values < 0.01) in each of the pollen leachate-C amended groups compared to their associated controls, indicating that the pollen leachate may have provided an energetically favorable C source or nutrients, that would have otherwise limited BP. For control groups, Total BR was also highest in CL incubations, with respiration significantly greater than at WL and GP (ANOVA, p value < 0.01), and again reflected published trends for the system (Raymond and Bauer 2000).

For control incubation groups from WL on the Pamunkey and CL near the middle of the estuary, BGE (averaging 24% and 30% respectively) was similar to the reported average for the York (~28%) as documented by Raymond and Bauer (2000) and the median BGE for rivers (~22%) and estuaries (~34%) as reviewd by del Giorgio and Cole (1998). The BGE at GP however, was significantly lower at ~7% (ANOVA, p value < 0.01). The review by del Giorgio and Cole (1998) indicates that the metabolism of OM

derived from marine phytoplankton or seaweed may coincide with a BGE of that order, however there is much overlap with regard to the BGE reflective of other OM sources. There was a weak negative relationship between BGE and DOC bioavailability (linear regression, r = -0.72) in the control groups, though this was not significant (p value = 0.100). Raymond and Bauer (2000) found a significant negative relationship between BGE and DOC bioavailability in the York River. Vascular plant material is associated with low bioavailability (Hedges 1992), and promoting higher BGEs (del Giorgio and Cole 1998), which suggests that decreases in the proportion of terrigenous OM relative to the bulk OM pool (McCallister et al. 2006b) may relate to the negative relationship between BGE and DOC bioavailability.

In pollen leachate-C amended incubations from all sites, BGE was higher than control groups indicating that the OM mixture of the pollen leachate-C and background C shifted bacterial metabolism so that a greater percentage of bulk C was incorporated into biomass. Proportionally the greatest increase in BGE of pollen leachate-C amended incubations relative to control treatments occurred in the GP sample waters (~35% relative to 7%), but BGE in amended groups from WL and CL exceeded 50% after two days, suggesting highly efficient use of C by the microbial community at these sites (Figure 2). Similar to BP, the increased BGE in amended treatment groups may relate to the availability of limiting nutrients and energy subsidies in the form of labile C (del Giorgio and Cole 1998), although it may also be related to the diversity of available OM compounds (Attermeyer et al. 2013; Fonte et al. 2013). Attermeyer et al. (2013) and Fonte et al. (2013) both found that the diversity of OM compounds enhanced BGE in incubations

with OM amendments relative to control groups with no amendments. Changes in the bacterial community composition could also be associated with the BGE within the pollen leachate-C amended treatments, as stimulation of different functional groups may result from OM subsidies and overall heterogeneity (Orwin et al. 2006). In the aforementioned study, Attermeyer et al. (2013) found community composition shifted when terrestrial OM (C3-plant leaves) and algal OM were mixed in an incubation, however treatments receiving different proportions of the mixture did not differ with respect to community composition, suggesting that overall OM heterogeneity was the driving factor. The diversity of the OM mixture in amended treatments (pollen leachate and background DOM), whether impacting the bacterial community composition or providing energetically favorable C sources and/or nutrients derived from the pollen leachate, may provide an explanation for the increased BGE in pollen leachate-C amended incubations in this study.

Aquatic Priming Effects

Labile C amended incubations from the sites along the York River Estuary displayed aquatic PEs ranging from -50% to 67%. This suggests that aquatic PE may be an important biogeochemical process mediating the flux of C in this system. Overall PEs for all sites in this study were well within the range of documented PE in terrestrial systems, from +1100% to -171% (Kuzyakov et al. 2000; Hamer and Marschner 2005) and marine benthic environments from +31% to -86% (Nugteren et al. 2009; Gontikaki et al. 2013). While assessments of aquatic PEs are limited in general, these values fall within the range

of those modeled by Hotchkiss and Hall (2014) for 10 western and Midwestern stream systems (-130% to +370%).

Positive Priming Effects in York River Water Incubations

The WL site, on the Pamunkey River, likely has the largest proportion of terrigenous OM comprising the bulk OM pool as a result of its strong linkages to the surrounding catchment. Previous work by McCallister et al. (2006b) in this system found a decrease in the percentage of lipid biomarkers diagnostic for terrestrial OM with increasing salinity and confirms the terrestrial nature of DOM at this location. Given that the WL amended treatment group experienced a positive PE (38% and 37% as calculated by DOC removal and BR respectively), these results indicate that terrestrial OM was likely primed by the addition of the labile pollen leachate-C. Guenet and others (2013) found tat soil organic matter mineralization in soil-water slurry incubations was enhanced by 12% with the addition of glucose compared to controls over a 45 day time period. These studies provide support for the notion that terrestrial OM in aquatic systems may become more bioavailable due to PE. Consequently, priming may mediate the export of terrestrial OM in riverine systems.

The residence time for the section of the Pamunkey River nearest WL averages ~90 days in duration, and during periods of high flow, the residence time drops to 46 days (Shen and Haas 2004). PE, as assessed through the isotopic mixing model calculations, was detected within the first seven days of incubation in the WL amended treatments and nearly all (>95%) of the bioavailable background DOC consumption had taken place by

this time. Furthermore, the amount of background C removal that took place in 4 days in the amended group for WL (~25 μM C) was equivalent to the total removal in the control group for the 28-day incubation (25 ± 4 μM C; Figure 7). In this temporal context, the evidence suggests that aquatic PE may be an important factor governing C fluxes in the Pamunkey River and that *in situ* PE in the Pamunkey River could result in an accelerated flux of C and remove a portion of terrestrial organic constituents before they are transported downstream. This hypothesis coincides with documented changes in the DOM regime along the York (McCallister et al. 2006b) wherein the proportion of terrestrial constituents relative to the total DOM pool decreases during transport to the ocean and provides a mechanism for the decreased terrestrial OM component near the mouth of the estuary. These results provide support for the hypothesis that aquatic PE may enhance terrestrial DOC flux from inland waterways to the atmosphere (Bianchi 2011), and in part be responsible for the lack of a terrestrial chemical or isotopic signature in DOC within the oceans (Hedges et al. 1997; Bianchi 2011; Flerus et al. 2012). However, this current project did not explicitly focus on this link and more studies are needed to establish the relationship between aquatic PEs and terrestrial C metabolism during transport within inland water systems.

