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Effects of nitrogen and dissolved organic carbon on microplankton abundances in four coastal South Carolina (USA) systems

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ABSTRACT: In blackwater systems of the southeastern US, dissolved organic carbon (DOC) contributes a major portion to the total dissolved organic matter pool. The primary DOC source is terrestrial vegetation, with phytoplankton contributing less. Thus, upland development may reduce terrestrial DOC inputs, thereby affecting bacterial abundances. Conversely, development and runoff may increase nitrogen (N) and phosphorus (P) inputs, fueling phytoplankton growth and algal-derived DOC. Yet, the variability of DOC, bacteria, and phytoplankton has not been fully assessed across diverse land uses. We investigated seasonal (July 2012 to May 2013) levels of DOC, bacteria, and phytoplankton biomass (chl *a*) in response to N and P additions at 4 coastal South Carolina sites: a forested/agricultural creek, an urbanized creek, a forested creek, and a detention pond. DOC concentrations were highest at the least developed site (forested creek), suggesting the influence of surrounding land. DOC was significantly and positively correlated with precipitation but negatively correlated with salinity, suggesting that rainfall affected DOC mobilization. Chl *a* was highest during summer and positively correlated with temperature, whereas bacterial abundances were generally negatively correlated with salinity. During experiments, chl *a* was often greater in addition treatments than controls, especially at the urbanized creek and detention pond. In certain N-amended treatments, particularly those containing urea, both DOC and chl *a* became elevated following incubation. These results indicate that urea stimulated phytoplankton biomass and possibly a greater contribution of phytoplankton-derived DOC to the total DOC pool. Our findings suggest that biogeochemical cycling of DOC may become altered in developing coastal regions.

KEY WORDS: Dissolved organic carbon · Bacteria · Phytoplankton · Estuaries · South Carolina

INTRODUCTION

Bacteria utilize dissolved organic matter (DOM) for growth and respiration, thereby remineralizing inorganic nutrients that are essential for phytoplankton production and serving as trophic links within coastal and marine systems (Azam et al. 1983, Carlson et al. 2007). In estuaries, DOM sources include allochthonous inputs from the land through rivers and groundwater as well as autochthonous inputs from primary producers (namely phytoplankton and plants) (Cauwet 2002, Aitkenhead-Peterson et al. 2003, Bertilsson & Jones 2003, Goñi et al. 2003, Teira et al. 2009). The chemical composition of the estuarine

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DOM pool includes dissolved carbon (C), nitrogen (N), and phosphorus (P) (Pinckney et al. 2001, Flynn 2008). Dissolved organic carbon (DOC) in particular provides reduced C for heterotrophic bacterial growth and respiration (e.g. Raymond & Bauer 2000, 2001). In fact, total DOM has a high C:N ratio, such that increased DOC fluxes can lead to bacteria competing with phytoplankton for inorganic N and P (Steelink 1985).

Major sources of DOC to estuaries include the breakdown of terrestrial vegetation, tannins, watershed runoff, zooplankton excretion, and phytoplankton cell leaching and lysis (Malinsky-Rushansky & Legrand 1996, Pinckney et al. 2001). Allochthonous DOC in particular has been shown to contribute substantially to the overall DOC pool in estuarine systems (Goñi et al. 2003, Chow et al. 2013). For example, many coastal regions in the southeastern US, including systems in South Carolina (SC), are influenced by colored 'blackwater' rivers (Smith & Benner 2005) that tend to have high DOC concentrations (Leff & Meyer 1991, Moran et al. 1999, Mallin et al. 2004, 2009). A primary source of allochthonous DOC to the SC coast is fresh and decomposed leaf litter from plants in surrounding forested wetlands (Goñi & Thomas 2000, Davis et al. 2006). For example, in Winyah Bay, the surrounding cypress-tupelo wetlands are a primary source of DOC to the estuary (Chow et al. 2013).

