Seasonal dynamics of dissolved, particulate and microbial components of a tidal saltmarsh-dominated estuary under contrasting levels of freshwater discharge.

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Abstract

Tidal *Spartina*-dominated saltmarshes and estuaries on the Southeast US coast are global hotspots of productivity. In coastal Georgia, tidal amplitudes and saltmarsh productivity are the highest along the Southeast US coast. Coastal Georgia is characterized by a humid subtropical seasonal climate, and inter-annual variability in precipitation, and freshwater discharge. The 2012-2013 timeframe encompassed contrasting levels of discharge for the Savannah River, a major Georgia river, with a 4.3-fold greater discharge in summer 2013 relative to summer 2012. In situ measurements of temperature, salinity, precipitation and Secchi depth, and water samples were collected weekly at high tide throughout 2012 and 2013 from the Skidaway River Estuary, a tidal saltmarsh-dominated estuary in coastal Georgia influenced by Savannah River hydrology. The effects of elevated discharge on the seasonal trends of water column components were evaluated. The shift from low discharge (2012) to high discharge (2013) led to decreased salinity in summer 2013, but no significant increases in inorganic nutrient (NH_4 , NO_x , SiO_2 and PO_4) concentrations. Dissolved inorganic carbon (DIC) concentrations decreased, and DIC stable isotopic signatures ($\delta^{13}\text{C}$ -DIC values) were depleted in summer 2013 relative to summer 2012. In 2013 dissolved organic carbon (DOC) concentrations, chromophoric and fluorescent dissolved organic matter (DOM: CDOM, FDOM) intensities, specific UV-absorbance (SUVA_{254}) and relative humic-like fluorescence were all higher than in 2012, indicating that, as discharge increased in 2013, estuarine water became enriched in terrigenous DOM. Secchi depth and particulate organic carbon (POC) and nitrogen (PON) concentrations displayed clear seasonal patterns that were not significantly altered by discharge. However, $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON isotopic signatures indicated higher terrigenous contributions at elevated discharge. Discharge influenced cyanobacterial composition, but did not affect total abundance of phytoplankton (<52

µm) or chlorophyll-a concentrations, a proxy for phytoplankton biomass. Phytoplankton community dynamics were primarily seasonally-driven. Bacterioplankton abundance and community composition, based upon flow cytometry, were affected by discharge, possibly due to decreased salinity and/or increased inputs of terrigenous DOM. Seasonal patterns in inorganic nutrient, POC, PON and chlorophyll-a concentrations, and Secchi depth were not significantly influenced by the 2013 increase in discharge. For other components, most notably $\delta^{13}\text{C}$ -DIC values, DOM and bacterioplankton, the influence of increased discharge in 2013 was superimposed upon their seasonal patterns. This study showed that in addition to tidal mixing and in situ saltmarsh and estuarine production and removal processes, the level of riverine freshwater discharge impacted the quantity and character of many water column components in this tidal saltmarsh ecosystem.

1.Introduction

Saltmarsh-dominated estuarine systems are globally relevant hotspots of productivity and critical mediators of the flux of dissolved inorganic and organic matter (DIM, DOM) and particulate organic matter (POM) between land and ocean (Hopkinson 1988; Cai 2011). These ecosystems represent an important component of global biogeochemical and carbon cycles (Bauer et al. 2013). In saltmarsh-dominated estuaries, the concentration and composition of DIM, DOM and POM are influenced by factors such as river discharge, tidal mixing, microbial activity and growth, and photochemical transformations (Moran et al. 1991; Mopper et al. 2015). DOM concentration and composition are also influenced by in situ autochthonous sources, such as saltmarsh vegetation, phytoplankton and benthic microalgae (Benner et al. 1985; Dame et al. 2000; Porubsky et al. 2008). The microbial communities within tidal saltmarshes and estuaries are adapted to semi-diurnal shifts in salinity associated with the tidal regime, and to diurnal and

seasonal variations in temperature, light availability and DOM quality (Buchan et al. 2003; Crump et al. 2004). Precipitation affects the concentration and composition of dissolved material and microbial communities, as it regulates wet deposition of aerosols (Kieber et al. 2006), riverine discharge of allochthonous, terrigenous DOM (Meybeck 1982), and inputs of low-salinity water into the estuary, both directly and through rivers (Thurman 1985).

Our study site, the Skidaway River Estuary in coastal Georgia, is a tidal saltmarsh-dominated estuary and is directly influenced by the hydrologic regime of Georgia Rivers, particularly the Savannah River (Blanton and Atkinson 1983; Verity 2002). This system is broadly representative of the saltmarshes that occupy 16,500 km² of the US coastal zone, mostly on the East coast between latitudes 30°N (northern Florida) and 44°N (northern Maine), as well as along the Alaskan coastline, and the Gulf of Mexico (Field et al. 1991; Hoekstra et al. 2010). The coast of Georgia accounts for 33% of the US East coast tidal saltmarsh area (Reimold 1977), exhibits the highest levels of marsh primary productivity, mainly from *Spartina alterniflora* (0.2 to 2.25 kg C m⁻² yr⁻¹), and experiences the highest (2 to 3 m) semi-diurnal tidal amplitudes (Hopkinson 1988; Wiegert and Freeman 1990) on the US East coast. The Georgia coast climate is generally warm and wet. However, drought can occur in the region due to inter-annual variability in precipitation (Karl et al. 2009), the time span between rainfall events, and air temperature, which drives ground and canopy evaporation and vegetation evapotranspiration (Vicente-Serrano et al. 2010). In 2012, the Southeast US experienced precipitation levels near the historical average for the area. However, mean annual air temperatures were the fifth warmest and the spring was the warmest since 1895 (NOAA 2013). These elevated air temperatures resulted in severe drought conditions in the Southeast (www.droughtmonitor.unl.edu). In contrast, in 2013, the region experienced the fifth wettest year

and the wettest summer on record (NOAA 2014). As a consequence of these climate patterns, the 2012-2013 timeframe represented a period of contrasting levels of freshwater discharge from major Southeast US Rivers into Georgia coastal waters. For example, Savannah River discharge from July through September, was 4.3-fold greater in 2013 relative to 2012 (USGS site #02198500, http://waterdata.usgs.gov/ga/nwis/uv?site_no=02198500), and the nearby Ogeechee River discharge was 8.4-fold greater in 2013 relative to 2012 (USGS site #02202190, http://waterdata.usgs.gov/ga/nwis/uv?site_no=02202190).

Previous studies have extensively investigated tidal, seasonal and long-term trends in water chemistry in the Skidaway River Estuary, and microbial controls on DOM composition in other saltmarsh-dominated estuaries in Coastal Georgia (Esham et al. 2000; Verity 2002; Buchan et al. 2003). However, the effects of variable freshwater discharge on the dissolved, particulate and microbial compartments of tidal saltmarsh-dominated estuarine ecosystems remain poorly understood. Present-day baseline data on hydrological impacts upon estuarine waters are needed in order to identify shifts attributable to future climate change in these globally relevant hotspots of productivity and biogeochemical cycling. Outlining seasonal versus inter-annual variability on dissolved and particulate organic matter, and on autotrophic and heterotrophic microbial communities is a critical approach in addressing these knowledge gaps.

The years 2012-2013 provided an exceptional opportunity to examine the effects of distinct hydrological conditions on saltmarsh estuarine systems in coastal Georgia. Recent work in the nearby Altamaha-Doboy-Sapelo estuary collected discrete samples in November 2012, and in March and September 2013 (Medeiros et al. 2015). Ultrahigh resolution mass spectrometry revealed that the molecular signatures of terrigenous DOM were elevated at high freshwater discharge in March and September 2013, while DOM molecular signatures indicative of marine

sources were prevalent during drought in November 2012 (Medeiros et al. 2015). In the present study, we report high temporal resolution (weekly) shifts in DIM, DOM, POM, and the autotrophic and heterotrophic microbial communities of the Skidaway River Estuary at a single tidal phase (high tide) between January 2012 and December 2013. The goal of this study was to gain a better understanding of the variations in these ecosystem components in response to seasonal cycles and across the transition from low to high freshwater discharge.