At CL near the middle of the estuary, the removal of DOC in the amended treatment group was three times greater than the removal in the control group (Table 2) suggesting that the mixture of the pollen leachate-C and bulk DOC from CL was highly labile. The positive PE on background DOC consumption (+67%) suggests that there was may have been a larger amount of "semi-labile" components in the DOM pool near this

site, relative to the Pamunkey River site. The DOM pool in the middle of the estuary during March contains a greater proportion of terrestrial constituents than the mouth, while also maintaining a larger proportion of phytoplankton and zooplankton derived organic matter than the Pamunkey (McCallister et al. 2006b); the concentration of Chl a at the time of sampling at CL (6.3 ± 0.4 μg L^{-1}) was nearly double that of WL (3.1 ± 0.2 μg L^{-1}) supporting the latter assessment. The PE assessments for BR and DOC consumption both indicate that the added pollen-leachate C enhanced the microbial processing of the background OM at CL near the middle of the York River. A further hypothesis, based upon these results and the OM assessments put forth by McCallister et al. (2006b), is that aquatic PEs may enhance the bioavailability of autochthonous OM sources in addition to allochthonous ones.

Negative Priming In Sample Waters from Gloucester Point?

The PE for GP amended incubations, calculated without the use of the isotope mixing-model, was determined to be negative; the negative PE represented a 9% and a 50% decrease in processing of background DOC as determined by BR and DOC removal, respectively. A negative PE, or the decreased removal of background C is believed to result from the preferential utilization of labile C sources over background sources (Kuzyakov et al. 2000; Guenet et al. 2010a; b; Gontikaki et al. 2013; Hotchkiss and Hall 2014). In an effort to conservatively calculate positive PEs an A=1 was used in calculations of equation 2. As a result, calculations of PE made without tracing the fate of

the pollen C may alternatively overestimate negative PEs and should not be viewed as conservative assessments.

Assuming the strength of the negative PE was not overestimated, the DOC priming results for GP imply a preference against the background C in bacterial metabolism. When considered with the high lability of the bulk DOC (25%), the low BGE (~7%) in the control group would indicate that the tendency of the bacterial community was to meet the energy demand for cellular upkeep rather than allocating C for growth. The pollen leachate addition may have provided limiting nutrients to the bacterial community, resulting in the enhancement of BP (as well as BGE) displayed in the amended group. In tandem, the high intrinsic lability of the bulk background DOC pool at GP may have alleviated the need to metabolize more recalcitrant OM sources in the amended treatment. The preferential metabolism of the pollen leachate-OM in an effort to relieve nutrient limitation, combined with the abundance of energetically favorable C sources, could be a mechanism for the negative PE in the GP amended group. From a methodological standpoint, the high lability of the bulk DOC in GP control incubations may have further impacted these results.

It should also be noted that in terrestrial systems, decreased and negative PEs have been associated with the proportion of added labile substrate relative to C stores already in a given system. In a meta-analysis, Blagodatskaya and Kuzyakov (2008) found an inverse relationship between PE and the amount of primer C added relative to the local microbial community C. Similarly, Guenet et al. (2010c) found in soil PE studies that the ratio of primer OM mineralization to primed background OM mineralization decreased when added labile substrates corresponded to a smaller proportion of the soil organic matter

stock. While *in situ* negative PEs may naturally occur at the estuarine mouth, it is possible that the proportion of added C relative to the background DOC pool may have been a factor influencing the nature of the PE in the GP incubations as the added pollen leachate concentration represented a proportionally larger addition compared to those received by sample waters from WL and CL (a 26% increase relative to 16% and 17% respectively). The high proportion of bioavailable background DOC in the control group coupled with the relative size of the labile C addition for this site highlight the limitations and the complications of aquatic PE studies done without determining the identity and fate of the OM sources intrinsically involved in the process.

Potential Impact of PE on C Dynamics in the York River Estuary

The nature of the PEs in relation to each of the three sites, positive in the lower (WL) and mid-salinity (CL) reaches of the York River, and negative near the estuarine mouth (GP), parallel both pCO_2 trends in the York River and CO_2 flux estimates along the salinity continuum (Raymond et al. 2000). Of the three sampled sites, PE may have the most impact on CO_2 outgassing in the middle of the estuary as incubations from CL displayed the largest quantity of positively primed C. Of the two sites that displayed positive PE (WL and CL), the middle of the estuary region has the largest proportion of evading CO_2 derived from the BR of DOC (43 ± 39%; Raymond and Bauer 2000). Raymond et al. (2000) indicated that the CO_2 flux from and pCO_2 levels in the system were strongly influenced by temperature and seasonality, with annual maxima in the summer and fall, and minima in winter. Aquatic PE (*in situ*) may also be linked to

temperature and seasonality through direct and indirect relationships such as the regulation of microbial metabolic processes and moderating primary production and labile DOC availability. Ylla et al. (2012) found at higher temperatures that bacterial abundance, BR, and DOC consumption increased in riverine biofilm communities. They also found that while the cycling of labile OM constituents occurred rapidly regardless of the temperature, recalcitrant OM metabolism was stimulated at higher temperatures with the enzyme activity of phenol oxidase linked to increases in the respiration of humics. The positive association between temperature and CO_2 evasion (Raymond et al. 2000), longer residence times occurring during the warmer months (Shen and Haas 2004) in the York River, and the potential for the enhanced microbial processing of recalcitrant OM (Ylla et al. 2012) at warmer temperatures, would suggests seasonality plays a large role in moderating the significance of aquatic PE in this system. Aquatic PE may contribute to the CO_2 flux from the less saline reaches of the estuary through the enhanced microbial metabolism of DOC with the greatest impact on C dynamics in the summer and fall months.