Surrounding land use further influences the source, quantity, and quality of DOC, thus mediating the levels and production rates of heterotrophic bacteria (Carlson et al. 2007, Nagata 2008, Wear et al. 2014). Certain regions along the southeastern US coastal zone are undergoing rapid expansion such that rates of land development often exceed rates of population growth (Allen & Lu 2003, DiDonato et al. 2009, Sanger et al. 2015). The urbanization of forested and agricultural land has been shown to strongly influence the magnitude and composition of terrigenous materials (Walsh et al. 2005, Hutchins et al. 2014). For example, DOC concentrations have been shown to be higher in runoff from forested compared to urbanized watersheds (Wahl et al. 1997), whereas runoff from urbanized watersheds typically exhibits higher concentrations of inorganic nutrients and total suspended solids than runoff from forested watersheds (Tufford et al. 2003, Mallin et al. 2009). Bacterial community composition in Winyah Bay was more correlated to DOM (including DOC) quality rather than quantity (Wear et al. 2014), suggesting that DOM source (i.e. composition) regulates bacterial assemblages. Thus, watershed development may affect both DOC delivery and bacterial community structure in receiving estuaries.

Leaching from phytoplankton cells also contributes to the overall DOC pool in coastal and marine systems, but the amount of phytoplankton-derived DOC varies spatially and temporally (Hitchcock et al. 2010). For example, phytoplankton extracellular release of DOC accounted for <40% of the bacterial C requirement (derived from bacterial production) across several freshwater and marine systems (Baines & Pace 1991) and ranged from 0 to 30% in several coastal Atlantic ecosystems (reviewed by Bertilsson & Jones 2003). In SC, estuarine phytoplankton contributes 20 to 50% of organic matter in Winyah Bay (Goñi et al. 2003). Since phytoplankton blooms are often associated with eutrophication (reviewed by Anderson et al. 2008, Heisler et al. 2008), it is plausible that phytoplankton-derived DOC levels would be greater in developed and nutrient-enriched regions than in less developed regions. However, the extent to which phytoplankton affects DOC concentrations and bacterial levels along developing coastlines, such as SC, has not been thoroughly investigated.

Despite the growing literature evaluating bacterial responses to DOC in coastal systems, fundamental questions remain relating DOC, bacteria, and phytoplankton across sites with differing land use characteristics. The present study examined seasonal levels of DOC, bacterial abundances, and phytoplankton biomass (chl *a*) across 4 coastal SC sites with distinct land uses: urban, stormwater detention pond, forested/agricultural, and forested/undeveloped. The overall goal was to investigate whether trends in DOC and bacterial concentrations followed phytoplankton biomass responses to nutrient additions. Specific objectives were to (1) quantify DOC concentrations, bacterial abundances, and chl *a* concentrations at each site and evaluate correlations between these parameters and with relevant environmental metrics (temperature, salinity, precipitation), and (2) determine whether bacterial abundances and/or DOC responses correspond with chl *a* changes in N and P additions.

MATERIALS AND METHODS

Site descriptions

This study was conducted in tandem with nutrient addition bioassays (Reed 2014) deployed at 4 tidally influenced sites along the SC coast (Fig. 1). These sites included 3 tidal creek habitats and 1 stormwater detention pond, as described below.

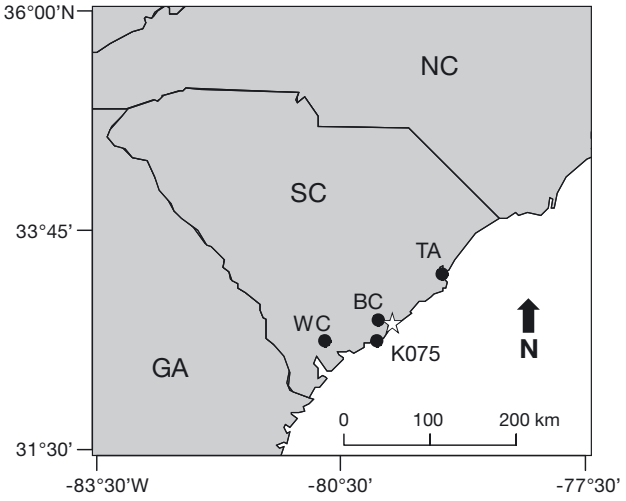


Fig. 1. Location of the 4 study sites: Thousand Acre (TA), Bull Creek (BC), Kiawah Island Pond number 075 (K075), and Wimbee Creek (WC). States are North Carolina (NC), South Carolina (SC), and Georgia (GA). Star indicates Charleston, SC