The four components of Skidaway River Estuary are defined in this study as (1) DIM, including inorganic nutrients (NH_4 , NO_x , SiO_2 and PO_4) concentration and ratios, and dissolved inorganic carbon (DIC) concentration and isotopic signature ($\delta^{13}\text{C}$ -DIC values); (2) DOM, including dissolved organic carbon (DOC) and nitrogen (DON) concentrations, and optical properties (chromophoric, CDOM, and fluorescent, FDOM); (3) POM, including particulate organic carbon (POC) and nitrogen (PON) concentrations and isotopic signatures ($\delta^{13}\text{C}$ -POC, $\delta^{15}\text{N}$ -PON), and Secchi depths; and (4) microbial dynamics, including measures of bulk chlorophyll-a concentrations, and autotrophic and heterotrophic microbial abundances and community composition, determined by flow cytometry. DIM, DOM and POM concentrations, ratios, isotopic and optical signatures provided information about the transport, changing sources, and processing of material across seasonal cycles and the hydrological transition. Bulk chlorophyll-a concentrations provided a proxy for bulk phytoplankton community biomass, and flow cytometric analysis of phytoplankton ($< 52 \mu\text{m}$) and bacterioplankton provided abundances and low resolution information on microbial community composition in response to seasonality and freshwater discharge. This high frequency, multi-disciplinary approach revealed seasonal patterns in these ecosystem components. Superimposed upon these seasonal trends, the transition

from low to high discharge had pronounced impacts upon the levels and composition of several of the measured parameters.

2. Material and Methods

2.1 Sampling, hydrology, and water chemistry.

In situ measurements of water temperature ($^{\circ}\text{C}$), salinity and Secchi depth (m) were collected weekly at high tide along with discrete water samples from the Skidaway River Estuary from January 10th 2012 to December 18th 2013 from the main dock at the University of Georgia Skidaway Institute of Oceanography (SkIO) ($31^{\circ}99''\text{N}$, $81^{\circ}02''\text{W}$, **Fig. A.1, Table A.1**), Savannah, GA, US, in association with the Skidaway River Monitoring Program (Verity 2002). Vertical profiles of water temperature and salinity were obtained by deployment of a multi-parameter probe (Manta-2, Eureka), calibrated following manufacturer's protocols. Based upon salinity and water temperature profiles the water column (~ 7 m max. depth at high tide) was always well mixed. Therefore, only data from 1 m, the depth at which discrete samples were collected, are presented. Secchi depths were used as a proxy for total suspended particulates, both live organisms and detritus, and for photosynthetic active radiation (PAR) attenuation. Water samples were collected from 1 m depth using a Niskin bottle and processed in SkIO labs within an hour of collection. Hourly recordings of local precipitation (mm) were obtained from the SkIO weather station (<http://weather.skio.uga.edu/>). Water temperature ($^{\circ}\text{C}$), precipitation (mm) and river water discharge ($\text{m}^3 \text{ sec}^{-1}$) for the Savannah River were obtained at 15 min intervals from the United States Geological Survey (USGS) database at the United States Army Corps of Engineers (USACE) dock site (data courtesy of USGS, site #0219897773, http://waterdata.usgs.gov/nwis/inventory/?site_no=021989773) and Savannah River near Clyo site (data courtesy of USGS, site #02198500,

http://waterdata.usgs.gov/ga/nwis/uv?site_no=02198500). Discharge data from the nearby Ogeechee River (not shown) is available at http://waterdata.usgs.gov/ga/nwis/uv?site_no=02202190.

2.2 Dissolved Inorganic Matter (DIM)

2.2.1 Nutrient analysis. Single samples for inorganic nutrient analysis were filtered through combusted 0.7 μm GF/F filters. The filtrate was collected into acid-washed polycarbonate bottles and immediately frozen at -20°C . Samples were analyzed for NO_x , NH_4 , PO_4 and SiO_2 using a Lachat Quickchem FIA+ 8000 nutrient analyzer, following established colorimetric protocols (EPA 1984; Mann et al. 2012).

2.2.2. DIC concentration and isotopic signature. Triplicate samples for DIC analysis were syringe-filtered through 0.2 μm nylon filters. The filtrate was collected directly into 2 mL crimp-seal vials. Each vial was overfilled with 2 volumes of sample, sealed immediately with butyl-rubber septa, refrigerated and analyzed within 2 months. DIC concentrations (mM-C) and isotopic signatures ($\delta^{13}\text{C}$ -DIC values) were obtained by liquid chromatography (LC-Isolink Thermo Electron) coupled with an isotope ratio mass spectrometer (IR-MS, Thermo-Fisher Delta V), with accuracy of 0.04‰ for triplicate injections (Brandes 2009). Blanks consisted of ultrapure water (Milli-Q, Millipore). Concentration and isotope calibration was accomplished using Na_2CO_3 standard solutions, made by weighing of Na_2CO_3 crystals directly into 25 mL volumetric flasks. The volume was made up with ultrapure water. Flasks were sealed with parafilm, inverted several times for gentle mixing and solutions were transferred to 2 mL crimp-seal vials and sealed with butyl-rubber septa. Standard solutions were made in triplicates for each concentration of the 5-point calibration curve, ranging from 1 to 3.5 mM-C. Triplicate injections were performed for each replicate concentration. Differences between first and last replicate

vials for each concentration were not significant ($p > 0.05$), and the r^2 of the total ion current peak values from injections across the standard series was always greater than 0.999, indicating no degassing during standard preparation. Both DIC concentrations and $\delta^{13}\text{C}$ -DIC values were calibrated with the laboratory standard Na_2CO_3 , which was cross-calibrated against the NBS 19 calcite standard, reference material #8455 from the National Institute of Standards and Technology (NIST). All isotope values are reported versus the vPDB scale.

2.3. Dissolved Organic Matter (DOM)

Samples for DOC quantification and DOM optical characterization were syringe-filtered through acid-washed 0.2 μm acrodisc filters directly into pre-combusted glass vials, acidified to pH 2 and refrigerated for analysis of DOC and total dissolved nitrogen (DN) concentrations (duplicates), and into acid-washed polycarbonate dark bottles and frozen at -20°C for optical characterization (single sample). DOC concentrations were used as a proxy for DOM concentrations, the mass of DOM being approximately double that for DOC (Krogh 1934). DOC and DN were quantified using a total organic carbon analyzer (TOC-Vcph, Shimadzu) coupled with a DN unit. DOC and DN were quantified as non-purgeable organic carbon (NPOC) and non-purgeable nitrogen by high temperature (720°C) combustion and conversion to CO_2 and NO , respectively. CO_2 was detected by non-dispersive infrared (NDIR) detection, and NO was mixed with ozone to produce chemiluminescence that was detected by a photomultiplier. Runs were calibrated with standard solutions of potassium hydrogen phthalate from 5 to 500 $\mu\text{M-C}$ (Stubbins and Dittmar 2012). Aliquots of deep seawater reference material (Batch 10, Lot# 05-10) from the Consensus Reference Material Project (CRM) were analyzed to check the precision and accuracy of the DOC analyses. Analyses of the CRM deviated by less than 5% from the reported value for these standards (41 to 44 $\mu\text{M-C}$;

<http://yyy.rsmas.miami.edu/groups/biogeochem/Table1.htm>). Dissolved organic nitrogen (DON) was calculated as the difference between DN and dissolved inorganic N ($\text{NO}_x + \text{NH}_4$).

Optical characterization of DOM was performed by analysis of the chromophoric and fluorescent fractions of DOM (CDOM and FDOM, respectively). Ultrapure water was used as blanks for CDOM and FDOM analyses. CDOM absorption spectra from 250 to 800 nm at 1 nm intervals were obtained using a UV-visible diode array spectrophotometer (Agilent 8453). Spectra were corrected for light scatter by subtracting absorption from 750 to 800 nm, and Napierian light absorption coefficients were calculated ($a \text{ m}^{-1}$) (Green and Blough 1994). Specific UV absorbance (SUVA_{254}) was calculated using the decadic light absorption coefficient at 254 nm (m^{-1}) normalized to DOC (mg-C L^{-1}) and used as a proxy for aromaticity (Weishaar et al. 2003). Emission-excitation matrix (EEM) spectra for FDOM analysis were determined using a spectrofluorometer (Aqualog, Horiba) (Spencer et al. 2007). Each EEM was comprised of 37 emission spectra from 280 to 500 nm in 1 nm intervals, at excitation from 240 to 420 nm in 5 nm intervals. EEMs were normalized to Raman units and corrected using instrument-specific excitation (xcorrect) and emission (mcorrect) correction files and for inner-filter effects using the Aqualog software tools. A parallel factor analysis (PARAFAC) model was developed for 94 corrected EEMs from samples collected over the study period using the DOM Fluor toolbox in Matlab version 7.14 (Stedmon and Bro 2008). The model was validated using random split-half analysis and the number of components was determined by visual examination of the residuals from models with gradually increasing number of components. Intensities of each component were calculated as the maximum fluorescent intensity of each component, i.e., using maxval values (Stedmon and Bro 2008).