Further Comments on this Study

This study attempted to balance changes in C stocks between the OC and IC pools, however there were discrepancies between the concentration of C respired (BR) and the change in DOC (C metabolized). This may relate to the use of DO consumption as a proxy for BR with a RQ of 1:1. This RQ ratio is widely used in the literature to evaluate respiration (del Giorgio and Cole 1998; Gulis and Suberkropp 2003; Middelboe et al. 2012; Franke et al. 2013). This RQ could be a confounding factor associated with the

discrepancies in the C balance between BR and DOC bioassays and, as a result, the subsequent PE calculations. Berggren et al. (2012) found negative relationships between bacterioplankton RQ and O_2 saturation as well as pH. Due to the nature of the filtration process used to isolate DOC in the collected sample waters, initial DO was above 100% saturation. This may have influenced the respiratory quotient, potentially resulting in it falling below the assumed 1:1 ratio in my experimental incubations. The pH of the sample waters, exceeded 8 for all three sites, and the oxidation level for the OM present (not investigated), may have also played a complimentary role influencing the RQ in a similar fashion (Berggren et al. 2012).

Pollen leachate lability was assessed through bioassays in DI water with inorganic nutrient amendments (10 μM Nitrogen as NH_4^+ and 1 μM as NO_3^-, and 0.5 μM Phosphorus as PO_4^+) at concentrations similar to those found in the York (McCallister unpublished data). Bioavailability of the pollen leachate-C was ~90% in 28 days incubations (data not shown), suggesting the leachate was highly labile. The two end-member mixing model used to evaluate PE based on the isotopic signature of the bulk OM in WL amended incubations indicated that the pollen leachate's lability was ~99% potentially as a result of co-metabolism of the background DOM from WL. While the focus of this current study was on the priming of background DOM, in a natural system, the enhanced metabolism of primer C through co-metabolism of other substrates would indeed increase the total flux of C in general.

Complications

An attempt was made to alternatively calculate aquatic PE through differences in the isotopic signature of the respired C between treatments at each site, measured as aqueous DIC. Due to the high concentration of the background DIC pool present at the three sites, isotopic analysis was not sensitive enough to detect changes in the $\delta^{13}C$ isotopic signature. As a result, inferences could not be made regarding the relationship of pollen-leachate derived aqueous DIC relative to background C derived DIC in incubated sample waters. In future studies modern techniques such as the Respiratory Carbon Recovery System (ReCReS) should be used to isolate and capture the microbially produced CO_2 for isotopic analyses in an effort to better determine the fate of the OM processed in PE mediated fluxes (see McCallister et al. 2006b for methodological considerations).

$DO^{13}C$ samples for treatments and controls from CL and GP have not yet been analyzed. This is due to the complications associated with the analysis of $DO^{13}C$ water samples that have a salinity greater than ~ 2 ppt. All samples from CL and GP must be run on a TOC analyzer modified to run dilute, low concentration samples. G.G. Hatch Stable Isotope laboratory in Ottawa is the only facility that receives samples for this specific analysis and their laboratory is in the process of relocating. Presently, they are not taking samples until they can ensure the quality of the results generated from their system after it is reassembled.

Recommendations and Considerations for Future Aquatic Priming Effect Research

The characteristics and mechanisms of aquatic PE in natural systems are not well understood, nor are the environmental variables that may influence the nature and degree of their occurrence. Through this research, the potential for aquatic PE within the DOC pools along the salinity gradient of a temperate estuarine system was identified. Due to the necessary constraints of bioavailability incubations, the influence of environmental factors such as discharge and temperature on PE was not assessed. These variables play distinct roles in moderating the processing and transport of OM throughout transport in the estuary. While the isotopic data and the mixing model generated for the WL amended treatment group provided useful insights into the potential timescale of aquatic PE, the effect of temperature, and to a lesser extent discharge, may mediate the importance of PE in the York and in other systems.

Other important facets of aquatic PE that should be addressed in future research efforts include but are not limited to; (1) the role of limiting nutrient availability as a co-varying factor or inherent mechanism of aquatic PE, (2) how variations in OM size class and age may impact aquatic PE, and (3) how bacterial community composition relates to aquatic PE. It has been suggested that microbial nutrient mining may be an associated factor and in some instances a mechanism of PE in terrestrial systems (see Kuzyakov 2010 for a review), although the significance of such a relationship in aquatic PE has yet to be adequately determined (Franke et al. 2013; Guenet et al. 2013; Bengtsson et al. 2014). In reference to the second, Amon and Benner (1996) suggested that OM reactivity follows a continuum related to size and that fresh high molecular weight materials are among the

most bioavailable to microbial communities. As such, it is likely that the availability of fresh OM as POC and HMW DOC would undoubtedly influence aquatic PE in inland water systems. Lastly, the relationship between bacterial community composition and PEs certainly remains a principal "unknown". Whether functional groups within the bacterial community benefit one another due to specific enzyme production, the bacterial community at large produces enzymes capable of breaking down a greater variety of substrates as a result of energy subsidies, or a combination of both scenarios functioning simultaneously is debated in the literature (Fontaine et al. 2003, 2011; Blagodatskaya and Kuzyakov 2008; Guenet et al. 2010a; Bianchi 2011; Thiessen et al. 2013; Pascault et al. 2013; Chen et al. 2014). I would encourage future researchers to place emphasis on these subject areas in an effort to elucidate the role and significance of aquatic PEs in inland water systems.