Thousand Acre (TA; 33°17′56″ N, 79°15′21″ W) is a forested and agricultural tidal creek situated within Winyah Bay, a coastal plain estuary that receives water from 5 rivers: Sampit, Black, Waccamaw, Little Pee Dee, and Yadkin-Pee Dee (Goñi et al. 2003, Buzzelli et al. 2004). Winyah Bay is the fourth largest estuary on the southeast coast (Goñi et al. 2003, 2009), and surrounding land is characterized by forested, natural, managed, industrial, and agricultural wetlands (Buzzelli et al. 2004).

Bull Creek (BC; 32°49′38″ N, 80°01′44″ W) is an urbanized tidal creek (Holland et al. 2004) located on the southeastern end of the Ashley River, a 48 km long coastal plain tidal river (South Carolina Department of Natural Resources 2003). The Ashley River basin (~2300 km^2) drains a variety of landscapes including marshes, forested wetlands, commercial, and residential development before reaching the city of Charleston (South Carolina Department of Natural Resources 2003, South Carolina Department of Health and Environmental Control 2005).

Kiawah Island (KI) Pond number 075 (K075; 32° 36′44″ N, 80°03′01″ W) is a stormwater detention pond (constructed water body acting as a catchment for runoff) that is bordered by homes, roadways, and a golf course (Brock 2006). Stormwater detention ponds accumulate nutrients, have high residence times, and tend to stagnate, thereby creating environments conducive to phytoplankton blooms, including harmful algal blooms (HABs) (Lewitus et al. 2003, 2008, Drescher et al. 2007). HABs reported in KI ponds have included several algal taxa, particularly raphidophytes (Lewitus et al. 2003, 2004, 2008), dinoflagellates (Lewitus et al. 2008), and cyanobacteria (Lewitus & Holland 2003, Brock 2006, Siegel et al. 2011, Greenfield et al. 2014).

Wimbee Creek (WC; 32°36′43″ N, 80°41′11″ W) is a forested tidal creek located on the Combahee River in the Ashepoo, Combahee, and Edisto Rivers (ACE) Basin. The ACE Basin drains an area of nearly 8000 km^2 (Noble et al. 2003). The area surrounding WC is forested, and the headwaters are characterized by a network of waterfowl impoundments.

Nutrient addition bioassays

Detailed methods for conducting nutrient addition bioassays considered here are provided in Reed (2014). Briefly, at each site described above, bioassays were deployed *in situ* seasonally (2011–2013): in summer (July to August), fall (October to November), winter (January to February), and spring (April to May). This study focused on Year 2 (July 2012 to May 2013) of the broader project. Bioassays were performed in 1 l acid-cleaned (10% hydrochloric acid, HCl, for 24 h) Nalgene® polycarbonate bottles, and treatments (in triplicate) were as follows: (1) no addition (control, C); (2) orthophosphate (PO_4^{3-}, P); (3) ammonium (NH_4^+, A); (4) NH_4^+ + PO_4^{3-} (AP); (5) nitrate (NO_3^-, N); (6) NO_3^- + PO_4^{3-} (NP); (7) urea (U); (8) urea + PO_4^{3-} (UP); and (9) all (NH_4^+ + NO_3^- + urea + PO_4^{3-}, ALL). N and P were added at Redfield ratios (16:1 as 20 µg per atom N and 1.25 µg per atom P; Redfield 1958). Bottles were randomized in mesh bags that were attached by a rope to a cement weight that anchored the experimental set-up. Bottles were incubated *in situ* for 48 h at subsurface depths (~0.2–0.3 m; Secchi depth at sites used here was typically 0.3–0.5 m) to avoid photoinhibition. This incubation depth corresponded to an irradiance of approximately 32% I_0 (irradiance at 0 m).