The three FDOM components identified by PARAFAC were distinguished by their excitation and emission (ex/em) spectra. Component 1 (C1) had ex/em maxima at 315/436 nm, component 2 (C2) had ex/em maxima at 270(380)/476 and component 3 (C3) had ex/em maxima at 285/359. The component's excitation and emission loadings and fluorescence intensities were tested against the OpenFluor database (www.openfluor.org). The database query returned 19 dataset matches and 23 component matches with similarity scores greater than 0.95 for C1 and C3, while C2 did not have a match. For C1 and C3, respectively, 3 and 2 components with similarity scores > 0.986 were used in aiding the interpretation and discussion of FDOM data in this study. Skidaway's C1 was identified as humic-like, terrigenous material, with potential agricultural influence, based on its high similarity to C1 in Sndergaard et al. (2003), C5 in Stedmon and Markager (2005) and BER-C1 in Walker et al. (2009). C3 was identified as a protein-like component, likely from microbial/autochthonous origin, based on its high similarity to C5 in both Stedmon et al. (2003) and Graeber et al. (2012). C2 had no matches, but appears to have spectral characteristics that fall between humic-like and fulvic-like (C2 and C3) components described in Stedmon et al. (2003) and Stedmon and Markager (2005), with terrigenous/autochthonous origins. Due to many uncertainties regarding the actual chemical composition and sources of these FDOM constituents (Maie et al. 2007; Stubbins et al. 2014) in the Skidaway River Estuary, the FDOM components are referred to as C1 (humic-like 1), C2 (humic-like 2) and C3 (protein-like) throughout this study.

2.4 Particulate organic matter (POM)

POM concentrations and isotopic signatures were obtained from single samples filtered onto pre-combusted 0.7 µm GF/F filters. Filters were dried at 60°C for 24 h, and packed into tin cups (Costech) for analysis. Blanks consisted of combusted GF/F filters, dried and packed into

tin cups. Concentration of POC and PON and their respective stable isotope ratios ($\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON) were obtained using a Thermo Flash elemental analyzer coupled to a Thermo-Fisher Delta V plus isotope ratio mass spectrometer (IR-MS) (Fry et al. 1992). Each run was calibrated with chitin standards (Sigma) ranging from 0.1 to 1 mg, cross-standardized to L-glutamic acid, NIST isotope reference material # 8573. Analytical precision was 0.2‰ for $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON, and 1% for C and N weight percentages.

2.5 Microbial communities

2.5.1 Chlorophyll-a concentration. Triplicate samples were filtered through 0.2 μm polycarbonate membranes, in the dark. Membranes were frozen at -20°C and samples were analyzed within 2 months. Blanks consisted of 0.2 μm membranes. Bulk chlorophyll-a was extracted in 90% acetone for 24 h and fluorescence emission was measured at $>665\text{ nm}$, with excitation at 340-500 nm, using a Turner 10-AU fluorometer calibrated following manufacturer's protocols. In order to measure phaeophytin fluorescence, chlorophyll-a extracts were acidified with 3 mM HCl (final concentration) and fluorescence was measured at the same excitation and emission settings (Parsons et al. 1984). Phaeophytin measurements were used as a proxy for degraded chlorophyll-a. Bulk chlorophyll-a concentrations and chlorophyll-a minus phaeophytin concentrations were calculated according to Parsons et al. (1984). Extracted bulk chlorophyll-a and chlorophyll-a minus phaeophytin refer to the whole phytoplankton community in the water column, thus these data were used as proxies for bulk phytoplankton biomass.

2.5.2 Microbial abundances and composition. Duplicate samples of unfiltered water were collected for flow cytometric analysis, preserved with 0.1% glutaraldehyde solution (final concentration) and frozen at -80°C . Blanks consisted of a 0.2 μm -filtered water aliquot. Microbial communities were analyzed using a flow cytometer (BD FACSCalibur) equipped with

a 15 mW air-cooled argon-ion laser tuned for blue excitation (ex 488 nm) and a red diode laser (ex 635 nm), with emission) detectors at 535, 585 and 650 nm, and forward (FSC) and side scatter (SSC) light detectors. Runs were calibrated with fluorescent polystyrene beads (1 μm , Spherotech) added to each sample to account for instrument reproducibility and fluorescence reference. Samples were pre-screened with a 52 μm mesh to preclude the introduction of larger particles into the flow cell. Sample volumes were estimated by sample weight before and after each run. Data were acquired using BD Cell Quest Pro software (v. 4.0.1) and analyzed with FlowJo software (v.10). Samples for analysis of phytoplankton <52 μm (referred to as Phyto-total) were run unstained and undiluted. Sub-communities within the Phyto-total community were distinguished by FSC and SSC, used as proxies for relative cell size, versus fluorescence intensity at ex/em 488/650 nm, used as a proxy for chlorophyll-a fluorescence per cell. In this study, FSC and SSC were used to differentiate sub-communities of microorganisms with distinct light scattering properties. These properties are, to a certain extent, dependent on cell size. However, FSC and SSC are also influenced by cell morphology, granularity and intracellular structures (Shapiro 2003), and thus are not used as measures of absolute cell size, biovolume or biomass without supporting cell metrics data from microscopy analysis. As we did not have these ancillary data, estimates of cell size, biovolume or biomass were not made. Furthermore, fluorescence intensity at ex/em 488/650 nm obtained by flow cytometry was used only to differentiate sub-communities while extracted chlorophyll-a concentrations (*refer to section 2.5.1*) were used as a proxy for bulk phytoplankton biomass. Fluorescence intensity at ex/em 630/650 nm and ex/em 488/585 nm were used as proxies for phycocyanin (PC) and phycoerythrin (PE) fluorescence per cell, respectively. PC and PE are phycobiliproteins, accessory light harvesting pigments found in cyanobacteria and some eukaryotic phytoplankton.

The ratios of PC to chlorophyll-a fluorescence per cell, and PE to chlorophyll-a fluorescence per cell were used to further characterize the sub-communities. Three sub-communities were defined as follows: sub-community 1 had low SSC and FSC values, low chlorophyll-a fluorescence per cell, and high ratio of PC to chlorophyll-a fluorescence per cell; sub-community 2 had intermediate values of SSC and FSC, intermediate chlorophyll-a fluorescence per cell, and high ratio of PE to chlorophyll-a fluorescence per cell; and sub-community 3 had high SSC and FSC values, high chlorophyll-a fluorescence per cell and low PC to chlorophyll-a and PE to chlorophyll-a ratios per cell. Sub-communities 1, 2 and 3 are referred to as Phyto-PC, Phyto-PE and Phyto-Euk, respectively.

Samples for analysis of heterotrophic bacteria (HB) were vortexed to disrupt bacterial assemblages and particle-attached bacteria, 10-fold diluted and stained with Sybr Green I, a nucleic acid (NA) binding stain (Marie et al. 1997). Total heterotrophic bacteria (HB-total) abundances represented the whole bacterioplankton community. Two sub-communities were distinguished within HB-total based on fluorescence intensities at ex/em 488/535 nm versus ex/em 488/585 nm. Low and high fluorescence intensities were used as proxies for low and high nucleic acid content bacterial sub-communities, and referred to as LNA and HNA, respectively (Gasol and Del Giorgio 2000).

2.6 Statistical analysis

Unpaired, nonparametric (Mann-Whitney test, with $p < 0.05$ threshold) tests were used to determine significant differences between years 2012 and 2013. Multiple linear regression (MLR) models were performed using the ordinary least squares (OLS) method in JMP Pro v 11.0.0 (SAS Institute Inc). MLR models were developed for data from 2012 and 2013 as a continuous dataset (2012-2013), and for years 2012 and 2013 as individual datasets (2012-only

and 2013-only, respectively). The former was used to identify significant environmental predictors throughout the entire study period and the latter approach was used to identify changes in environmental predictor(s) associated with hydrological shifts. Water chemistry data (water temperature and salinity) collected at the Skidaway River Estuary and local precipitation data from the SkIO weather station were selected as potential environmental predictors in the MLR model. Water temperature, salinity and local precipitation were selected as abiotic factors to describe the variability in DIM, DOM, POM and microbial communities for two reasons. First, their own variability is controlled by seasonal climatic and/or hydrological factors, thus they are suitable as independent variables in the context of this study. Second, they were measured in the Skidaway River Estuary water at the same time that the samples for DIM, DOM, POM and microbial analyses were collected. Therefore, water temperature, salinity and precipitation were an integral part of the snapshot that each sampling excursion represented through the study period.