Conclusion

This study, to the author's knowledge, represents the first empirical, intra-system analysis for aquatic PE in an estuarine system. PE was assessed in bioassays from the oligohaline Pamunkey River, and at mesohaline sites along the tidal York River estuary. The shift in the nature of PE across the system, from WL and CL, both positive PE, to GP, potentially negative PE, lends some support to the hypothesis of veritable PE "hot spots" within or along inland water systems (Guenet et al. 2010a; Bianchi 2011), and may be a factor associated with CO_2 outgassing and high pCO_2 levels in the York (Raymond et al. 2000). Further, the isotopic evidence from this study suggests that aquatic PE may take

place on timescales such as hours to days rather than weeks to months as documented in many terrestrial PE studies (Kuzyakov et al. 2000; Fontaine et al. 2007; Blagodatskaya and Kuzyakov 2008; Guenet et al. 2012). This is the first insight into the timeframe of aquatic PEs and their relative importance in relation to these transient natural inland water systems. The results of this study highlight the importance of sampling multiple sites in an effort to better understand PE at the system scale. Finally, aquatic PE may be an important biogeochemical factor mediating the flux of C in the York River estuary, and potentially inland waterways.

Results Figures

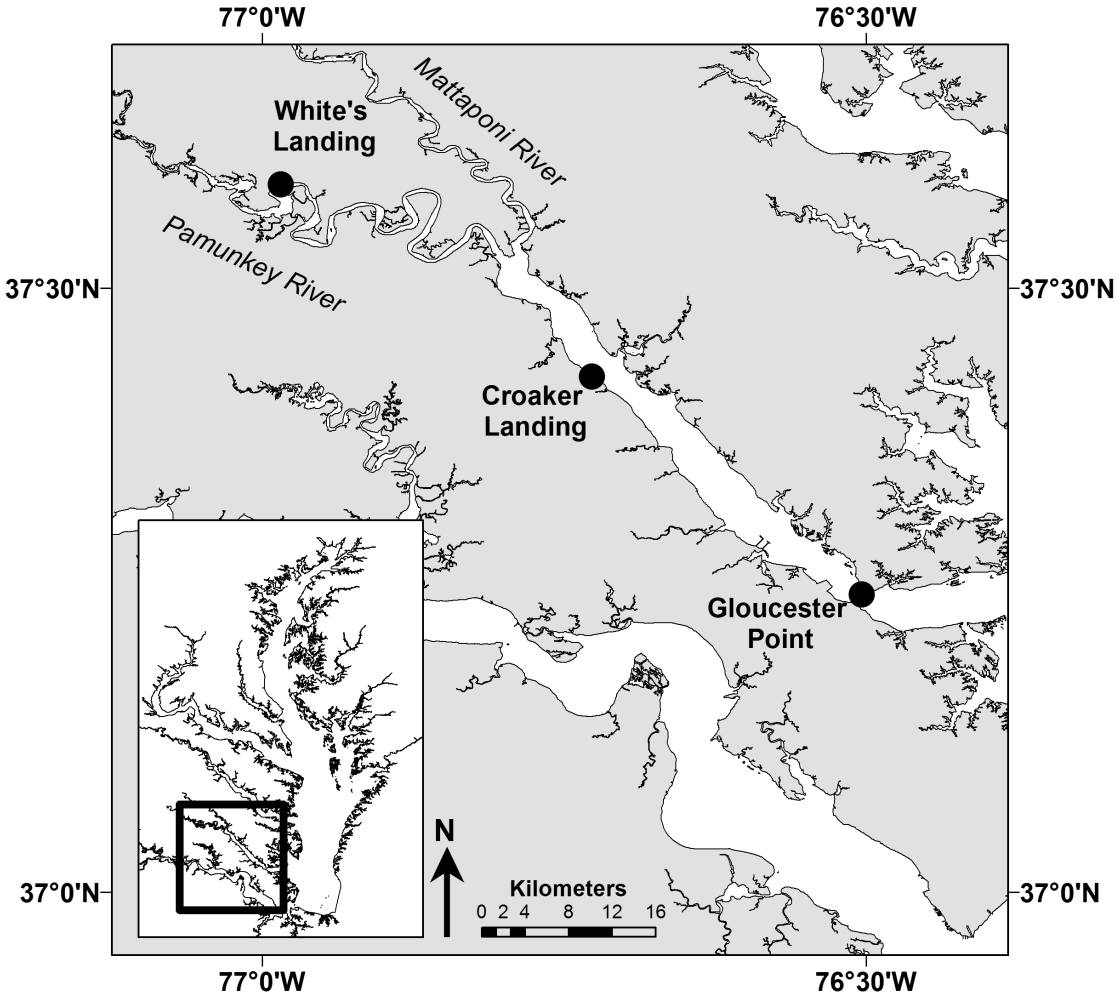

Figure 1. The York River Estuary and Sampling Site Locations. The map displays the York River Estuary and portions of the fresh water tributaries the Pamunkey River and the Mattaponi River in coastal Virginia. The black circles denote sampling site locations. Inset displays the York River estuary relative to the greater Chesapeake Bay.

