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Biogeochemistry of Natural Organic Matter

Dissolved Organic Matter Composition in a Marsh-Dominated Estuary: Response to Seasonal Forcing and to the Passage of a Hurricane

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Key Points:
- Changes in DOM composition over a year at the Altamaha River and Sapelo Sound are strongly correlated with river discharge
- High river discharge conditions are characterized by DOM with stronger terrigenous signature and higher microbial utilization rates
- Passage of Hurricane Matthew resulted in large input of terrigenous DOM to Sapelo Sound, greatly increasing DOC concentrations

Supporting Information:
- Supporting Information S1

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Abstract Dissolved organic matter (DOM) is a large and complex mixture of compounds with source inputs that differ with location, season, and environmental conditions. Here, we investigated drivers of DOM composition changes in a marsh-dominated estuary off the southeastern United States. Monthly water samples were collected at a riverine and estuarine site from September 2015 to September 2016, and bulk, optical, and molecular analyses were conducted on samples before and after dark incubations. Results showed that river discharge was the primary driver changing the DOM composition at the mouth of the Altamaha River. For discharge higher than ~150 m^3/s, dissolved organic carbon (DOC) concentrations and the terrigenous character of the DOM increased approximately linearly with river flow. For low discharge conditions, a clear signature of salt marsh-derived compounds was observed in the river. At the head of Sapelo Sound, changes in DOM composition were primarily driven by river discharge and possibly by summer algae blooms. Microbial consumption of DOC was larger during periods of high discharge at both sites, potentially due to the higher mobilization and influx of fresh material to the system. The Georgia coast was hit by Hurricane Matthew in October 2016, which resulted in a large input of carbon to the estuary. The DOC concentration was ~2 times higher and DOM composition was more aromatic with a stronger terrigenous signature compared to the seasonal maximum observed earlier in the year during peak river discharge conditions. This suggests that extreme events notably impact DOM quantity and quality in estuarine regions.

Plain Language Summary Dissolved organic matter (DOM) is a crucial component of aquatic ecosystems and characterizing how its composition and concentration change is important to better understand the carbon cycle. Composition and quantity of DOM can vary spatially and temporally due to a variety of factors, including biological activity, precipitation patterns, and proximity to source inputs such as rivers, salt marshes, and the open ocean. To track these changes, monthly water samples were collected and analyzed over the course of a year in a marsh-dominated estuary off the Georgia coast, USA. River flow was shown to be an important factor controlling the amount and type of DOM present at both riverine (Altamaha River) and estuarine (Sapelo Sound) locations. In months with high river flow, organic matter contents were higher and had more terrestrially derived compounds compared to months with low river flow. Additionally, Hurricane Matthew was shown to significantly alter the organic matter at Sapelo Sound, suggesting that extreme events greatly impact DOM quantity and quality in estuarine regions.

1. Introduction

Dissolved organic matter (DOM) is a key component of the carbon cycle in aquatic settings. This DOM pool is critical for bacterial production, biogeochemical transformations, and nutrient availability, and it influences bacterial and phytoplankton community structure and functions (Crump et al., 2009). The amount of carbon held in the marine DOM pool is comparable to the atmospheric carbon pool (Walther, 2013), which makes it an important part of the global carbon cycle. This DOM can come from many sources, each source contributing organic matter that is unique in its composition and concentration (Hopkinson, 1985), resulting in thousands of different molecular compounds (Moran et al., 2016) and attributing a highly complex and variable chemical makeup to its composition. Allochthonous inputs to the DOM pool include terrestrial runoff, river discharge, and groundwater flushing (Aitkenhead-Peterson et al., 2003), whereas autochthonous inputs include phytoplankton metabolism and excretion, viral lysis, and releases

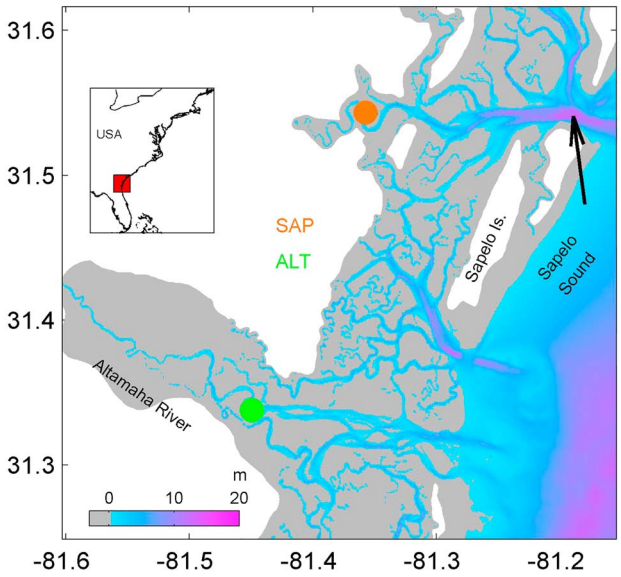

Figure 1. Sampling location at the head of Sapelo Sound (orange circle) and at the Altamaha River (green circle). Salt marshes and uplands are shown in gray and white, respectively. Colors indicate bottom topography.

associated with zooplankton grazing (Nagata, 2000). These differing inputs vary over seasonal and spatial gradients and add an additional layer of complexity to the problem of quantifying and characterizing the DOM pool.

Coastal Georgia, USA, is a unique setting due to its short but ecologically diverse coastline. There are five large rivers in the area, the Savannah, Satilla, St. Marys, Ogeecheee, and the Altamaha River. The Altamaha River watershed covers an area of 36,718 km^2 and is the third largest contributor of fresh water to the Atlantic Ocean from North America (Schaefer & Alber, 2007). Nearby saltmarsh areas along the estuary can vary substantially in their source inputs, DOM composition and processing (Moran et al., 1999). The Altamaha River has been shown to have a strong allochthonous terrestrial signature and a general low dissolved organic carbon (DOC) bioavailability over a time scale of a few days (Wiegner et al., 2006). Medeiros, Babcock-Adams, et al. (2017) observed that part of the DOC from the Altamaha River can be exported all the way to the South Atlantic Bight shelf break, especially in late spring.

The transport of terrestrial material to aquatic systems is often enhanced following major rainfall events, where the export of DOM can account for the majority of the annual carbon export budget (e.g., Inamdar et al., 2006; Raymond & Saiers, 2010). Yoon and Raymond (2012) reported an export of 43% of the annual DOC flux in only 5 days in a forested watershed in New York following Hurricane Irene. Similarly, 3 days following the passage of the same hurricane, both DOC and chromophoric dissolved organic matter (CDOM) nearly tripled in the Neuse River estuarine system in North Carolina (Miller et al., 2016). Because of possible changes in hurricane activity in the future (Bender et al., 2010), it is important to have a better understanding of how storms currently affect coastal watersheds and the transport and processing of material in associated aquatic systems.