The response of each DIM, DOM, POM and microbial parameter to the variables selected as environmental predictors was modeled independently from one another. The significance of the MLR model itself was evaluated by the ratio between the variance accounted for by the regression and the variance in the residuals (F-test, with $p < 0.05$ threshold). Adjusted r^2 values indicated the proportion of variation explained by MLR models. For models with F-test results where $p < 0.05$, the predictor with the highest statistical significance (α) (with $p < 0.05$ threshold) was defined as the principal predictor of variability in each DIM, DOM, POM and microbial parameter. For MLR analyses of the continuous dataset (2012-2013) where a secondary predictor for which α was $p < 0.05$, this secondary predictor is also reported.

To estimate DOC loss rates from the system due to combined net-removal processes (e.g. dilution, bio-degradation, photo-degradation), DOC decay rates were calculated from time series for each year. The time series had starting points on July 24th 2012, and July 23rd, 2013, at the maxima summer DOC concentration measured, and ending points on December 18th, 2012 and December 17th, 2013, at minima winter DOC concentrations before the end of each calendar year ($n = 22$ for both years, **Table A.2**). DOC half-lives ($t_{1/2}$) were calculated as $\text{Ln}(2)/\lambda$, using the three-parameter decay function: $y_t = y_0 \exp(-\lambda t) + b$, where y_0 = DOC value at time 0 (i.e., maximum DOC concentration each year); y_t = modeled DOC value at a given time; λ = decay constant; t = time (d); b = DOC-projected value at time equals infinity, representing the ‘residual’ DOM fraction; and $\exp$ = the base of the natural logarithm (Westrich and Berner 1984) using Sigma Plot v. 12.0 (Systat Software Inc). In this study, these half-lives represent the combined influence of the temporally dynamic differential between DOM sources (e.g. terrigenous inputs, marine inputs, autochthonous inputs) and removal processes (e.g. biological mineralization, photochemical mineralization, flocculation, sorption and hydrological flushing). Due to limitations in the data available regarding those processes, DOC rates were determined only for comparative purposes between years.

3. Results

3.1 Hydrology and water chemistry (Fig. 1). Seasonal patterns were evident throughout 2012-2013 at the Savannah River and at the Skidaway River Estuary locations, with water temperature maxima between 26 and 31°C in summer and minima between 12 and 15°C in winter (**Fig. 1A-B**). Local precipitation measured at the Skidaway River Estuary was greater than at the Savannah River location (**Fig. 1A-B**). At both locations, water temperature and precipitation were not significantly different between the years ($p > 0.05$). The magnitude of Savannah River discharge

was significantly greater in 2013 relative to 2012 ($p < 0.0001$). The cumulative freshwater discharge of the Savannah River in late February and late August was 25% and 146% higher in 2013, respectively, relative to same periods in 2012 (**data not shown**). During 2013, freshwater pulses occurred in February, March, May, June, July, August and September (**Fig. 1C**). The transition from low to high Savannah River discharge resulted in a significant decrease in salinity ($p < 0.0001$) in 2013 relative to 2012 in the Skidaway River Estuary. Salinity in the estuary averaged $32 (\pm 1.2)$ during 2012, and decreased to $27.1 (\pm 3.8)$ during 2013. Maximum river discharge was observed on July 17th 2013, coinciding with a dip in salinity in the estuary, which reached a minimum of 16 on July 30th (**Fig. 1C**). In October 2013, Savannah River discharge decreased, and salinity increased in the estuary, levelling off at 30 on average through to the end of the year.

3.2 Dissolved Inorganic Matter (Fig. 2). Concentrations of inorganic N (NO_x and NH_4) peaked in late summer in both years (**Fig. 2A**). NO_x concentrations were similar between years ($p > 0.05$) and NH_4 concentrations were significantly greater in 2012 relative to 2013 ($p < 0.0001$). PO_4 concentrations peaked synchronously with inorganic N in 2012 and 2013, but also peaked in winter 2012/13 (**Fig. 2B**). SiO_2 concentrations peaked in both summers (**Fig. 2B**). PO_4 and SiO_2 concentrations were elevated in 2013 relative to 2012, but the difference between years was not significant ($p > 0.05$). N/P, N/Si and P/Si ratios were greater in 2012 relative to 2013 ($p < 0.01$) (**data not shown**).

DIC concentrations exhibited minimal variability during 2012, and markedly decreased during the first (February/March) and the greatest freshwater discharge events (July) in 2013 (**Fig. 2C**). $\delta^{13}\text{C}$ -DIC values became more depleted during summer relative to spring, fall and

winter in both years, and were more depleted in summer 2013 relative to summer 2012, also following the pulses in freshwater discharge (**Fig. 2C**).

3.3 Dissolved Organic Matter (Figs. 3-4). Bulk DOC and DON concentrations were greater in summer, peaking in late July in both years, and both exhibited greater concentrations in 2013 relative to 2012 ($p < 0.0001$) (**Fig. 3A**). DOC peaked synchronously with pulses of freshwater discharge in March and July 2013. DOC half-lives ($t_{1/2}$) were $42.5 (\pm 1.9)$ and $42.3 (\pm 1.9)$ days for 2012 and 2013, respectively, and not significantly different between years. As for DOC concentration, CDOM absorbance (a_{254} , a_{350}) and intensity of FDOM components C1, C2 and C3 were all greater in summer in both years, greater in 2013 relative to 2012 ($p < 0.0001$), and peaked synchronously with freshwater discharge pulses, most markedly in March, July and September 2013 (**Fig. 3B-C**). Within FDOM, the humic-like components C1 and C2 displayed greater magnitude of increases relative to the protein-like component C3 at high discharge (**Fig. 3C**).

DOM character shifted from 2012 to 2013. DOC/DON ratios were slightly greater in 2013, but not significantly different ($p > 0.05$, **Fig. 4A**). $SUVA_{254}$ values (used as a proxy for DOM aromaticity), were significantly greater in 2013 relative to 2012 ($p < 0.0001$, **Fig. 4A**). The ratio of humic-like 1 to humic-like 2 (C1/C2) fluorescence peaks was constant over the whole time series ($p > 0.05$), while the ratios of humic-like C1 to protein-like C3 and humic-like C2 to protein-like C3 were significantly greater ($p < 0.0001$) in 2013 relative to 2012, peaking with freshwater discharge pulses (**Fig. 4B**).

3.4 Secchi depth and Particulate organic matter (Fig. 5). Secchi depths were shallower in the summer and deeper in the winter (**Fig. 5A**), and not significantly different between 2012 and 2013 ($p > 0.05$). These trends in Secchi depth values indicated that total particulates in suspension in the water column and light attenuation were greater in the summer, but similar at low and high

discharge (i.e. between years). POC and PON exhibited the highest concentrations in summer in both years (**Fig. 5B**). POC concentrations displayed similar values between the two years ($p>0.05$), and PON concentrations were greater in 2012 relative to 2013 ($p<0.05$). $\delta^{13}\text{C}$ -POC values were more depleted in 2013 (-23.8‰) relative to 2012 (-22.9‰) ($p<0.001$) and $\delta^{15}\text{N}$ -PON values were more enriched in 2013 (5.6‰) relative to 2012 (5.3‰) ($p<0.001$) (**Fig. 5C**). The depletion in $\delta^{13}\text{C}$ -POC values and enrichment in $\delta^{15}\text{N}$ -PON values across the hydrological transition is clearer when $\delta^{13}\text{C}$ -POC versus $\delta^{15}\text{N}$ -PON values are plotted for each year (**Fig. 5D**).

3.5 Microbial communities (Fig. 6). In both years, the onset of phytoplankton growth, based upon increasing chlorophyll-a concentrations and cell abundance, occurred in spring (**Fig. 6A-B**), coinciding with rising water temperatures (**Fig. 1B**). Bulk chlorophyll-a concentrations, chlorophyll-a minus phaeophytin concentrations and Phyto-total abundance were not significantly different between 2012 and 2013 ($p>0.05$). The abundance of Phyto-PC started to increase in May in both years. The bloom started to decay in late June 2012, but persisted until August in 2013 (**Fig. 6B**). In 2012, the collapse of the Phyto-PC bloom was followed by a Phyto-PE bloom in July. This Phyto-PE bloom did not occur in 2013 (**Fig. 6B**). Phyto-Euk peaked in spring in both years and was the most abundant group from late summer through early spring before and after the Phyto-PE and Phyto-PC blooms in both years (**Fig. 6B**).