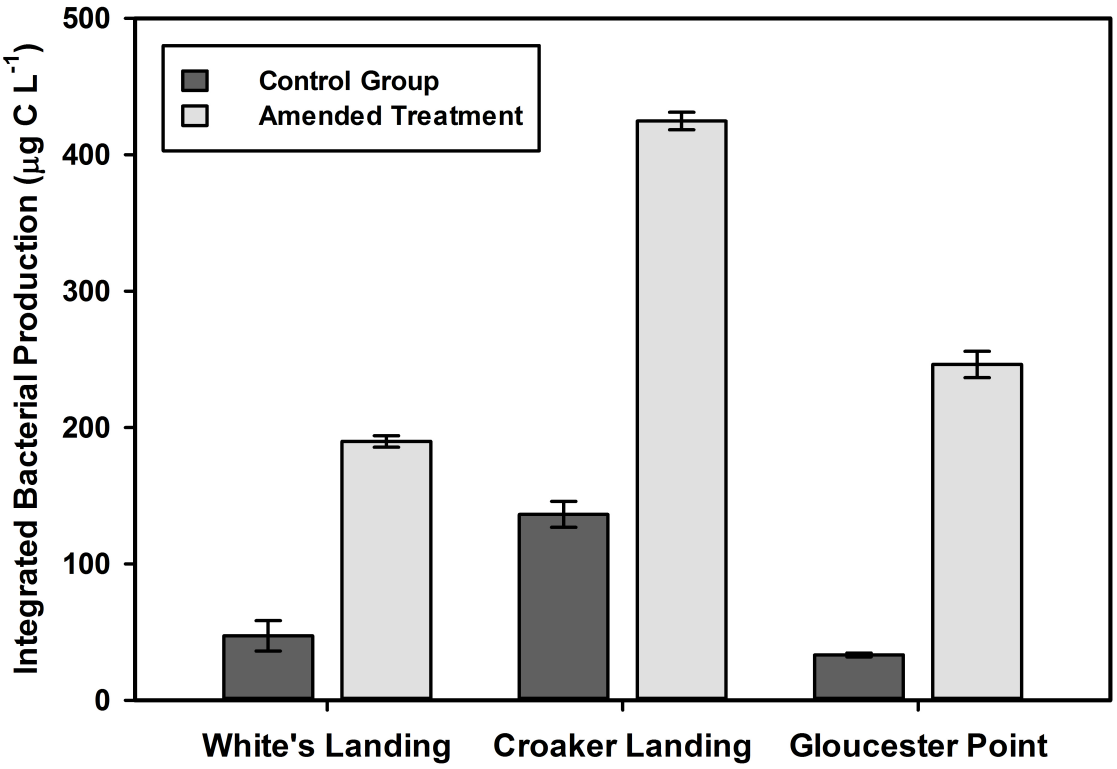

Figure 2. Integrated Bacterial Production after 48 hours. Side by side comparison of the integrated bacterial production for control (dark gray) and pollen carbon amended groups (light gray) for all three sites. Error bars represent ± 1 standard deviation of the mean.

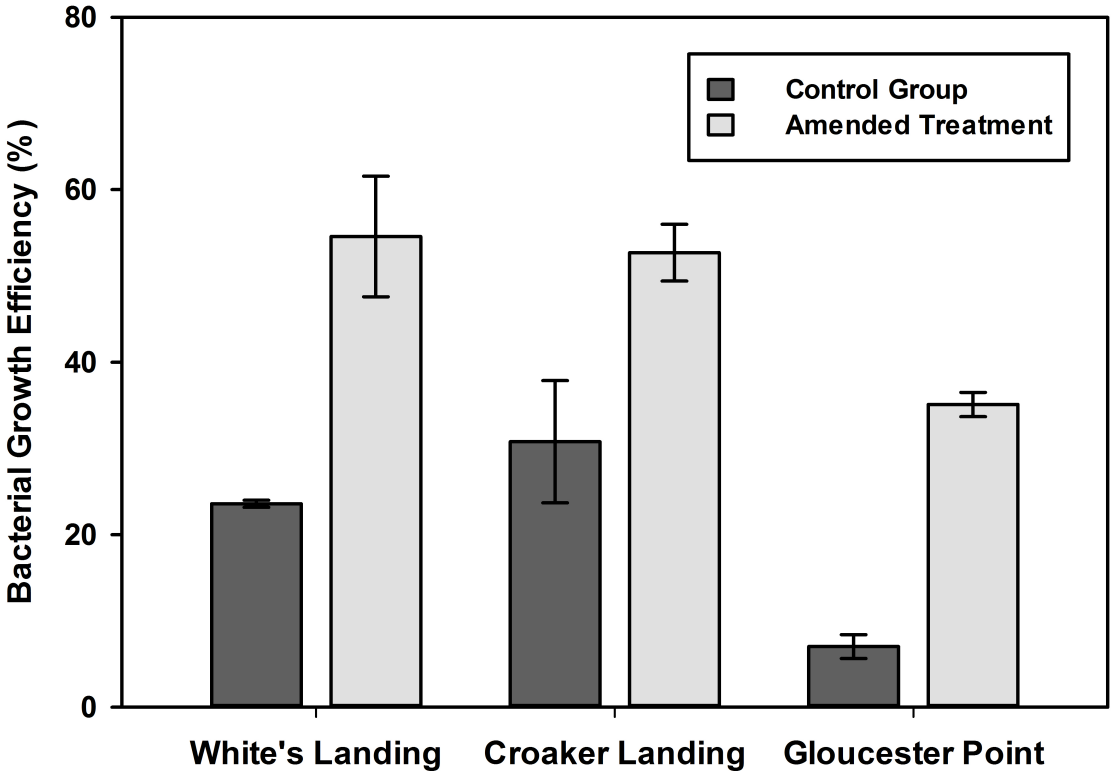

Figure 3. Bacterial Growth Efficiency after 48 hours. Side by side comparison of the bacterial growth efficiency for control (dark gray) and pollen carbon amended groups (light gray) for all three sites. Error bars represent ± 1 standard deviation of the mean.