Through the use of ultrahigh resolution mass spectrometry, in particular Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), the chemical nature of the DOM has been investigated (e.g., Kujawinski et al., 2002; McIntyre et al., 1997; Sleighter & Hatcher, 2007) and compared over different seasons (Herzsprung et al., 2017; Medeiros, Seidel, Dittmar, et al., 2015; Singh et al., 2014) and locations (Kim et al., 2006; Koch et al., 2005; Seidel et al., 2015). Here, we used untargeted approaches to investigate the molecular composition of riverine and estuarine DOM in a typical marsh-dominated estuary off the U.S. East Coast. Using an untargeted approach is useful because it is not known *a priori* which compounds (or classes of compounds) will dominate the changes in DOM composition in the system. We focused our analyses at the mouth of the Altamaha River and at the head of the Sapelo Sound estuary off Georgia (Figure 1). While the Altamaha site is directly influenced by a river, Sapelo Sound is located 25 km to the north and is characterized by a larger influence of local precipitation (Wang et al., 2017) and marine inputs, which can potentially increase the complexity of patterns in DOM changes. We identified the DOM molecular signatures that are associated with the different organic matter sources as well as changes in DOM composition and bacterial processing that arose from variations in hydrological conditions over a year, including the passage of Hurricane Matthew. Comparing the evolutions of DOC concentration and DOM composition in these two sites with contrasting characteristics allows for investigating the relative contributions of multiple drivers, including variations in hydrology and microbial biodegradation, to compositional changes in this marsh-dominated system.

2. Materials and Methods

2.1. Sample Collection

Monthly surface water samples were collected over the course of a year (September 2015 to September 2016) during high tide conditions at two locations, the Altamaha River (31.337°N, 81.449°W) and the head of Sapelo Sound (31.539°N, 81.423°W) in coastal Georgia, USA (Figure 1). These two sampling locations were chosen due to their distinct characteristics in terms of riverine and marine influence, despite their

geographical proximity. Temperature and salinity measurements were taken at the time of sampling and are shown in Table S1 in the supporting information. On 7 October 2016, approximately a month after the end of our year-long collection, Hurricane Matthew hit the coast of Georgia resulting in strong rainfall and in a storm surge of >2 m. Five days following the passage of the hurricane, a sample was collected at Sapelo Sound site after a change of water color was noticed in several parts of the estuary.

River discharge data were obtained from the U.S. Geological Survey (http://waterdata.usgs.gov) at the nearest monitoring station at Doctortown, GA, roughly 20 km upstream from the Altamaha River sampling site. Precipitation data were collected at Sapelo Island (Figure 1) as part of the Georgia Coastal Ecosystem Long Term Ecological Research program. Water level measurements for Fort Pulaski, GA, located about 70 km to the north of Sapelo Island, were obtained from National Oceanic and Atmospheric Administration (https://tidesandcurrents.noaa.gov).

2.2. Sample Extraction and Dark Incubations

Immediately after collection, inorganic nutrients (20 μM Na_2PO_4; 50 μM NH_4Cl) were added to all samples prior to filtration in order to sustain microbial communities through long-term incubations (see below). To remove any photosynthetically active organisms, triplicate samples were filtered through 0.7 μm Whatman GF/F filters (precombusted at 450 °C for 5 hr) into acid-washed 1 L polycarbonate bottles. Samples referred to as T_0 were then filtered through prewashed 0.2 μm Pall Supor membrane filters into 60 mL amber bottles for DOC and CDOM analyses. Samples for DOC and CDOM analyses were immediately frozen (−20 °C) and refrigerated (4 °C), respectively, and analyzed within 5 days. The remaining filtrates of T_0 samples (~1 L) were acidified to pH 2 (concentrated HCl), and DOM was extracted using solid phase extraction (SPE) with cartridges filled with a styrene divinyl benzene polymer (Agilent Bond Elut PPL) as described by Dittmar et al. (2008). The DOM extracts (SPE-DOM) from each month were eluted using methanol, concentrated using ultrapure nitrogen gas, and stored at −20 °C in the dark for FT-ICR MS analysis that were pursued at the end of the field sampling. Additional triplicate riverine and estuarine samples underwent in-lab dark incubations to track the temporal bacterial degradation. Samples were filtered through 0.7 μm Whatman GF/F filters and incubated during 2-, 5-, 10-, 20-, and 80-day intervals at temperature of collections. After incubations, samples were filtered through 0.2 μm Pall Supor membrane filters, collected, and stored for DOC and CDOM analyses as described previously. Samples collected in October 2015 and January, April, and July 2016 after the 80-day incubation (T_{80}) were also analyzed using FT-ICR MS.

2.3. Bulk DOC

Concentrations of DOC from both water samples and SPE-DOM (completely dried and resuspended in ultrapure water) were measured with a Shimadzu TOC-L_{CPH} analyzer using potassium hydrogen phthalate as a standard for the DOC calibration curve. Prior to and alongside sample analysis, both internal blanks and Milli-Q water blanks were run on the instrument. Analytical accuracy and precision were tested against the Consensus Reference Material (Hansell, 2005) and were better than 5%. SPE extraction efficiency across all samples, defined as DOC concentration in the SPE extract versus DOC concentration in the original sample (Seidel et al., 2014), was 74 $\pm$ 5% of the DOC. Bacterial utilization of DOC was determined as

$$[(DOC_{T0}-DOC_{T80})/DOC_{T0}\times 100] \quad (1)$$

where DOC_{T80} is the concentration of DOC after 80-day incubations were complete and DOC_{T0} is the initial concentration before incubations. Additional DOC measurements have been collected at the Altamaha River site from October 2000 to April 2009 as part of the Georgia Coastal Ecosystem Long Term Ecological Research monitoring efforts. The time interval between sampling during that period was 5.5 $\pm$ 6.6 days (Medeiros, Babcock-Adams, et al., 2017).

2.4. Chromophoric DOM

Absorbance measurements of water samples were taken at room temperature on an Agilent 8453 UV-visible spectroscopy system. Prior to sample measurement, blank calibrations were performed with Milli-Q water to achieve a baseline background reading. Absorbance was measured from wavelengths 190 to 1,100 nm and was converted to absorption coefficients as in D'Sa et al. (1999).

Spectral slope ($S_{275\text{-}295}$) was calculated for the absorbance spectra between 275 and 295 nm as

$$\alpha_g(\lambda) = \alpha_g\ (\lambda_{ref}) e^{-S(\lambda - \lambda ref)} \quad (2)$$

where $\alpha_g(\lambda)$ is the absorption coefficient of CDOM at each wavelength, λ_{ref} is a reference wavelength of 275 nm, and S is the slope fitting parameter (Helms et al., 2008; Spencer et al., 2008). Spectral slope has been shown to have a correlation with DOM molecular weight and a negative correlation with terrigenous DOM (Fichot & Benner, 2012; Helms et al., 2008). The ratio of absorbance at $\lambda = 250$ nm to $\lambda = 365$ nm ($a_g(250)$:$a_g(365)$) was also calculated for each sample; this ratio is used as an inverse proxy for DOM aromaticity and molecular weight (Peuravuori & Pihlaja, 1997).