Heterotrophic bacterioplankton (HB-total) community abundance peaked earlier in June 2012 and in August 2013 (**Fig. 6C**). Average abundances of HB-total and HNA were greater in 2013 relative to 2012 ($p<0.001$), while abundance of LNA was similar between years ($p>0.05$). During spring and summer 2012, the bacterioplankton community became increasingly dominated by the LNA sub-community. Throughout 2013, the bacterioplankton community had

more equal proportions of LNA and HNA, with a slightly greater proportion of HNA, except for the month of June, when LNA was slightly more abundant (**Fig. 6D**).

3.6 Multiple linear regression (MLR) models

When 2012-2013 data were modelled as a continuous dataset, either water temperature or salinity were identified as the primary predictors of DIM, DOM, POM and microbial parameters (**Table 1**). Phyto-Euk abundance was the only parameter for which the MLR model was not statistically significant (F-test, $p > 0.05$). Inorganic nutrient concentrations were primarily predicted by water temperature, except NH_4 that was best predicted by salinity. Because NH_4 represented the largest fraction of inorganic N (**Fig. 2A**), the ratios N/P and N/Si were also best predicted by salinity. DIC concentrations and $\delta^{13}\text{C}$ -DIC values were best predicted by salinity. DOM quantitative and qualitative parameters were best predicted by salinity, except those representatives of the DON pool (DON concentrations, DOC/DON and FDOM C3 protein-like material), that were better predicted by water temperature. The best predictor for POM concentrations and POC/PON was water temperature, while $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON values were best predicted by salinity. HNA abundances were best predicted by salinity. All other microbial abundances and chlorophyll-a concentrations (bulk and phaeophytin-discounted) were best predicted by water temperature. Precipitation was not the primary predictor for any parameters, but was a significant secondary predictor for NO_x , N/P, N/Si and POM concentrations.

When 2012 and 2013 datasets were modelled separately as independent datasets, a shift from water temperature in 2012 to salinity in 2013 as the primary predictor of most DIM, DOM, POM and microbial parameters was detected (2012-only and 2013-only, **Table 1**). This shift reflected the transition from low discharge (2012) to high discharge conditions (2013). The

exceptions were DIC concentrations, best predicted by salinity in both years, and DOC/DON and Phyto-PC and Phyto-PE abundances, best predicted by water temperature in both years. NH_4 , N/P and POC concentrations were best predicted by local precipitation in 2012 and by salinity in 2013.

4. Discussion

4.1 Hydrology

Annual mean discharge in 2012 ($141 \text{ m}^3 \text{ sec}^{-1}$) was the lowest on record for the Savannah River (1930-2013, USGS site#02198500). At the Skidaway River Estuary, annual mean salinity measured at high tide in 2012 (32 ± 1.2) was the highest on record (1986-2013; average 26.8 ± 2.2) (Verity 2002) (<http://www.bco-dmo.org/project/2192>). Therefore, the high salinity detected consistently throughout 2012 was unusual for the Skidaway River Estuary. Mean annual discharge in 2013 ($319 \text{ m}^3 \text{ sec}^{-1}$) was the same as the average for 1930 to 2013. However, since discharge of Georgia Rivers is usually higher between late winter and early spring, and lowest between summer and fall (Blanton and Atkinson 1983; Alber and Sheldon 1999), the high discharge in July and August 2013 was atypical (**Fig. 1C**). Consequently, in the Skidaway River Estuary, the average salinity in July and August 2013 (22.1 ± 3.8) was the lowest on record (1986 – 2013, average 28.4 ± 3.15 ; Verity 2002, <http://www.bco-dmo.org/project/2192>). In this historic context, the shift in Savannah River discharge and Skidaway River Estuary salinity from 2012 to 2013 represents an extreme inter-annual variability in hydrological dynamics for this site. Between 1930 and 2013, the amplitude of variation in Savannah River annual average discharge was close to 5-fold, with no clear evidence of increasing or decreasing trends over time. Historical data for precipitation in the Southeast US also indicates no significant trends over the last century (Karl et al. 2009). However, predictions for the next century in association with

global climate change include shifts in low versus high-salinity water intrusions in the Southeast US coast. Predicted scenarios include increased drought severity and/or duration (Karl et al. 2009; Seager et al. 2009), increased net precipitation and/or intense storm frequency (Seager et al. 2009; IPCC 2013), and sea level rise (Weston et al. 2006). Therefore, a better understanding of the interactions between freshwater discharge and the multiple components of saltmarsh-dominated estuaries is imperative in order to predict and manage the response of these ecosystems to climate change in the Southeast US.

4.2 Dissolved Inorganic Matter

The driving factors behind the association between inorganic nutrients and water temperature (**Table 1**) are likely underpinned by other seasonal factors, such as day-length and growing season, along with ecological successions that occur during the year, as well as adsorption and desorption from sediments (Conley and Malone 1992; Jordan et al. 2008; Cornwell et al. 2014). For example, the variability in inorganic N concentrations can be linked with uptake by eukaryotic phytoplankton in fall (*refer to section 4.4.1 for further discussion*), remineralization of DON, the competition between phyto- and bacterioplankton for inorganic N in this system (Hanson et al. 1990), and the high rates of nitrification and denitrification typical of marsh dominated waters and sediments (Cai et al. 2000).

Local precipitation seemed to play a role in the variability of inorganic nutrients (NO_x , N/P and N/Si in 2012-2013, and NH_4 and N/P in 2012, **Table 1**). NH_4 is the dominant form of inorganic N in groundwater (Krest et al. 2000), thus changes in groundwater flow pressure and flushing of marsh pore waters caused by rainfall and storm events could be among the underlying mechanisms leading to NH_4 inputs into the estuary water column (Whiting and Childers 1989; Verity 2002). Precipitation can also result in an increase in NO_x , as well as PO_4

and SiO_2 , by increasing saltmarsh surface runoff and drainage into adjacent estuaries (Imberger et al. 1983).

PO_4 and SiO_2 are sourced from weathering of rocks, so the concentrations of these inorganic constituents are expected to increase with discharge. PO_4 and SiO_2 appeared enriched in 2013 (**Fig. 2B**), although the differences in concentrations between years were not significant. The main sinks of these inorganic constituents is phytoplankton uptake. The phytoplankton community is thought to be light limited and/or N limited in these estuarine waters (Cloern 1987; Hanson and Robertson 1988), which explains the apparent low drawdown of PO_4 and SiO_2 in the water column in the Skidaway River Estuary (*refer to section 4.4.1 for further discussion*).

DIC concentrations and isotopic signatures in estuarine waters are influenced by four main processes: freshwater versus marine water intrusions, rates of respiration and photosynthesis (both in the water column and within marsh and estuarine sediments), and outgassing (Atekwana et al. 2003; Jiang et al. 2008). In this study, salinity was the primary predictor of variability in DIC concentrations and $\delta^{13}\text{C}$ -DIC values (except $\delta^{13}\text{C}$ -DIC values when 2012 was treated as an independent dataset, **Table 1**). Rates of respiration and photosynthesis were not measured, which precludes the determination of the significance of these factors as drivers for DIC and $\delta^{13}\text{C}$ -DIC values in this study. DIC concentrations and $\delta^{13}\text{C}$ -DIC values are likely influenced by marsh and estuarine respiration and photosynthesis rates in this system, as suggested by the depletion in $\delta^{13}\text{C}$ -DIC values in summer at low discharge (**Fig. 2C**), and best predicted by water temperature in 2012 (**Table 1**). However, these signals were likely swamped out by the salinity signal, which was the primary predictor based on the statistical approach used, and at the temporal (weekly) resolution captured in this study. These results are supported by previous studies that suggest that marsh primary production balances

local respiration in the tidal marsh-dominated systems along the Georgia coast (Cai 2011). In this context, marsh productivity and respiration include primary production and organic matter utilization beyond the free flowing water column of the estuary and encapsulates the whole hydrologically linked system of fluvial channels, and tidally pumped marsh pore waters and estuarine sediments.

4.3 Dissolved Organic Matter

In 2012, DOC had lower concentration and variability throughout the year, with a single summer peak, and DOM had lower absorbance (CDOM) and fluorescence (FDOM) intensities, relative to 2013 (**Fig. 3A-C**). In 2012, of the environmental parameters considered, DOM was primarily predicted by water temperature and displayed autochthonous or marine characteristics, particularly regarding SUVA₂₅₄ and FDOM composition, relative to 2013 (Jaffé et al. 2008; Vazquez et al. 2010). The relatively lower levels of SUVA₂₅₄ (2.5 and lower) and high relative content of protein-like (C3) relative to humic-like (C1 and C2) FDOM (**Fig. 4A-B**) suggested that the DOM was predominantly produced in situ or imported from coastal waters in spring and summer 2012 (Ya et al. 2015). Autochthonous sources of DOM within the marsh system could have included *Spartina* leachates (Turner 1993; Wang et al. 2014) and phytoplankton primary productivity and exudation (Dame et al. 2000; Bittar et al. 2015). Leaching of *Spartina*-DOM is greater in summer and at high salinities, and is also considered an important source of organics that fuel secondary productivity in saltmarsh estuaries (Wang et al. 2014). In addition, algal DOM could have originated from benthic algal mats that cover the marsh sediment surface, and leached into the water column (Porubsky et al. 2008).