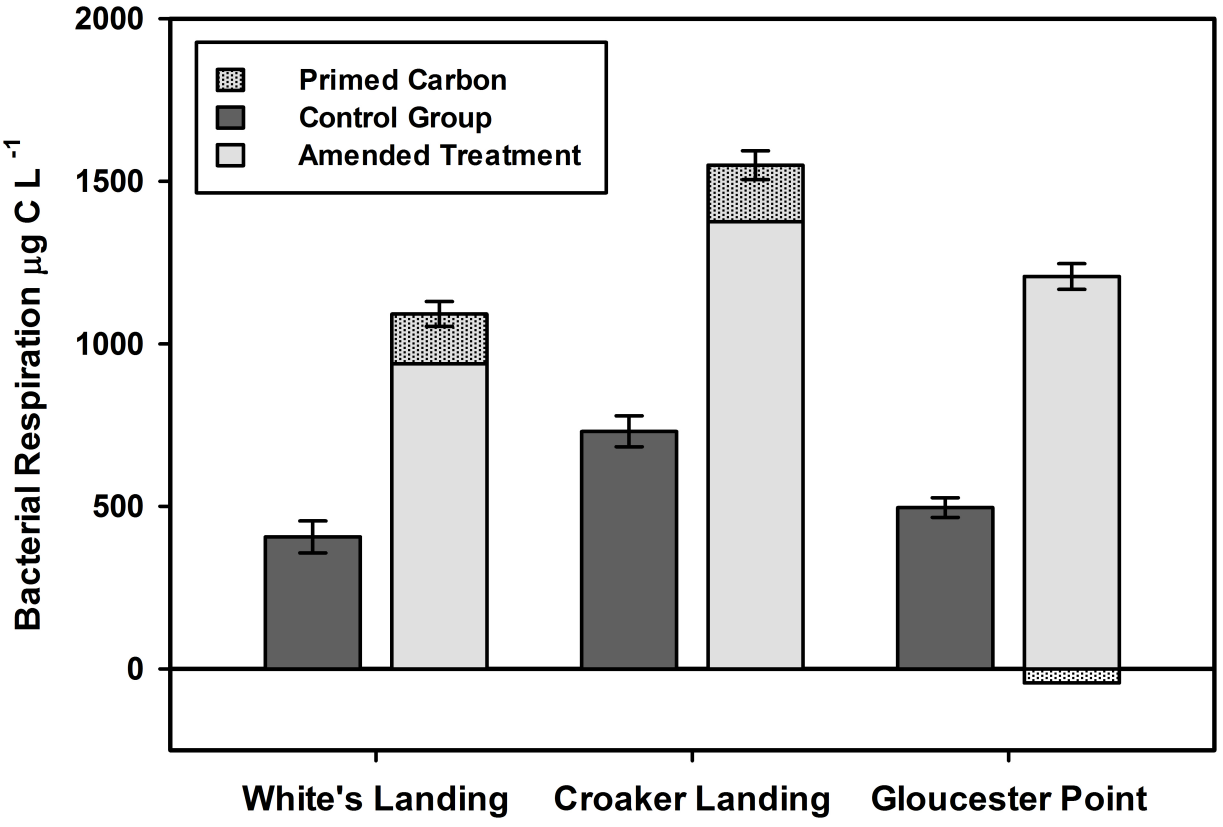

Figure 4. Total Bacterial Respiration and Conservative Priming Effects. Side by side comparison of the total bacterial respiration for control (dark gray) and pollen carbon amended groups (light gray) for all three sites after 28 days of incubation. Gray patterned sections represent the portion of primed carbon relative to the total respired C, as calculated using equation 2. Error bars represent ± 1 standard deviation of the mean of total bacterial respiration.

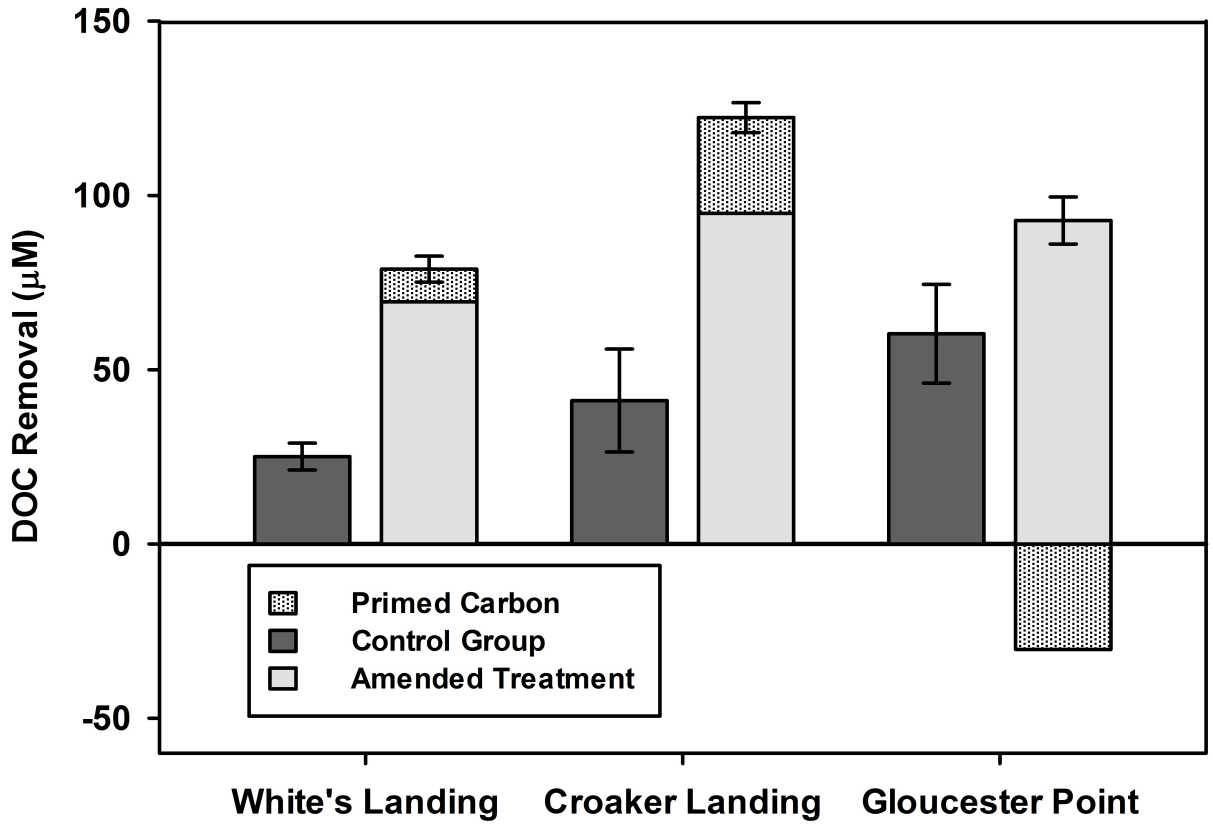

Figure 5. DOC Removal and Conservative Priming Effects. Side by side comparison of dissolved organic carbon removal for control incubations (dark gray) and pollen carbon amended groups (light gray) for all three sites after 28 days. Gray patterned sections represent the portion of primed carbon relative to the total, calculated using equation 2. Error bars represent ± 1 standard deviation of the mean of the total change in dissolved organic carbon.