2.5. FT-ICR MS Analysis

The molecular composition of the DOM extracts (200 mg C/L in methanol) was analyzed on a 9.4 T FT-ICR MS with electrospray ionization (negative mode) at the National ICR Users' Facility at the National High Magnetic Field Laboratory (Florida State University, Tallahassee, FL). Sample processing was done as described in Vorobev et al. (2018). A total of 150 scans was accumulated for each sample. Each m/z spectrum was internally calibrated with respect to an abundant homologous alkylation series whose members differ in mass by integer multiples of 14.01565 Da (mass of a CH_2 unit) confirmed by isotopic fine structure (Savory et al., 2011), achieving a mass error of <0.4 ppm. Molecular formulae were assigned for masses in the range of 150 and 750 Da by applying the following restrictions: $^{12}C_{1\text{-}130}\ ^{1}H_{1\text{-}200}\ O_{1\text{-}150}\ ^{14}N_{0\text{-}4}\ S_{0\text{-}2}\ P_{0\text{-}2}$. Assignment of molecular formulae was performed by Kendrick mass defect analysis (Wu et al., 2004) with PetroOrg software (Corilo, 2015) and using the criteria described by Rossel et al. (2013). Only compounds with a signal-to-noise ratio of 6 or higher were used in the analysis to eliminate intersample variability based on peaks that were close to the limit of detection. The peak intensity of each molecular formula was normalized to the sum peak intensities of the total identified peaks in each sample. Peaks with molecular formulae assigned accounted on average for ~90% of the sum of the intensities of all peaks in the final spectra. Repeated analysis of several of these samples revealed that differences in DOM composition due to instrument variability were substantially smaller than variability between samples.

2.6. Statistical Analyses

The variability of DOM molecular composition at each location was analyzed using principal component (PC) analysis of the FT-ICR MS data. All peaks with molecular formulae assigned were used in the PC analysis. All modes shown here are significantly different (95% confidence level) from results obtained by pursuing a PC analysis of random processes that are spatially and temporally uncorrelated. This indicates that the signals in the modes described here are significantly greater than the level of noise (Overland & Preisendorfer, 1982). Spearman's rank correlation analysis (α level 0.05) was used to test correlations between environmental conditions, bulk (DOC concentrations and DOC biodegradation rates), and optical (spectral slope and $a_g(250)$:$a_g(365)$) parameters as well as DOM molecular composition (FT-ICR MS). The Wilcoxon rank-sum test was used for comparisons between samples, as in Osterholz et al. (2016).

3. Results and Discussion

The Altamaha River discharge is characterized by strong seasonality, generally peaking in March or April and reaching a seasonal minimum during fall (Medeiros, Babcock-Adams, et al., 2017). The peak in discharge in 2016 occurred a few months earlier in January and February (Figure 2a), however, due to increased rainfall associated with the positive phase of the El Niño–Southern Oscillation (Hansen et al., 1997; Keener et al., 2010).

3.1. Seasonality of Riverine and Estuarine DOM Composition

Time series of DOC concentrations and of optical characteristics for the Altamaha River and for Sapelo Sound are shown in Figures 2 and 3, respectively. Concentrations of DOC at the Altamaha River ranged from 362 to 965 μM (Table S1) and were strongly modulated by river discharge ($r = 0.87$). Peak concentrations were observed in winter and early spring, when river discharge was high (Figures 2a and 2b and Table S1). The maximum correlation between river discharge and DOC concentrations occurred with no lag, indicating a rapid response to pulses in river flow. Climatologies of river discharge and

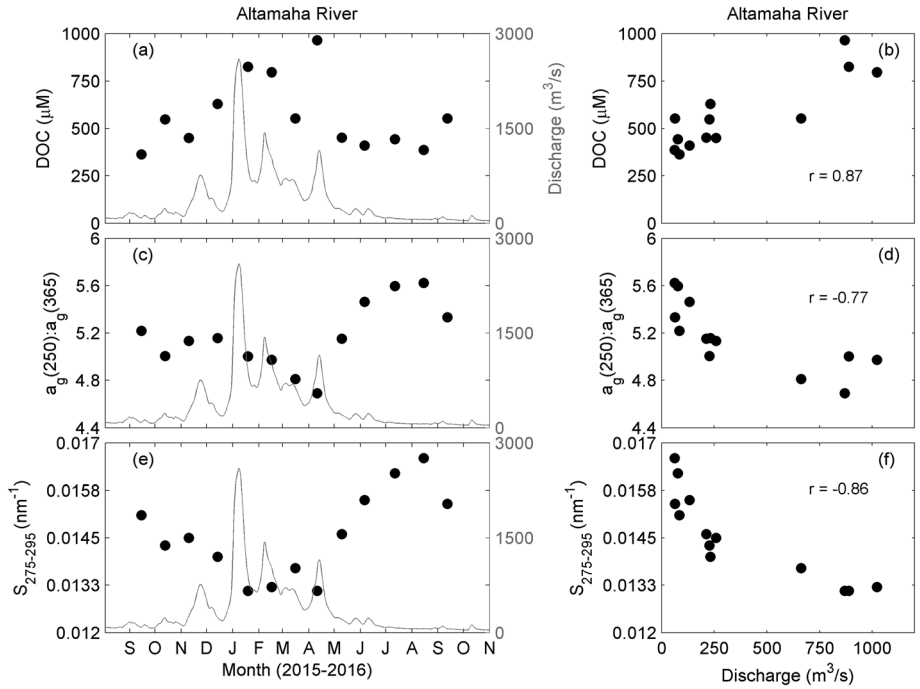

Figure 2. Time series of (a) dissolved organic carbon (DOC) concentration, (c) ratio of absorbance at $\lambda = 250$ nm to $\lambda = 365$ nm ($a_g(250):a_g(365)$), and (e) spectral slope of absorbance spectra between $\lambda = 275$ nm and $\lambda = 295$ nm ($S_{275\text{-}295}$) at the Altamaha River. River discharge is shown in gray. Scatterplots and correlation coefficients between parameters and river discharge are shown on the right panels (b, d, and f).