Water quality measurements

A YSI 6600 data sonde was attached to the cement weight to record environmental parameters (temperature, salinity, dissolved oxygen, and turbidity) at 15 min intervals throughout the 48 h incubation. At the beginning of each deployment (t_0), 3 water samples (1 l each) were collected to assess initial concentrations of DOC, chl *a*, and bacterial abundances. After 48 h, incubation bottles were retrieved and immediately transported to the laboratory in the dark

in coolers for analyses (≤2 h to transport samples). Cumulative precipitation (mm) data 5 d prior to deployments were obtained from the National Climatic Data Center for the Georgetown Airport (TA), the Charleston International Airport (BC), the Marine Corps Air Station, Beaufort (WC), and the Kiawah Island Community Association (K075).

DOC analyses

A Shimadzu TOC-V_{CSN} analyzer with autosampler ASI-V was used to determine DOC concentrations. All glassware used for DOC analyses was acid-washed (10% HCl), rinsed with distilled (DI) water, dried, and combusted at 475°C for 4.5 h in a Barnstead Thermolyne 30400 furnace to remove residual organic C. Subsamples (~17 ml) of each t_0 and bioassay replicate were filtered through pre-combusted (450°C for 4 h) 0.7 µm pore size Whatman™ glass fiber (GF/F) filters into 20 ml acid-washed (10% HCl) scintillation vials; samples were then acidified with 1 to 2 drops of 10% HCl and stored (4°C) until analysis. Prior to each analysis batch (30–60 samples), a 6 point calibration curve was generated according to standard protocols (Shimadzu TOC-$V_{CSH/CSN}$ User Manual) as follows. A 1000 mg l^{-1} total organic carbon (TOC) stock solution was prepared by combining 2.125 g potassium acid phthalate with 2 ml 36.5–38.0% HCl and then diluting this solution to 1 l with DI water in a volumetric flask. TOC standards were then serially diluted to 100, 50, 25, 10, 5, and 2.5 mg l^{-1}. Calibration curves were accepted if the R^2 value was 0.99 or higher. Analysis batches consisted of blanks (DI water) and C standards (low C standard of 41–44 µM DOC, high C standard of 25 mg l^{-1} (2081 µM) from the stock solution, and an inorganic C standard prepared by combining 2.202 g Na_2CO_3 and 1.7485 g $NaHCO_3$ in a volumetric flask followed by dilution to 500 ml with DI water) to monitor instrument drift and accuracy.

Phytoplankton biomass

Whole water samples (up to 40 ml) from each replicate were filtered through 0.7 µm pore size Whatman™ GF/F filters for total chl *a* as a proxy for phytoplankton biomass. Filters containing samples were placed into acid-washed (10% HCl) 25 ml scintillation vials, and 1 ml of magnesium carbonate ($MgCO_3$) was added as a buffer to prevent acid degradation of chl *a*. Samples were frozen (–20°C) until analysis, at which time 9 ml of high-performance liquid chromatography (HPLC) grade acetone (90%) were added to each replicate, and chl *a* was extracted (–20°C for 36 h). Following extraction, chl *a* concentrations (µg l^{-1}) were quantified according to Welschmeyer (1994) using a Turner TD 700 fluorometer.

Microbial quantification

Subsamples (~5 ml) from triplicate bioassay and t_0 bottles were syringe-filtered through 5 µm Nitex® cloth, and 1.5 ml of the filtrate was collected in 2 ml cryovials. Formaldehyde (0.2 µm-filtered, 10% solution) was added to each cryovial to yield a 1.0% final concentration. Samples were placed in a 4°C refrigerator to fix (~30 min); fixed samples were then flash frozen in liquid nitrogen and stored at –80°C. Prior to analysis, fixed samples were stained with SYBR® Green I (SYBR-I) Nucleic Acid Gel Stain (Molecular Probes®) to a final concentration of 10^{-4} of the commercial stock solution. Samples were then incubated in the dark for 30 min at room temperature. Just before analysis, 14 µl of a bead stock (2.0 µm diameter fluorescent beads) suspension were added to the samples (Marie et al. 1997, 1999). Flow cytometry was conducted with a MoFlo *Astrios* High Speed Cell Sorter (Beckman Coulter®), and green fluorescence was collected in the FL1 channel (600 nm).