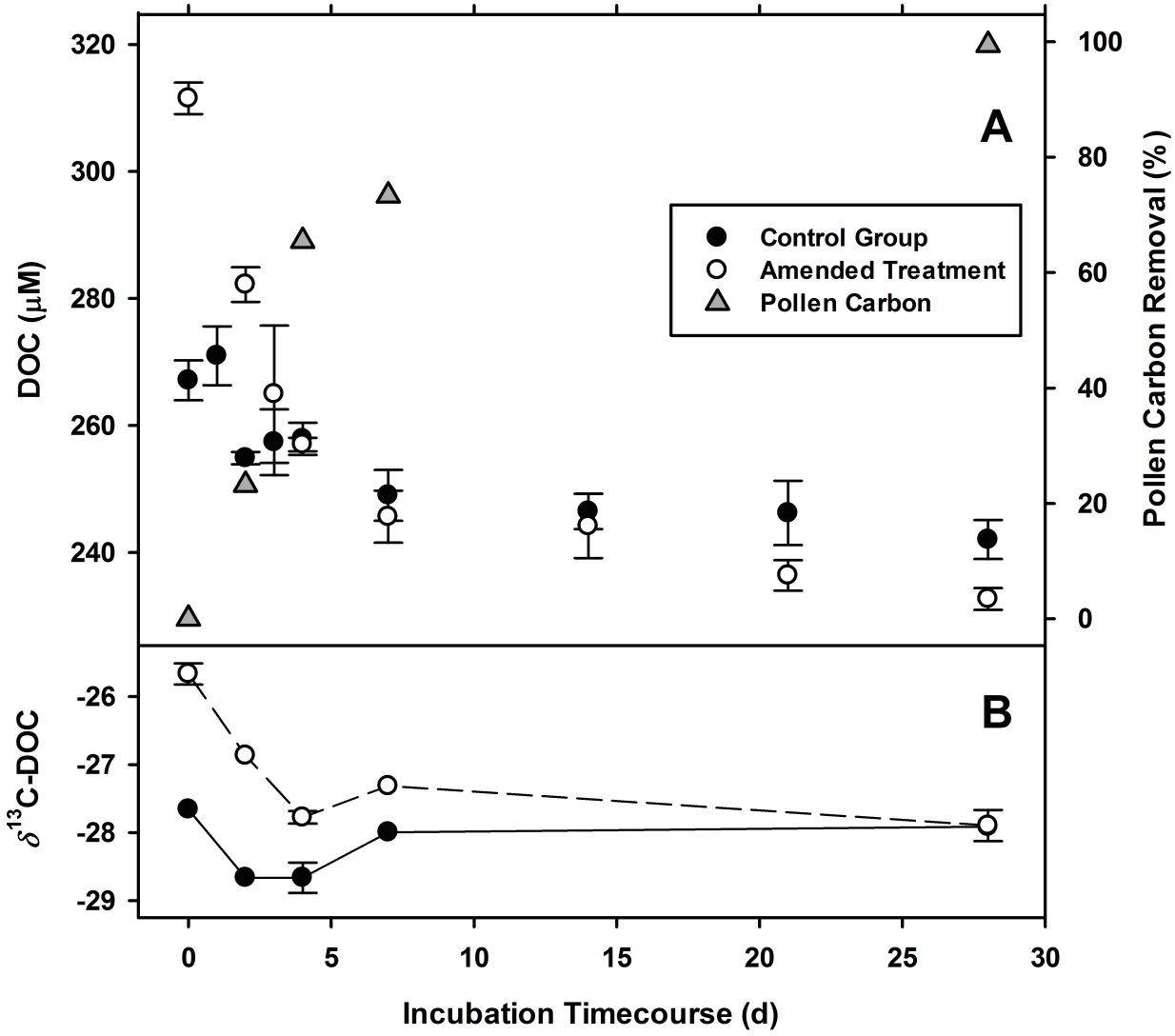

Figure 6. DOC Concentration, Pollen Carbon Removal, and the DOC Isotopic Signature of the White's Landing Groups. (A) Dissolved organic carbon concentration in White's Landing control incubations (black circles) and the pollen leachate-C amended incubation group (white circles) over the 28-day incubation period. Gray triangles represent pollen leachate carbon removal within the amended group as estimated through equations 3 & 4. **(B)** Isotopic signature of the dissolved organic carbon present in the control incubations (black circles), solely background carbon, and pollen leachate carbon amended group (white circles), representing a mixture of background carbon and pollen carbon, at select time points during the incubation period. All error bars represent ± 1 standard deviation of the mean. Calculated values for pollen have not associated error.