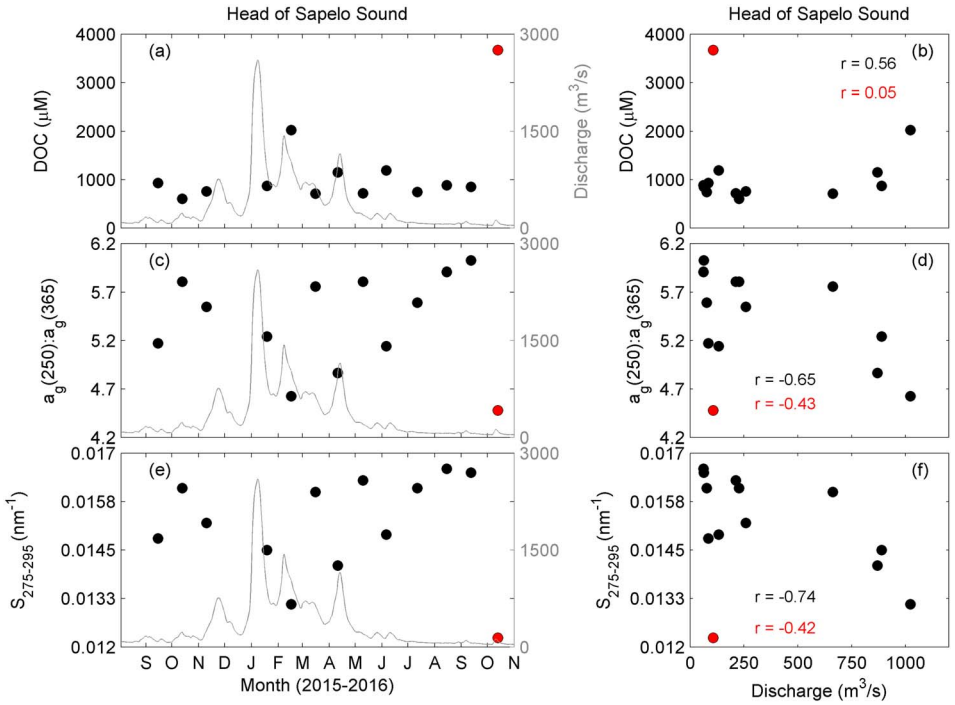

Figure 3. Time series of (a) dissolved organic carbon (DOC) concentration, (c) ratio of absorbance at $\lambda = 250$ nm to $\lambda = 365$ nm ($a_g(250):a_g(365)$), and (e) spectral slope of absorbance spectra between $\lambda = 275$ nm and $\lambda = 295$ nm ($S_{275\text{-}295}$) at the head of Sapelo Sound. River discharge is shown in gray. Scatterplots and correlation coefficients between parameters and river discharge are shown on the right panels (b, d, and f). Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm.

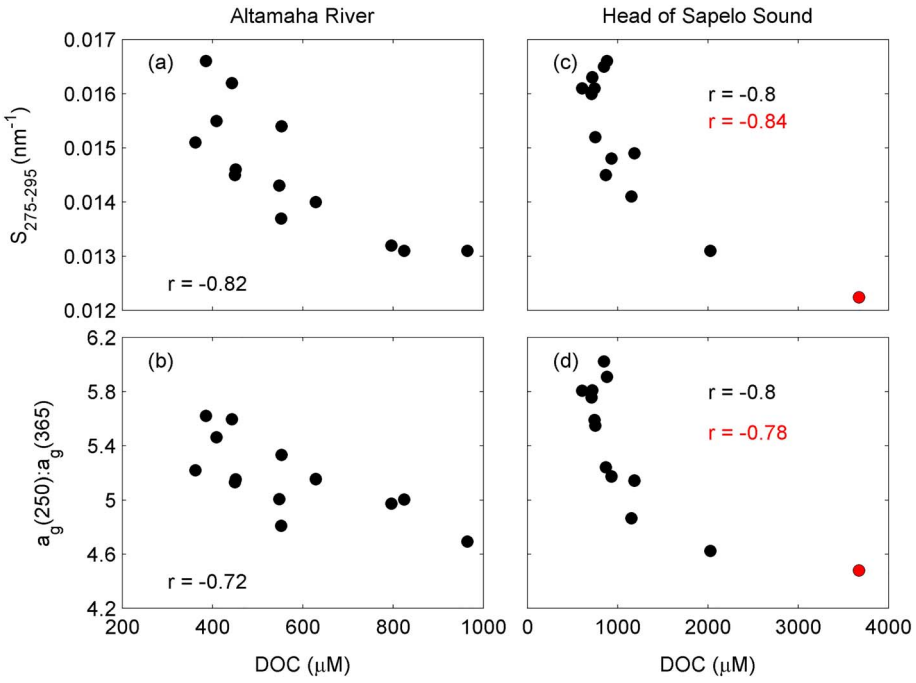

Figure 4. Scatterplot of dissolved organic carbon (DOC) concentration and (a, c) spectral slope ($S_{275-295}$) and (b, d) ratio of absorbance at $\lambda = 250$ nm to $\lambda = 365$ nm ($a_g(250):a_g(365)$) for (left) Altamaha River and (right) head of Sapelo Sound. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm.

DOC concentration at the Altamaha River, built using observations from 2000 to 2009, are also correlated ($r = 0.63$, $p < 0.05$), indicating that this is a robust pattern (Figure S1). Strong relationships between river discharge and DOC concentrations have been reported for other riverine and watershed systems (e.g., Raymond & Saiers, 2010; Ward et al., 2013).

The seasonal change in DOC concentration at the Altamaha River was accompanied by changes in DOM composition as determined by optical properties (Figures 2c–2f). During high discharge conditions, both the $a_g(250):a_g(365)$ ratio and the spectral slope parameter ($S_{275-295}$) at the Altamaha River decreased ($r = -0.77$, $p < 0.05$ and $r = -0.86$, $p < 0.05$, respectively). Previous studies have revealed that the $a_g(250):a_g(365)$ ratio is related to changes in the aromaticity and molecular size of the DOM, with decreasing values indicating higher aromaticity and higher molecular size (Peuravuori & Pihlaja, 1997). Spectral slope ($S_{275-295}$) has been shown to be closely related to DOC-normalized lignin yields in rivers and thus to be a good tracer of terrigenous DOM (Fichot & Benner, 2012). Shallower slopes indicate a higher terrigenous signature with a higher aromatic content and higher molecular weight (Del Vecchio & Blough, 2002; Helms et al., 2008). Concentration of DOC at the Altamaha River was correlated with both $S_{275-295}$ ($r = -0.82$, $p < 0.05$) and $a_g(250):a_g(365)$ ($r = -0.72$, $p < 0.05$; Figures 4a and 4b), suggesting that the increase in DOC concentration observed during high river flow conditions was at least in part related to terrigenous DOM input of high molecular weight aromatic compounds. The seasonal evolution of DOC concentration and DOM composition at the Altamaha River was consistent with the evolution reported for other riverine systems (e.g., Yukon River, Spencer et al., 2009; Kolyma River, Mann et al., 2012). Concentration of DOC and optical characteristics were not correlated with local precipitation ($p > 0.05$).

At the head of Sapelo Sound, both DOC concentration and DOM composition were also correlated with river discharge, as long as the sample collected in October 2016 shortly after the passage of Hurricane Matthew was not considered in the analysis. The magnitudes of the correlations between river discharge and DOC or optical parameters were somewhat lower than in the Altamaha River, ranging between 0.56 and 0.74 (Figure 3). Similarly to results from the Altamaha River, high river discharge resulted in higher DOC concentrations, which were associated with a stronger terrigenous signature and with higher

aromaticity and molecular weight (Figures 4c and 4d). Even though the Altamaha River is located farther south (see Figure 1), Wang et al. (2017) showed that increased river discharge leads to decreased salinity over the entire estuarine area, including at Sapelo Sound. The lower correlation coefficients indicate that the control of DOM composition variability was more complex at Sapelo Sound, however, with factors other than river discharge presumably playing a larger role than at the Altamaha River. Precipitation was not found to be correlated with DOC concentration or with optical properties at the head of Sapelo Sound. This is surprising, since salinity at that location has been shown to be correlated with precipitation data convoluted with a one-sided, exponentially decaying filter (Austin & Barth, 2002) with a decay scale of 26 days (Wang et al., 2017). Although tidal variation likely plays a role in the variability of DOM composition at the head of Sapelo Sound, that process cannot be resolved by our sampling, which was restricted to high tide conditions.