Statistical analyses

Statistical analyses were performed using R (v. 2.14.2) statistical software. Data were initially tested for normality (Shapiro-Wilk), and those that were not normally distributed were log-transformed before further analyses. All t_0 data (DOC, bacterial abundances, and chl *a*) were normally distributed. Following incubation, most data remained normally distributed with the exceptions of spring DOC concentrations at WC; bacterial abundances at TA during fall, BC and K075 during winter and spring, and all seasons at WC; and chl *a* concentrations at TA during summer, K075 during winter, and WC during fall and spring. Differences in t_0 DOC, bacterial abundances, and chl *a* across sites and seasons were determined using 2-way ANOVA. Pearson's product-moment correlation analyses were performed on mean t_0 DOC, chl *a*, bacterial abundances, and water quality parameters (temperature, salinity, precipitation) at each site as well as across all bioassays (pooled). Separate 1-way ANOVAs were conducted on mean

treatment DOC, bacterial abundances, and chl *a* followed by post hoc Tukey's HSD tests to determine significant differences in treatment levels compared to the control during each bioassay.

RESULTS

Ambient water quality

Seasonal differences in mean ± SD water temperature were observed across all sites: summer (29.6 ± 1.3°C), fall (16.0 ± 3.3°C), winter (13.1 ± 1.7°C), and spring (21.7 ± 4.0°C), with K075 being the warmest site and WC typically the coolest (Table 1). Sites were polyhaline with comparable salinities, except for winter at K075, when salinity was elevated (24.6 ± 2.3 psu) relative to the other 3 sites. The lowest salinities usually followed periods of highest 5 d cumulative rainfall. Warm temperatures were generally associated with low dissolved oxygen, as mean dissolved oxygen was lowest during the summer at TA, BC, and WC and during spring at K075. Mean turbidity was lowest overall at K075 (4.2 ± 2.5 NTU) and highest at TA (165.4 ± 288.2 NTU), coincident with several extremely high values recorded during the summer 2012 TA bioassay.

Initial (t_0) DOC concentrations did not differ significantly across sites or seasons ($p > 0.05$). However, the least developed site (WC) had the highest levels (mean ± SD) of DOC (1648.2 ± 34.4 µM) compared to the lowest value at BC (549.6 ± 121.5 µM), both recorded during winter (Fig. 2A). Bacterial abundances (cells ml^{-1}) differed significantly across sites ($p < 0.05$), but not seasons ($p > 0.05$), such that they were highest at TA followed by BC, WC, and K075 (Fig. 2B) and did not coincide with trends in DOC concentrations (Fig. 2). Chl *a* concentrations differed significantly across sites ($p < 0.05$) and seasons ($p < 0.05$) and were highest at K075 and TA, lowest at WC, and generally greatest during the summer across sites (Fig. 2C).

Correlations between DOC, bacteria, phytoplankton biomass, and water quality

The t_0 DOC, bacterial abundance, and chl *a* values were at times highly, but not significantly, correlated (Table 2; $p > 0.05$). Specifically, DOC and bacterial abundances had a strong negative correlation at TA ($r = -0.87$), but they were positively correlated at all other sites, including a strong correlation ($r = 0.80$) at WC. DOC and chl *a* were negatively correlated at the less developed sites (TA and WC) but positively correlated at the more developed sites (BC and K075). Bacterial abundances and chl *a* were weakly and positively correlated at TA and BC, but negatively correlated at K075 and WC. DOC was negatively correlated with salinity at all sites ($r = -0.58$). For sites

Table 1. Mean (±SD) and ranges of ambient temperature (T), salinity (S), dissolved oxygen (DO), and turbidity (TB) throughout each 48 h incubation (n range: 191–198), as well as precipitation (P) values. nd: no data. Study sites are shown in Fig. 1