DOC concentrations and freshwater discharge were higher in 2013 relative to 2012 (**Fig. 3A**), suggesting inputs of terrigenous DOC in 2013. High discharge also corresponded with

changes in DOM quality that were indicative of greater terrigenous DOM inputs, including a slight increase in DOC/DON (**Fig. 4A**), an enrichment in chromophoric, aromatic DOM (i.e., significant increase in SUVA₂₅₄, **Fig. 4A**), and an elevated proportion of humic-like FDOM (i.e., significant increase in C1/C3 and C2/C3, **Fig. 4B**), which typically indicates increase in concentrations of lignin-phenols (Walker et al. 2009). These shifts suggest that discharge was a primary driver of terrigenous DOM inputs into the estuary (Stedmon and Markager 2005; Walker et al. 2009) and, ultimately, a relevant factor shaping DOM character (Meybeck 1982; Medeiros et al. 2015). While the first pulse of freshwater discharge (February/March 2013) was 4-fold lower than the greatest pulse (July 2013), shifts in the concentration and quality of DOM, CDOM and FDOM were apparent in March (**Fig. 3-4**). It is possible that the early spring discharge events led to the release of significant amounts of material that had been trapped during the preceding drought period, as predicted by hydrological modelling (Grieve 1991). In addition, the concomitant production of autochthonous DOM in summer 2013 possibly counteracted the terrigenous DOM inputs via discharge. As seasonal trends in water temperature and light availability for autochthonous DOM production are similar between years than those of freshwater discharge, the latter was identified as the primary driver for the balance between autochthonous and terrestrial (allochthonous) DOM in the estuary .

In both years, net increases in DOM concentrations occurred between early spring and late summer, while the autumn was characterized by a period of decreasing DOM concentrations (**Fig. 3A**). The multiple processes that drove these seasonal trends are unclear. Focusing upon the decline in DOC concentrations post-summer, multiple processes should be considered. These include a reduction of the DOC source term to the system and subsequent hydrological flushing from the system (Dixon et al. 2014); photochemical and biological mineralization of DOC (Ward

et al. 2013; Marín-Spiotta et al. 2014; Mopper et al. 2015); and auto- and photo-flocculation (Sholkovitz 1976; Helms et al. 2013). Despite uncertainties in the removal processes at play and the clear distinctions in DOM character between the two years, the decay constant of DOC in the autumn was similar for 2012 and 2013, with half-lives of 42.5 and 42.3 days, respectively (**Fig. 3A**).

It is likely that microbes and DOM dynamics are tightly linked (Marín-Spiotta et al. 2014), as suggested by the greater concentrations of DOM, and greater abundances of HB-total and HNA in summer 2013 relative to 2012 (*refer to section 4.4.2 for further discussion*). However, increases in bacterioplankton abundance cannot be attributed to DOM alone. The coincidence between increased DOC concentrations and HB-total /HNA abundances may be the result of other factors that influence both DOC and microbial standing stocks. For instance, salinity has a strong influence upon both DOM (Ya et al. 2015) and the microbial community composition in estuarine systems (Crump et al., 2004).

The winter was characterized by minima in DOC concentrations each year. Both winters had similar DOC concentrations ($\sim 180 \mu\text{M-C}$), but CDOM absorbance (a_{254} and a_{350} , $p < 0.01$), aromaticity index (SUVA_{254} , $p < 0.01$) and the ratio of FDOM humic C1 to protein-like C3 ($p < 0.01$) were greater in 2013 relative to 2012. These results indicate that the distinct sources of DOM influenced the character of the winter DOM. Therefore, regardless of similar DOC removal rates and concentrations, the characteristics of the DOM in the system in winter are likely to resemble those of their sources, i.e., autochthonous-like features (likely from in situ primary production) at the end of a low discharge year, and terrigenous-like features at the end of a high discharge year.

4.4 Particulate organic matter and Secchi depth

Seasonally-driven increases in day-length and temperature generally result in increased metabolic activity and biomass accumulation, thus Secchi depths were shallower (**Fig. 5A**) and POC and PON reached greater concentrations (**Fig. 5B**) in summer relative to the rest of the year. Shallower Secchi depths indicate not only greater amounts of total particulates (i.e., live organisms and detritus), but also greater light attenuation in the water column in summer. High discharge led to a subtle, but statistically significant shift in the isotopic signatures of $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON (**Fig. 5C-D**), suggesting a shift from a more estuarine-phytoplankton signature in 2012 to a more terrigenous signature in 2013 (Cloern et al. 2002). Although the isotopic signature of POM in the water indicated terrigenous-like particulates during high discharge, POM concentrations and Secchi depths were not significantly altered by discharge. This observation is likely associated with specific characteristics of the Savannah River watershed land use, and the complex hydrography of the Skidaway River Estuary (Blanton and Atkinson 1983; Blanton et al. 2010). Dams and large artificial reservoirs built along the Savannah River tend to significantly decrease the particle load transported by the River (Wiegert et al. 1999). Georgia estuaries are characterized by fine sediments and tidal mixing is the most relevant control on particle resuspension and deposition in the Skidaway River Estuary (Blanton and Atkinson 1983; Wiegert et al. 1999). Therefore, the amount of POM suspended in the water column at any given time is most likely a function of tidal amplitude and local wind stress, with discharge only influencing the quality of the sediment that is suspended.

4.5 Microbial communities

4.5.1 Phytoplankton

The main controls on phytoplankton physiology are temperature, light- and nutrient availability (Reynolds 1989). Spectral characteristic of underwater light affect coexistence and

competition among phytoplankton, particularly cyanobacteria with distinct accessory pigments (Stomp et al. 2007). During 2012-2013, seasonal shifts in water temperature in the estuary were greater than inter-annual variability, and the transition from low to high freshwater discharge did not cause significant changes in inorganic nutrients concentrations (**Fig. 2A-B**), POM concentrations, or light attenuation in the water column (based on Secchi depths) (**Fig. 5A-C**). The lack of a connection between these factors and discharge may therefore explain the lack of a significance differences in phytoplankton total abundance and biomass (based on chlorophyll-a concentrations) between years of low and high discharge (**Fig. 6A-B**). Salinity shifts did not affect phytoplankton biomass or Phyto-total abundance, but it did affect the composition of cyanobacterial sub-communities within Phyto-total (**Fig. 6B**). The small relative cell size and presence of the cyanobacterial accessory pigments PE and PC (*refer to section 2.4.3*) suggest the Phyto-PC and Phyto-PE sub-communities are primarily comprised of cyanobacteria. Another indication is the bloom-like growth dynamics of these groups in summer, as cyanobacteria display competitive advantages over eukaryotic phytoplankton under higher temperatures (Paerl and Huisman 2008). The main shift in sub-community succession detected in this study was the co-occurrence of Phyto-PC and Phyto-PE in summer 2012, in contrast with the longer-lasting bloom of Phyto-PC and absence of Phyto-PE in 2013. These results suggested that Phyto-PC is likely a euryhaline cyanobacteria, while Phyto-PE is likely a marine, high-salinity *Synechococcus*-like cyanobacteria (Mitbavkar et al. 2012). Since marine *Synechococcus* have an obligate salt requirement (Waterbury et al. 1986), the decreases in salinity below 75% of full salinity that occurred in summer 2013 are likely the primary cause of the low competitive advantage of Phyto-PE in 2013. The taxonomic composition of the Phyto-Euk sub-community, on the other hand, is more difficult to determine without a higher resolution taxonomic approach,

such as extensive microscopy-based analyses, or DNA sequencing data. In other estuaries, non-cyanobacteria groups are comprised of multiple taxa such as chlorophytes, diatoms and cryptophytes, that generally have greater competitive advantages in spring, autumn and winter (Valdes-Weaver et al. 2006).