The sample collected at the head of Sapelo Sound in October 2016 was substantially different from those collected in previous months, indicating that the passage of Hurricane Matthew had a dramatic effect in the system. Despite the low river discharge at that time, DOC concentration reached ~3,700 μM, which is almost twice as high as the seasonal maximum that occurred earlier in the year in February and is approximately 4 times higher than the average concentration for the year (Figure 3). Optical characteristics also indicated a considerable input of highly aromatic terrigenous material shortly after the passage of the hurricane, even though river discharge was at a seasonal minimum.

3.2. Molecular Characterization of DOM Composition

Composition of DOM was also investigated at the molecular level using FT-ICR MS analysis. Over 6,000 molecular formulae were assigned to the complex DOM mixture. PC analysis was used to identify the dominant modes of variability in DOM composition in the system. All initial samples (T_0) from each location were used in the PC analysis, covering 13 months at the Altamaha River and 14 at the head of Sapelo Sound.

At the Altamaha River, the dominant PC accounted for 23% of the total variance in DOM composition in the system. PC 1 scores were highest from January to April and were lowest from June to September (Figure 5a), which is consistent with the time variability in river discharge (see Figure 2). PC 1 was correlated with $S_{275\text{-}295}$ ($r = -0.93$, $p < 0.05$; Figure 5d), suggesting that PC 1 was related to the terrigenous character of the DOM. This is supported by analysis of a van Krevelen diagram of the loading of PC 1, which showed a tendency for high positive loadings (i.e., red dots in Figure 5b) to cluster at low H/C ratios. Terrigenous DOM is generally enriched with formulae with low H/C ratios (Medeiros, Seidel, Ward, et al., 2015; Sleighter & Hatcher, 2008), which are indicative of more aromatic compounds (Kim et al., 2003; Koch & Dittmar, 2006, 2016). Thus, from January to April (i.e., when PC 1 scores are positive), the Altamaha DOM was enriched with compounds with more terrigenous characteristics, corroborating the results obtained based on optical analysis (Figure 2).

At months when the PC 1 score is negative, the DOM at the Altamaha River was relatively enriched with molecular formulae with negative loading of PC 1 (i.e., blue dots in Figure 5b). Marshes have been shown to be important sources of DOC to estuaries (e.g., Bauer et al., 2013; Moran & Hodson, 1994; Peterson et al., 1994). Medeiros, Seidel, Dittmar, et al. (2015) compared the DOM composition in water immediately before and after exposure to a nearby marsh to identify changes in DOM composition that were associated with the addition of new organic compounds and with transformation processes occurring in the marsh. The molecular formulae enriched after marsh exposure (see Figure 4 in Medeiros, Seidel, Dittmar, et al., 2015) occupied a region in van Krevelen space similar to that occupied by formulae with negative loading of PC 1 (Figure 5b). This suggests that during low discharge conditions, when PC 1 is negative, the DOM at the Altamaha River was imprinted with the signature of marsh-derived compounds. A quantitative assessment of this input can be obtained by selecting the 506 molecular formulae that were identified by Medeiros, Seidel, Dittmar, et al. (2015) as being enriched after marsh exposure and quantifying their contribution to the total intensity of the sum of all peaks with molecular formulae assigned for samples collected at the Altamaha River. The relative contribution of marsh-derived compounds at the Altamaha River (Figure 5c) approximately mirrored the PC 1 score (Figure 5a), suggesting that the increase in the relative abundance of formulae with negative loading of PC 1 (blue dots in Figure 5b) from June to September was possibly related to inputs from salt marshes.

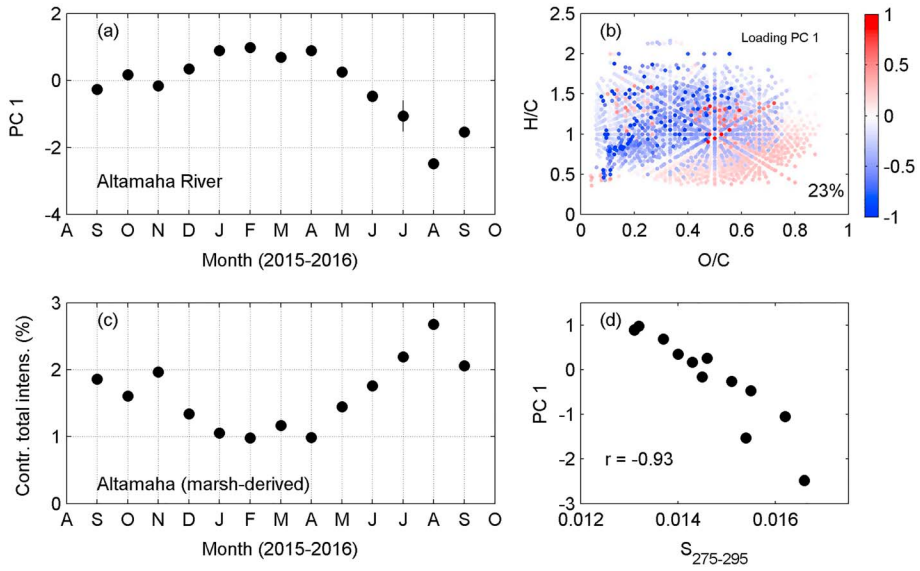

Figure 5. Principal component (PC) analysis of dissolved organic matter (DOM) composition at the Altamaha River. (a) Time series of first principal component. (b) Van Krevelen diagram with loading of PC 1 color coded. (c) Percentage contribution of marsh-derived compounds to the sum of the magnitude of all peaks with molecular formula assigned in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) spectra. Marsh-derived compounds identified by Medeiros, Seidel, Dittmar, et al. (2015). (d) Scatterplot of spectral slope $S_{275\text{-}295}$ and scores of PC 1 (from panel a).

Collectively, these results indicated that during high river discharge conditions, the DOM at the Altamaha River became more aromatic due to the input of terrigenous material. During low discharge conditions, on the other hand, the terrigenous signature of the DOM decreased, and the relative importance of marsh-derived compounds increased. A scatterplot of river discharge versus PC 1 scores revealed a large change in the slope of the curve at about 150 m^3/s (Figure 6). Marsh-derived compounds made an increasingly important contribution for the DOM composition when river flow was lower than that threshold. Indeed, marsh-derived compounds have been previously shown to imprint a distinct signature on the riverine DOM during drought conditions (Medeiros, Seidel, Dittmar, et al., 2015). For river discharge larger than ~150 m^3/s, the signature of marsh-derived compounds was presumably overwhelmed by the input of terrigenous DOM. In that limit of high discharge, the terrigenous character of the DOM increased approximately linearly with river flow.