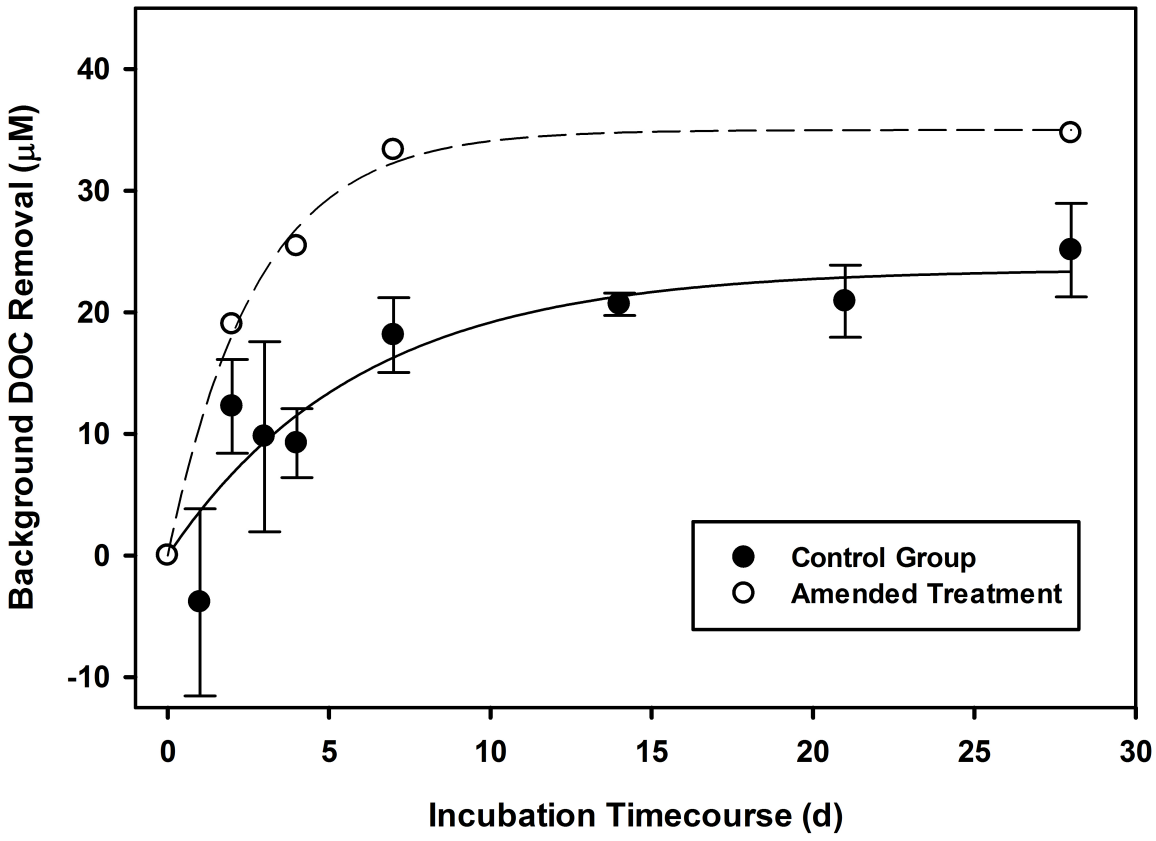

Figure 7. Background Carbon Removal in the White's Landing Groups. Dissolved organic carbon removal the in White's Landing control group (black circles) and the calculated values of background dissolved organic carbon removal in the amended group (white circles) at select time points over the 28-day incubation period. Error bars for the control group removal represent ± 1 standard deviation of the mean. Calculated values for background carbon have no associated error.

Table 1. York River Water Collection Site Characteristics

Site	Salinity*	pH*	Distance# (rkm)	Chlorophyll a (µg L^{-1})* Mean ± SD	DOC (µM)$ Mean ± SD
White's Landing	0.5	8.05	93	3.1 ± 0.2	267 ± 3
Croaker Landing	10	8.47	42	6.3 ± 0.4	336 ± 12
Gloucester Point	21	8.67	13	5.3 ± 0.9	245 ± 12

* *Assessed made relative to surface waters at each site at the time of sampling.*
# *Relative to the mouth of the estuary by river.*
$ *Assessments are reflective of the dissolved organic carbon in control incubations for each site.*

Table 2. Dissolved Organic Carbon Characteristics Incubation Groups

Sampling Site	Treatment Group	DOC (µM) Mean ± SD		|ΔDOC| (µM) Mean ± SD (%)*	Carbon Addition (%)$
		Initial	Final		
White's Landing	Control	267 ± 3	242 ± 3	25 ± 4 (9)	-
	Amended	312 ± 3	233 ± 2	79 ± 4 (25)	17
Croaker Landing	Control	336 ± 12	295 ± 3	41 ± 14 (12)	-
	Amended	390 ± 7	268 ± 7	122 ± 4 (31)	16
Gloucester Point	Control	245 ± 12	185 ± 3	60 ± 14 (25)	-
	Amended	308 ± 4	215 ± 6	93 ± 7 (30)	26

**Percentage as relative to the total initial concentration of dissolved organic carbon.*
#Calculated as the difference between initial concentrations in amended and control groups.
$Addition of pollen carbon received by treatment groups relative to the initial concentration of dissolved organic carbon in corresponding sites control groups.

Table 3. Conservative Priming Effect Assessments for BR and DOC Consumption[§]

Sampling Site	Treatment Group	Background Carbon Respiration			Background DOC Consumption			
		Respired Carbon[*$] (µM)	Primed Respiration (µM)[#]	Observed Priming Effect (%)[#]	DOC Consumed[*] (µM)	Primed DOC (µM)[#]	Observed Priming Effect (%)[#]	95% CI for Priming Effect (%)[&]
White's Landing	Control	34	-	-	25	-	-	-
	Amended	47	13	38	34	9	37	± 25
Croaker's Landing	Control	60	-	-	41	-	-	-
	Amended	75	15	24	69	27	67	± 36
Gloucester Point	Control	41	-	-	60	-	-	
	Amended	37	-4	-9	30	-30	-50	± 22

[§] *All assessments are in reference to total carbon after 28 days of incubation.*
[*] *Control values represent measured values as bacterial respiration and dissolved organic carbon concentration, amended treatments values represent the conservatively calculated estimates for priming using equation 2 wherein A=1.*
[$] *Respired carbon concentration assessments (µg C L^{-1}) have been converted to µM carbon for ease of comparison.*
[#] *Values correspond to background carbon metabolism relative to control metabolism in control groups.*
[&] *Intervals were assessed as the difference between the final concentrations of carbon in the control and amended treatments.*

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