4.5.2 Bacterioplankton

Bacterial responses, both in terms of abundance and community composition, to multiple environmental factors are highly complex (Moran et al. 2013). Both salinity and DOM concentration and composition are important drivers of bacterioplankton dynamics (Crump et al. 2004; Landa et al. 2014), and both these factors changed substantially in the Skidaway River Estuary in response to high discharge (*refer to sections 4.1 and 4.3 for further discussion*). Early studies exploring the significance of LNA and HNA sub-communities suggested that HNA represent the active portion of the microbial community, whereas LNA represent dormant or inactive bacteria (Li et al. 1995). Further studies revealed that the activity rates of these sub-communities relative to bulk bacterial activity varied with environmental settings, with LNA displaying greater activity in oceanic oligotrophic waters, whereas HNA was the most active sub-community in coastal waters (Servais et al. 2003; Mary et al. 2006). In line with this pattern, assuming that HNA sub-community represents the most active fraction of the bacterioplankton in the coastal Skidaway River Estuary, the increase in HNA abundances detected in 2013 could be interpreted as an increase in bulk bacterioplankton activity. In that context, the increase in HNA abundance in 2013 implies that high discharge has the potential not only to increase bacterioplankton abundance, but also enhance specific activity, respiration and CO₂ outgassing (Fasching et al. 2014). Aside from activity levels, high-resolution community structure studies have demonstrated that bacterioplankton LNA and HNA sub-communities represent bacterial

assemblages with distinct phylogenetic composition, and thus play distinct ecological and biochemical roles in natural waters (Schattenhofer et al. 2011; Vila-Costa et al. 2012; Read et al. 2015). In this study, only two major components of the bacterioplankton community could be discerned by flow cytometry. High proportions of LNA coincided with periods when Phyto-total displayed highest abundances and DOM displayed more autochthonous character (throughout summer 2012 and in June 2013, **Fig. 4A-B, 6D**). This pattern suggested that LNA could represent bacterial taxa that have competitive advantages over HNA taxa when freshly-produced, autochthonous DOM is more likely to occur. The association between LNA and phytoplankton-DOM has been detected previously (Scharek and Latasa 2007), but contrast with other reports (Wetz and Wheeler 2004; Huete-Stauffer and Morán 2012). Alternatively, this trend could also be interpreted as LNA being favored by consistently higher salinities. Larger genomes of HNA, and thus greater metabolic versatility, have been associated with greater competitive advantages of this sub-community in environments with greater diversity of ecological niches, e.g. in coastal versus open ocean waters (Pommier et al. 2010; Schattenhofer et al. 2011). The results presented here are consistent with these patterns, as the increase in HB-total abundance was driven by increases in HNA abundances in 2013. Moreover, HNA was the only sub-community best predicted by salinity in 2012-2013, thus it could be a sub-community comprised of taxa with greater tolerance to changing salinity, relative to the LNA sub-community.

4.6 Significance of MLR modeling trends

The components of the water column in subtropical tidal estuaries dominated by saltmarshes are influenced by hydrological forces, seasonality and by in situ processes, such as biological and photochemical mineralization, flocculation and aggregation. The hydrology of a tidal estuary is complex as many factors influence the mixing of water masses with distinct

characteristics, including tidal pumping, riverine discharge and flushing, precipitation and evaporation, groundwater discharge, and sediment pore water intrusions. The combination of these factors affect the residence time of the water column, which in turn complicates the interpretation of variability attributable to in situ processes. The MLR modelling approach used in this study does not have the statistical power to disentangle or describe all relevant mechanisms that have the potential to affect DIM, DOM, POM and microbial communities. In addition, the efforts to model the datasets presented in this study are limited by the lack of data on spatial distribution, and rates of production and removal attributable to specific processes. Therefore, the MLR model can only be interpreted as descriptive of the relative relevance of seasonality versus hydrology on the water column components in the timeframe of this study.

An important consideration that must underpin the interpretation of water temperature, salinity and local precipitation as environmental predictors in the MLR model developed in this study is that, in most cases, these predictors do not necessarily have a direct effect on DIM, DOM, POM and microbes. For example, water temperature does not have a direct effect on DON concentrations. Rather, temperature affects metabolic activity, growth and biomass accumulation of primary producers (phytoplankton and *Spartina*) and zooplankton, which in turn determines rates of DON release through exudation, leaching and grazing/excretion. The statistically significant relationship between a given environmental predictor (e.g., water temperature) and a given variable (e.g., DON concentration) is often mediated by a process that was not directly measured (e.g., metabolic and release rates). As with any statistically significant correlation, cause and effect cannot be assumed, particularly in such a complex ecosystem. In addition, MLR is limited as it does not account for potential predictors other than the ones selected to represent seasonal (water temperature), inter-annual (salinity) or stochastic (local

precipitation) forces. The absence of these other potential predictors from the model could explain the low percentages of variability explained by the model (i.e., low r^2 values) for some of the variables, for example NO_x and PO_4 (**Table 1**).

Keeping these limitations in mind, the MLR model is informative on general trends of DIM, DOM, POM and microbial communities relative to the environmental factors selected as predictors. Two specific examples of ecologically and biogeochemically relevant trends that can be extracted from the continuous dataset (2012-2013) are the overall association of DOC concentrations and DOM optical signatures with salinity, and the association of phytoplankton abundances and biomass (extracted chlorophyll-a) with water temperature (2012-2013, **Table 1**). As discussed in section 4.3, DOM has many potential sources and sinks in this saltmarsh estuarine system, many of which are associated with water masses mixing, and thus DOM is likely to follow the conservative mixing trends in salinity. At the same time, while phytoplankton are also subject to mixing of water masses with different salinities, these organisms have intrinsic growth rates in each of these water masses, which are directly influenced by temperature through metabolic and enzymatic activity, growth and biomass accumulation, and grazing/mortality rates.

Within the limits of the MLR models, relevant trends in response to the hydrological shifts from low to high discharge can also be detected from the 2012 and 2013 datasets modelled individually (2012-only and 2013-only, **Table 1**). Overall, this modelling approach indicated that at low discharge and relatively constant, high salinity (2012-only, **Table 1**), seasonality trends are robust and determine the variability in most parameters in this system. Inter-annual shift in salinity (associated with discharge) can be interpreted as a pulsing disturbance in this seasonally-driven variability (2013-only, **Table 1**). Examples of the mechanisms by which these low

salinity water pulses affect the system are (1) conservative mixing of salinity-defined endmembers, particularly for the dissolved compartment, and (2) shifts in community composition based on physiological salinity-tolerance of microbial autotrophic and heterotrophic sub-communities.

In summary, the results presented in this study showed that changes in water temperature, concentrations of inorganic nutrients and POM, and Secchi depths displayed conservative seasonal cycles and were relatively independent from inter-annual variability in freshwater discharge and salinity. Consequently, and in spite of the changes in cyanobacterial composition likely caused by salinity, the variability in phytoplankton (Phyto-total abundances and biomass) was also more strongly affected by seasonality than by inter-annual variability in discharge. At the same time, DIC isotopic signatures, DOC concentrations, DOM optical signatures, and bacterioplankton community dynamics were more strongly affected by high discharge via the loading of low salinity water and terrigenous-derived organics. Collectively, these results suggest that the phytoplankton community, which represents a fraction of the micro-autotrophic compartment of the estuary, appears less affected by hydrology shifts relative to the micro-heterotrophic compartment, represented in this study by the bacterioplankton community.

Riverine discharge has been previously described as a mechanism of DOM delivery to coastal waters (Meybeck 1982). The findings that discharge strongly influenced DOM and DIC characteristics and the bacterioplankton community at the Skidaway River Estuary is relevant considering the hydrology, seasonal and landscape features of this site. The high tidal amplitude, dense saltmarsh coverage area and high levels of grass primary productivity create a hydrologically and chemically dynamic environment, representing significant inputs of material with very distinct character, from multiple sources. One important point that this study brings

forward is that tidal and marsh pumping of water with distinct DOM, DIC and microbial characteristics did not have enough of a buffering and/or diluting capacity to diminish the influence of high riverine discharge on the estuary.

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Figure 1. Water temperature and precipitation (A) at the Savannah River (SR) (USGS site #0219897773) and (B) at the Skidaway River Estuary (SRE), and (C) Savannah River discharge (USGS site #02198500) and salinity at SR and SRE from January 2012 to December 2013.

Figure 2. Dissolved inorganic matter in the Skidaway River Estuary in 2012-2013. Concentrations of (A) dissolved inorganic nitrogen (NH_4 and NO_x); (B) dissolved phosphate (PO_4) and dissolved silicate (SiO_2); (C) dissolved inorganic carbon (DIC) concentrations and $\delta^{13}\text{C-DIC}$ signatures.