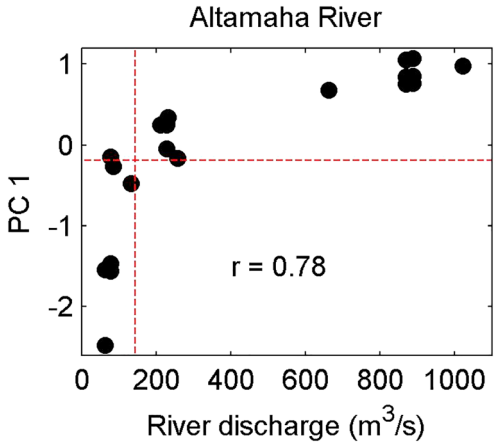

Figure 6. Scatterplot of Altamaha River discharge and scores of first principal component (PC) of dissolved organic matter (DOM) composition at the Altamaha River (from Figure 5a). Red dashed lines emphasize point where large change in the slope of the curve is observed.

Two dominant modes of variability in DOM molecular composition were statistically significant ($p < 0.05$) at the head of Sapelo Sound (Figure 1), and they each explained approximately the same fraction of the total variance (25% and 21% for PCs 1 and 2, respectively). We begin by presenting and discussing results from the second mode (Figures 7c and 7d), which are comparatively easier to interpret. The time series of PC 2 scores (Figure 7c) was correlated with river discharge (especially if the sample influenced by Hurricane Matthew is neglected; Figure 8a), and it was highly correlated with $S_{275\text{-}295}$ (Figure 8b). The pattern captured by the loading of PC 2 (Figure 7d) was typical of terrigenous/marine gradients in DOM composition, and it has been previously observed in river to ocean transects in this (Medeiros, Babcock-Adams, et al., 2017; Medeiros, Seidel, Gifford, et al., 2017) and in other river systems such as the Amazon River plume (Medeiros, Seidel, Ward, et al., 2015). It is also consistent with the pattern of variability observed along a river to ocean transect at the lower Chesapeake Bay, where H/C ratios were found to increase from more riverine to more oceanic samples (Sleighter & Hatcher, 2008). Thus, during high discharge conditions, DOM at the

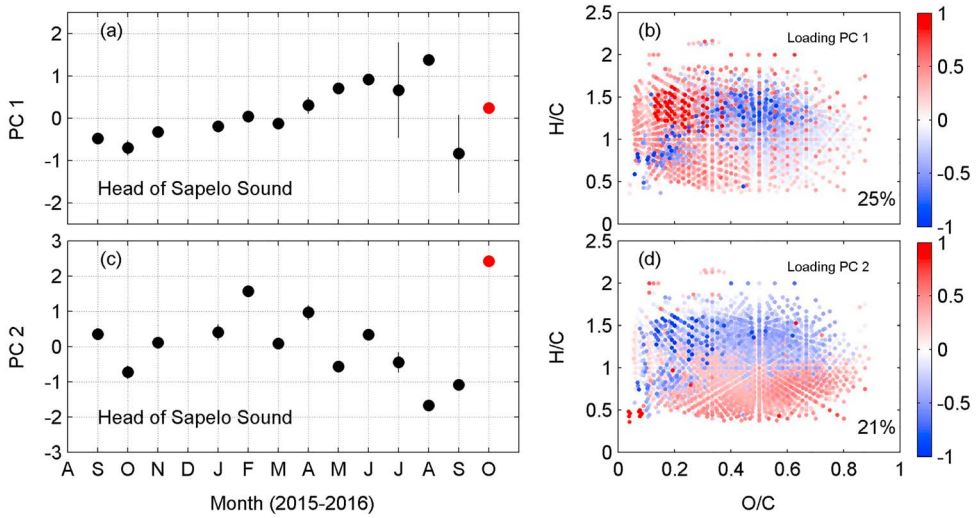

Figure 7. Principal component (PC) analysis of dissolved organic matter (DOM) composition at the head of Sapelo Sound. Time series of (a) first and (c) second principal components. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Van Krevelen diagrams with loading of (b) PC 1 and (d) PC 2 color coded.

head of Sapelo Sound had a stronger terrigenous signature. Although river discharge remained low after the passage of Hurricane Matthew (Figure 3), the storm resulted in a substantial input of terrigenous DOM to the system (Figure 7c). In contrast to the Altamaha River site, however, the DOM at the head of Sapelo Sound had a more marine signature (instead of marsh-derived signature) when discharge was low. Time series of salinity measured during the sampling period (Table S1) indicated that salinity at the Altamaha River station hovered around zero year-round, indicating that the input of marine DOM should indeed be small. At Sapelo Sound, on the other hand, salinity varied from 10–13 during peak discharge to around 30–32 when river flow was at a minimum in early fall. This was consistent with a larger contribution of marine DOM during that period (Figures 7c and 7d).

Results from PC 1 at Sapelo Sound are more difficult to interpret. PC 1 scores were either approximately 0 or negative over the entire period, except during summer (May to August 2016) when they were positive (Figure 7a). The time series was not correlated with river discharge or with precipitation. The pattern revealed by the loading of PC 1, shown color coded in a van Krevelen diagram (Figure 7b), was different from the pattern typically observed in river to ocean transects (Medeiros, Babcock-Adams, et al., 2017; Medeiros, Seidel, Dittmar, et al., 2015; Medeiros, Seidel, Gifford, et al., 2017; Medeiros, Seidel, Ward, et al., 2015; see

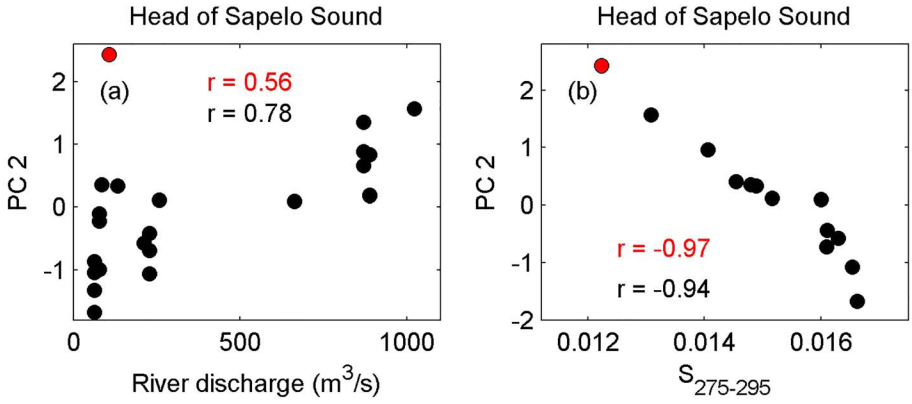

Figure 8. (a) Scatterplot of river discharge and score of PC 2 (from Figure 7c) at the head of Sapelo Sound. (b) Scatterplot of spectral slope $S_{275\text{-}295}$ and score of PC 2. Sample collected shortly after the passage of Hurricane Matthew in October 2016 is shown in red. Correlation coefficients shown in red include sample collected shortly after the storm. PC = principal component.

also Figure 7d). This suggests that the mode was not related to the varying contribution of terrigenous vs marine sources to the estuarine DOM pool.