Site	Season	T (°C)		S (psu)		DO (mg l^{-1})		TB (NTU)		P (mm)
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Total
TA	Summer	28.9 (1.4)	26.4–32.2	14.0 (1.9)	10.4–18.3	4.9 (1.6)	3.0–8.7	165.4 (288.2)	18.8–1291.2	19.8
	Fall	14.8 (1.3)	12.2–17.3	22.2 (3.2)	15.6–28.1	7.3 (0.5)	6.0–8.0	35.1 (21.4)	9.9–119.9	0.0
	Winter	10.9 (1.9)	7.2–14.1	5.9 (1.2)	3.5–8.3	8.8 (1.0)	6.4–11.8	31.6 (32.3)	8.0–147.8	5.1
	Spring	18.9 (1.7)	16.6–24.2	2.2 (0.9)	0.6–4.6	7.4 (1.1)	4.9–9.7	60.6 (49.3)	12.3–235.4	36.8
BC	Summer	30.1 (0.7)	28.5–31.8	15.5 (3.0)	0.2–20.8	3.9 (1.3)	2.1–8.4	17.5 (8.5)	3.1–55.4	1.3
	Fall	21.3 (2.0)	14.7–25.4	16.3 (7.3)	0.1–23.3	5.7 (1.6)	4.2–10.4	22.1 (23.3)	0.6–294.4	0.0
	Winter	14.1 (0.7)	13.1–16.6	9.6 (6.9)	0.0–21.8	8.1 (0.8)	6.8–11.0	nd	nd	45.2
	Spring	18.8 (1.0)	16.2–21.4	7.5 (3.4)	0.0–14.5	6.2 (0.9)	4.7–10.4	19.9 (8.5)	0.0–71.1	21.8
K075	Summer	31.5 (1.0)	29.9–33.5	14.5 (0.5)	13.9–17.9	7.7 (1.4)	5.3–10.5	4.2 (2.5)	3.0–33.6	46.7
	Fall	15.4 (0.8)	14.1–16.8	25.2 (0.3)	24.2–26.0	7.3 (0.5)	5.8–8.4	0.4 (0.3)	0.0–1.9	9.7
	Winter	15.3 (1.5)	12.3–17.7	24.6 (2.3)	21.0–28.5	7.7 (0.9)	6.1–9.5	0.0 (2.5)	0.0–33.0	0.0
	Spring	27.4 (0.6)	26.6–29.4	16.0 (3.4)	11.4–24.8	4.0 (0.9)	2.4–6.1	6.6 (2.3)	4.1–17.3	0.0
WC	Summer	28.0 (0.3)	27.5–28.5	22.2 (2.9)	17.8–27.1	3.2 (0.3)	2.8–4.0	32.0 (43.0)	6.7–339.1	9.7
	Fall	12.3 (0.2)	11.9–12.8	26.7 (1.7)	24.0–30.0	7.5 (0.2)	7.2–7.8	10.1 (3.4)	3.0–30.9	0.0
	Winter	12.1 (0.7)	11.1–13.4	3.4 (3.0)	0.5–11.0	9.0 (0.2)	8.6–9.3	30.4 (11.2)	7.2–56.2	65.3
	Spring	nd	nd	nd	nd	nd	nd	nd	nd	34.3

Table 2. Pearson's product-moment correlation coefficients between mean initial measurements from each site (n = 4) and across all bioassays (N = 16). Values include bacterial abundances (BA), salinity (S), temperature (T), and precipitation (P). Numbers in **bold** represent significant correlations ($p < 0.05$). Study sites are shown in Fig. 1. DOC: dissolved organic carbon

Site	Response measure	Chl *a* (µg l^{-1})	BA ($\times 10^5$ cells ml^{-1})	S (psu)	T (°C)	P (mm)
TA	DOC	−0.15	−0.87	−0.95	−0.71	0.39
	Chl *a*		0.23	−0.14	0.67	0.45
	BA			0.73	0.88	0.08
BC	DOC	0.65	0.53	−0.81	0.66	−0.44
	Chl *a*		0.09	0.09	0.79	−0.41
	BA			−0.74	−0.26	0.50
K075	DOC	0.20	0.42	−0.91	0.94	0.65
	Chl *a*		−0.56	0.12	0.14	0.24
	BA			−0.76	0.56	−0.28
WC	DOC	−0.27	0.80	−0.86	−0.15	**0.97**
	Chl *a*		−0.69	0.63	0.74	−0.51
	BA			**−0.99**	−0.24	0.90
Pooled	DOC	−0.07	−0.22	−0.58	0.08	**0.51**
	Chl *a*		0.05	0.04	0.43	−0.21
	BA			−0.40	0.16	0.10