Figure 3. Dissolved organic matter concentrations and optical intensities in the Skidaway River Estuary in 2012-2013. (A) Dissolved organic carbon (DOC) and nitrogen (DON) concentrations; DOC half-lives were 42.5 (± 1.9 d) in 2012 and 42.3 (± 1.9 d) in 2013; (B) intensity of chromophoric DOM (CDOM) absorbance at 254 and 350 nm; and (C) intensity of fluorescent DOM (FDOM) components 1, 2 and 3 derived from PARAFAC modelling.

Figure 4. Dissolved organic matter character in the Skidaway River Estuary in 2012-2013. (A) DOC/DON ratios and SUVA₂₅₄; and (B) ratios of FDOM components (C1/C2, C1/C3, C3/C2).

Figure 5. Particulate matter in the Skidaway River Estuary in 2012-2013. (A) Secchi depths; (B) particulate organic carbon (POC) and nitrogen (PON) concentrations; (C) $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PON; and (D) $\delta^{13}\text{C}$ -POC versus $\delta^{15}\text{N}$ -PON for each year.

Figure 6. Microbial communities at the Skidaway River Estuary in 2012-2013. (A) Bulk chlorophyll-a $[\text{chl-a}]$ and chlorophyll-a minus phaeophytin $[\text{phaeo}]$ concentrations; (B) phytoplankton total and sub-communities abundances; (C) bacterioplankton total and sub-community abundances; and (D) bacterioplankton relative composition.

Table 1. Multiple linear regression (MLR) models with salinity, water temperature and local precipitation defined as predictors of variability in each measurement of dissolved inorganic and organic matter, particulate matter and microbial communities from the Skidaway River Estuary water collected weekly at high tide during years 2012 and 2013. Data were modelled as continuous (2012-2013) and individual (2012-only, 2013-only) datasets. Adjusted R^2 = proportion of variability explained by MLR models; p values = significance of prediction; n/a = MLR not significant.

Parameter	Measurement	2012-2013			
		Adjusted R^2	Primary Predictor	p	Secondary predictor
Dissolved Inorganic Matter (DIM)	[NH ₄]	0.44	salinity	3.8E-08	water temperature
	[NO _x]	0.27	water temperature	1.1E-04	local precipitation
	[PO ₄]	0.15	water temperature	2.9E-04	n/a
	[SiO ₂]	0.49	water temperature	7.7E-10	salinity
	N/P	0.28	salinity	1.3E-04	local precipitation
	N/Si	0.35	salinity	3.5E-07	local precipitation
	P/Si	0.22	water temperature	1.6E-04	salinity
	[DIC]	0.83	salinity	3.1E-37	water temperature
Dissolved Organic Matter (DOM)	$\delta^{13}\text{C}$ -DIC	0.81	salinity	8.0E-27	water temperature
	[DOC]	0.73	salinity	5.0E-23	water temperature
	[DON]	0.59	water temperature	1.8E-13	salinity
	CDOM a254	0.85	salinity	8.7E-34	water temperature
	CDOM a350	0.84	salinity	3.2E-32	water temperature
	FDOM C1	0.85	salinity	2.2E-29	water temperature
	FDOM C2	0.75	salinity	7.2E-21	water temperature
	FDOM C3	0.81	water temperature	1.9E-25	salinity
	DOC/DON	0.29	water temperature	3.8E-07	n/a
	SUVA ₂₅₄	0.65	salinity	5.3E-14	water temperature

	C1/C2	0.35	salinity	2.2E-08	water temperature	2.6E-04
	C1/C3	0.84	salinity	4.3E-35	water temperature	3.4E-04
	C2/C3	0.61	salinity	7.3E-19	<i>n/a</i>	0.0E+00
Particulate Organic Matter and Secchi depth	Secchi depth	0.30	water temperature	4.6E-07	local precipitation	1.7E-02
	[POC]	0.30	water temperature	2.4E-05	local precipitation	4.8E-03
	[PON]	0.39	water temperature	7.3E-09	local precipitation	1.0E-02
	$\delta^{13}\text{C}$ POC	0.13	salinity	1.1E-03	<i>n/a</i>	
	$\delta^{15}\text{N}$ PON	0.22	salinity	1.8E-05	<i>n/a</i>	
	POC/PON	0.14	water temperature	6.7E-04	salinity	1.9E-02
Microbial communities	[Chl-a]	0.42	water temperature	5.8E-10	salinity	6.0E-04
	[Chl-a]-[phaeo]	0.27	water temperature	1.0E-05	salinity	2.6E-03
	Phyto-PC	0.35	water temperature	6.5E-08	salinity	1.1E-02
	Phyto-PE	0.35	water temperature	1.0E-07	salinity	3.3E-05
	Phyto-Euk	-0.01	<i>n/a</i>			
	LNA	0.34	water temperature	3.6E-08	<i>n/a</i>	
	HNA	0.39	salinity	1.8E-09	water temperature	3.5E-02

Table 1. (continuation)

Parameter	Measurement	2012-only			2013-only		
		Adjusted R ²	Primary Predictor	p	Adjusted R ²	Primary Predictor	p
Dissolved Inorganic Matter (DIM)	[NH ₄]	0.20	local precipitation	8.1E-03	0.41	salinity	3.8E-04
	[NO _x]	0.32	water temperature	2.0E-03	0.20	salinity	8.2E-03
	[PO ₄]	0.24	water temperature	1.2E-02	-0.01	<i>n/a</i>	
	[SiO ₂]	0.49	water temperature	7.6E-06	0.21	salinity	9.8E-03
	N/P	0.11	local precipitation	3.0E-02	0.17	<i>n/a</i>	
	N/Si	0.13	water temperature	2.8E-02	0.40	salinity	7.2E-04
	P/Si	0.25	water temperature	3.8E-04	0.04	<i>n/a</i>	
	[DIC]	0.36	salinity	4.5E-05	0.90	salinity	2.0E-24
Dissolved Organic Matter (DOM)	δ ¹³ C-DIC	0.42	water temperature	8.7E-07	0.75	salinity	6.1E-12
	[DOC]	0.49	water temperature	6.4E-07	0.75	salinity	1.1E-13
	[DON]	0.58	water temperature	6.7E-10	0.66	salinity	1.8E-05
	CDOM a254	0.79	water temperature	1.0E-16	0.80	salinity	1.8E-16
	CDOM a350	0.74	water temperature	1.3E-14	0.79	salinity	5.8E-16
	FDOM C1	0.72	water temperature	3.2E-13	0.77	salinity	6.3E-13
	FDOM C2	0.67	water temperature	8.2E-12	0.63	salinity	4.4E-09
	FDOM C3	0.68	water temperature	7.4E-12	0.72	salinity	1.5E-09
	DOC/DON	0.43	water temperature	5.2E-07	0.25	water temperature	1.5E-02
	SUVA ₂₅₄	0.50	water temperature	1.4E-08	0.59	salinity	1.3E-09
	C1/C2	0.29	water temperature	4.2E-04	0.15	salinity	1.3E-02
	C1/C3	0.21	water temperature	1.1E-03	0.82	salinity	1.5E-16
	C2/C3	-0.05	<i>n/a</i>		0.50	salinity	1.2E-07
	Secchi depth	0.16	<i>n/a</i>		0.32	salinity	9.3E-03
Particulate Organic Matter and Secchi depth	[POC]	0.24	local precipitation	2.8E-03	0.32	salinity	2.0E-03
	[PON]	0.44	water temperature	1.5E-03	0.27	salinity	1.2E-03
	δ ¹³ C POC	0.05	<i>n/a</i>		-0.05	<i>n/a</i>	

	$\delta^{15}\text{N}$ PON	-0.07	<i>n/a</i>		0.22	salinity	1.4E-03
	POC/PON	0.26	water temperature	2.7E-04	-0.01	<i>n/a</i>	
Microbial communities	[Chl-a]	0.30	water temperature	9.49442E-05	0.26	salinity	1.2E-03
	[Chl-a]-[phaeo]	0.17	water temperature	1.8E-02	0.22	salinity	1.0E-03
	Phyto-PC	0.17	water temperature	1.9E-02	0.13	water temperature	3.2E-02
	Phyto-PE	0.39	water temperature	4.4E-05	0.22	water temperature	1.1E-03
	Phyto-Euk	0.13	<i>n/a</i>		0.00	<i>n/a</i>	
	LNA	0.41	water temperature	4.8E-04	0.13	salinity	4.8E-03
	HNA	0.02	<i>n/a</i>		0.28	salinity	2.7E-05