Several additional processes are known to transform the DOC pool and to result in changes in DOM composition in aquatic environments, including photochemical reactions (e.g., Chen et al., 2014; Medeiros, Seidel, Powers, et al., 2015; Stubbins et al., 2010), microbial degradation (e.g., Kujawinski et al., 2004; Moran & Zepp, 1997; Obernosterer & Benner, 2004; Seidel et al., 2015), inputs from phytoplankton (e.g., Landa et al., 2014; Medeiros, Seidel, Ward, et al., 2015), and flocculation (e.g., Hernes & Benner, 2003; Sholkovitz et al., 1978). The molecular signatures of DOM transformations due to some of these processes have been previously identified using FT-ICR MS. For example, photodegradation is generally associated with the consumption of compounds with low H/C ratios and with the enrichment of compounds with high H/C and low O/C ratios (Medeiros, Seidel, Powers, et al., 2015; Seidel et al., 2015; Stubbins et al., 2010). Microbial biodegradation is also generally associated with the consumption of compounds associated with molecular formulae with high O/C and low H/C ratios, and with the enrichment in relative abundance of compounds associated with formulae with low O/C and high H/C ratios (Medeiros, Seidel, Ward, et al., 2015; Seidel et al., 2015). Thus, the pattern of transformation captured by PC 1 (Figure 7b) was different from the pattern reported many times in the literature in multiple environments as characteristic of the transformation in DOM composition associated with photochemistry or microbial degradation. This suggests that photooxidation and microbial degradation were likely not the main drivers of the DOM transformation captured by PC 1.

Chlorophyll concentration, a proxy for phytoplankton abundance, was not measured simultaneously with the DOM analysis. However, historical observations at seasonal intervals (2014–2017) at the head of Sapelo Sound revealed that chlorophyll concentration was generally high during summer and was substantially lower during the remaining seasons (Figure S2). This time variability is somewhat similar to the time variability captured by PC 1 (Figure 7a), suggesting that the pattern of DOM composition variability captured by mode 1 may be related to phytoplankton-derived DOM. Analysis of cultures grown in laboratory has revealed phytoplankton-derived DOM enriched with compounds with $H/C > 1$ and $O/C < 0.5$ (Landa et al., 2014). Additionally, analysis of DOM composition using FT-ICR MS in the Amazon River plume has revealed a pattern in which samples characterized by high chlorophyll concentrations and high phytoplankton cell counts were enriched with compounds with $H/C > 1$ and low O/C, while samples with low chlorophyll concentrations and low phytoplankton cell counts were enriched with compounds with $H/C > 1$ and high O/C ratios (see Figure 2 in Medeiros, Seidel, Ward, et al., 2015). The loading of PC 1 (Figure 7b) was consistent with that description. Thus, it is possible that summer algae blooms had a detectable impact driving variability in DOM composition at the head of Sapelo Sound. The interpretation of the process(es) that may have been responsible for the seasonal change in DOM composition captured by the first PC (Figures 7a and 7b) is characterized by high uncertainty. More detailed studies are thus needed to identify what is (are) the dominant mechanism(s) driving seasonal changes in the composition of the DOM at the head of Sapelo Sound.

3.3. Microbial Consumption of Riverine and Estuarine DOC

Dark incubations were pursued at both sites for all months to investigate microbial consumption of riverine and estuarine DOC. Concentration of DOC and optical parameters was measured in all cases. For October 2015 and January, April, and July 2016, FT-ICR MS analysis was also pursued at the end of the incubation after 80 days. No incubation was pursued for October 2016 following the passage of Hurricane Matthew. We note that variability in DOC consumption between the different months may be related both to DOM composition (i.e., to its lability) and to changes on microbial community composition.

Utilization of DOC, defined as the fraction of the DOC that is consumed during each incubation experiment (see equation (1)), was correlated with river discharge at the Altamaha River ($r = 0.65$, $p < 0.05$) and at Sapelo Sound ($r = 0.89$, $p < 0.05$; Figure 9). Thus, microbial consumption of DOC at both sites was enhanced in months of high river discharge. Mann et al. (2012) observed that the contribution of humic-like fluorescence indicative of terrigenous DOM input was positively correlated with DOC utilization in an Arctic river. The increase in DOC in Arctic rivers during peak discharge is often young and rich in lignin (Raymond et al., 2007; Spencer et al., 2008), indicating that fresh terrestrial DOM can be highly biolabile possibly due to its short degradation history (Mann et al., 2012). To further investigate how river discharge influences

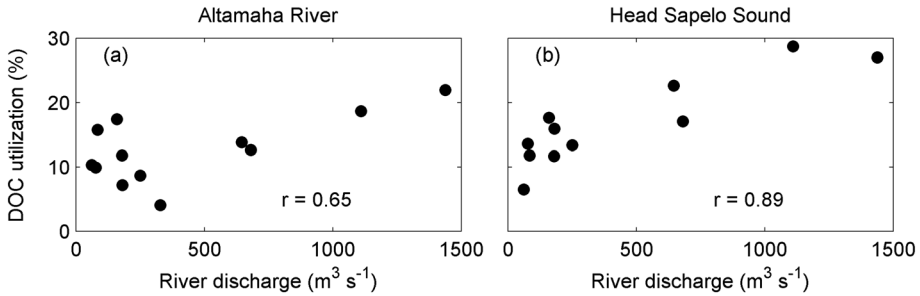

Figure 9. Scatterplot of river discharge and dissolved organic carbon (DOC) utilization (as a fraction of the initial DOC concentration for each incubation; see equation (1)) for each month at the (a) Altamaha River and at the (b) head of Sapelo Sound.

microbial degradation in the Altamaha River and at Sapelo Sound, we computed the average change in DOC concentration and optical parameters during the incubations for high and for low discharge conditions. January to April 2016 were defined as high discharge months (discharge larger than average plus 1 standard error of the mean), while the remaining months (September to December 2015 and May to September 2016) were characterized by low discharge conditions. Consistent with Figure 9, DOC concentration (expressed as a percentage of the initial concentration) decreased faster during high discharge conditions, especially at Sapelo Sound (Figures 10a and 10d). This suggests that increased discharge may have changed the DOM pool by mobilizing fresh terrestrial DOM from the soil and transported it into the coastal system (Vazquez et al., 2011). These changes were seen in both the Altamaha River and in the Sapelo Sound estuarine area, indicating a far-reaching effect of this environmental condition. Optical parameters were also different between high and low discharge conditions in both the Altamaha River ($p < 0.05$) and in Sapelo Sound ($p < 0.05$). At both sites the spectral slope $S_{275\text{-}295}$ and the ratio $a_g(250):a_g(365)$ were lower during high discharge conditions, which was indicative of stronger terrigenous signature, higher aromaticity, and higher molecular size (Del Vecchio & Blough, 2002; Fichot & Benner, 2012; Helms et al., 2008; Peuravuori & Pihlaja, 1997).

It is interesting to note that $S_{275\text{-}295}$ and $a_g(250):a_g(365)$ remained approximately constant throughout the incubations (Figure 10), with values at the initial condition (T_0) not being statistically different from values measured after 80 days (T_{80}) at the end of the incubation (Wilcoxon ran-sum test, $p > 0.05$). We also note that repeating the PC analysis of FT-ICR MS data described before (Figures 5a, 5b, and 7) but including the samples collected at the end of the incubation (for October 2015 and January, April, and July 2016) produced nearly identical results for both sites (Figure S3). Moreover, for the months in which FT-ICR MS analyses were pursued at the end of the incubations, the PC scores for T_0 and T_{80} samples were not different from each other (Wilcoxon ran-sum test, $p > 0.05$). This indicates that any alteration in DOM composition that may have occurred during the incubations due to microbial degradation was smaller than the seasonal changes in DOM composition observed due to other processes (e.g., changes in hydrology, phytoplankton-derived inputs). This is consistent with biodegradation not explaining the dominant patterns of DOM composition variability observed in the system (Figures 5a, 5b, and 7). We note that a fraction of the decreased DOC measured during the experiments likely encompassed components of the DOM pool that cannot be detected by the techniques used here (Vorobev et al., 2018). For example, saccharides are known to be labile (Kirchman et al., 2001; Rich et al., 1996), but they are not well retained by SPE.

Using our measurements of DOC and DOC utilization at the Altamaha River, we can estimate the total export of DOC and of biolabile DOC out of the system. Using historical DOC data from the Altamaha River from October 2000 to April 2009, Medeiros, Babcock-Adams, et al. (2017) calculated an average export of 69 Gg C/year. For the current sampling period, the total DOC export was larger at 108 Gg C/year. Medeiros, Babcock-Adams, et al. (2017) showed that DOC flux is highly correlated to discharge at the Altamaha River. Thus, the 50% increase in DOC export compared to the long-term average is likely related to increased river discharge during the study period associated with El Niño conditions (Sheldon & Burd, 2014). The residence time at the Altamaha River has been estimated to be about 5 days during low discharge conditions, decreasing to less than 2 days during high discharge (Wang et al., 2017). Since most of the DOC

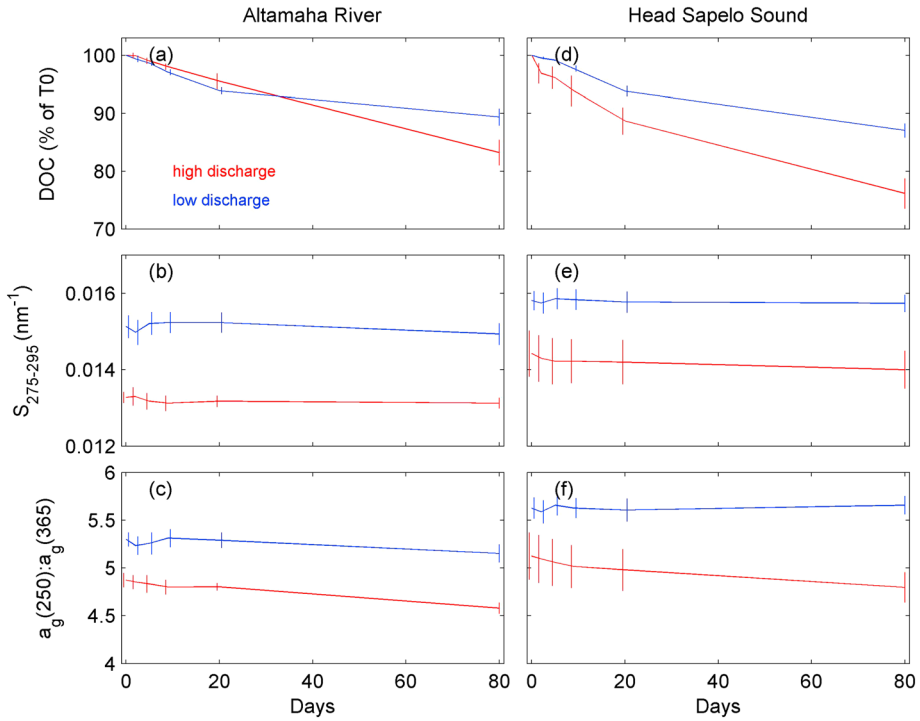

Figure 10. Time series of (a, d) dissolved organic carbon (DOC) concentration, (b, e) spectral slope $S_{275\text{-}295}$, and (c, f) $a_g(250):a_g(365)$ during the course of incubations at the (left) Altamaha River and at the (right) head of Sapelo Sound. Values have been averaged for high (red) and low (blue) discharge conditions. Error bars are standard errors of the mean.

utilization occurred on time scales longer than that (Figure 10), it is reasonable to assume that most of the biolabile DOC from the Altamaha River can be exported out of the system to the coastal ocean. If that is true, then the Altamaha River exported 18 Gg C/year of biolabile DOC to the shelf during our study period, or about 16.5% of the total DOC flux, which is comparable to the labile DOC flux as a percentage of total annual DOC flux out of other rivers (e.g., Holmes et al., 2008). If that fraction is approximately constant from year to year, then a climatological export of biolabile DOC can be estimated at 11 Gg C/year. Most of this biolabile DOC is presumably exported during spring, since river discharge, DOC concentration, and DOC lability all peak during that season.

4. Conclusions

Bulk, optical, and molecular analyses of riverine and estuarine samples of a typical salt marsh-dominated estuary off the southeastern United States revealed that DOC concentration and DOM composition were constantly being altered by various processes throughout the year. River discharge strongly modulated changes in DOM composition at the Altamaha River. When discharge was higher than ~150 m^3/s, the Altamaha was characterized by higher DOC concentrations and DOM with a strong terrigenous character. At low discharge conditions, a clear imprint of marsh-derived compounds was observed in the river. At Sapelo Sound, the composition of DOM was also altered by river discharge through the entrainment of freshwater into narrow channels and streams that carve the estuarine area, showing the far-reaching effect of discharge on the DOM pool across this aquatic setting. Another seasonal pattern of variability in DOM composition was observed at the head of Sapelo Sound, which is possibly related to phytoplankton-derived inputs during summer. Higher DOC utilization by bacteria was observed during months of high discharge levels and DOM with a stronger terrigenous signature, potentially due to the higher mobilization and influx of fresh material to the system. Lastly, the effects of a severe weather event, Hurricane Matthew, were shown to have a great impact at Sapelo Sound. Concentrations of DOC were greatly increased, and DOM had a higher terrestrial and aromatic content directly following the hurricane compared to other months. This demonstrates that hurricanes can impact not only the DOC content but also the molecular composition of DOM potentially influencing its cycling in estuarine environments.

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