pooled, DOC and precipitation were significantly and positively correlated ($r = 0.51$, $p < 0.05$), with a pronounced and significant relationship at WC ($r = 0.97$, $p < 0.05$). Bacterial abundances tended to be negatively correlated with salinity (except at TA), but this correlation was only statistically significant at WC (Table 2; $p < 0.05$). Bacterial abundances were weakly and negatively correlated with temperature at BC and WC, but positively correlated at TA and K075. Chl *a* was positively, but not significantly, correlated with temperature across sites, particularly at BC ($r = 0.79$) and WC ($r = 0.74$).

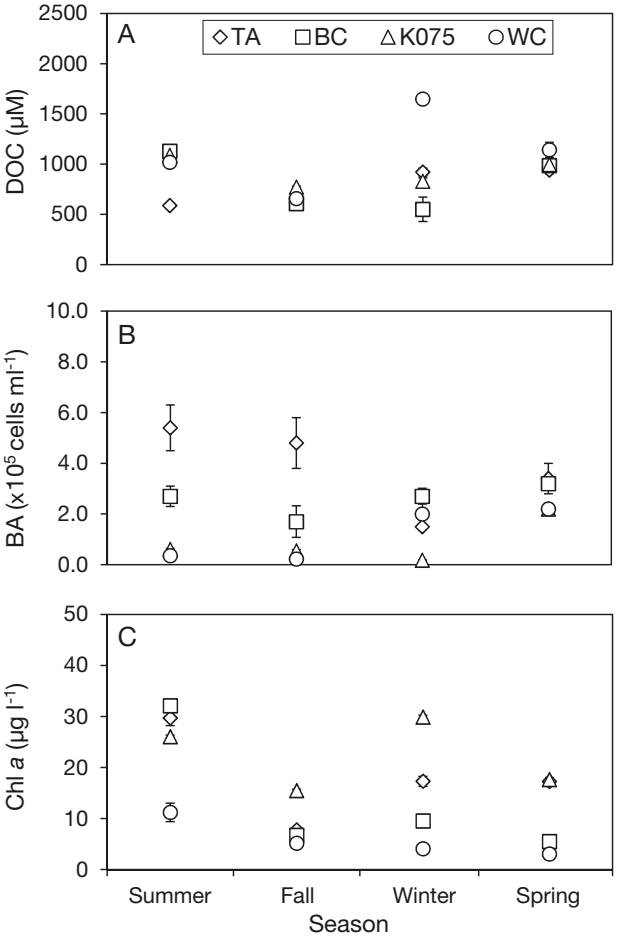


Fig. 2. Mean (n = 3) (±SD) initial (A) dissolved organic carbon (DOC) concentrations, (B) bacterial abundances (BA), and (C) chl *a* during each bioassay at each site. Study sites are shown in Fig. 1. Symbols obscure each other in some instances

Bioassays

DOC. Final control concentrations were lower than t_0 concentrations at all sites except K075 during the summer and WC during fall and spring (Figs. 3–6), suggesting utilization of DOC (likely by bacteria) during experimentation. DOC concentrations in treatments containing urea (U, UP, and ALL) were significantly greater than the controls during all bioassays except the summer U and UP treatments at K075 and the fall U treatment at WC (Table 3; $p < 0.05$). DOC concentrations were also significantly greater in several inorganic nutrient additions compared to the controls (e.g. A treatment at BC during spring; P, A, AP, N, and NP treatments and A, AP, N, and NP treatments at K075 during summer and winter, respectively; AP and NP treatments at WC during winter and spring, respectively; Table 3; $p < 0.05$).

Bacterial abundances. Final control bacterial abundances were lower than t_0 abundances at all sites except TA and WC during the fall and K075 during the winter (Figs. 3–6). However, final bacterial abundances in treatments containing urea were significantly higher than controls at BC and K075 during the summer, the UP treatment at BC during the winter, and the ALL treatment at K075 during the winter (Table 3; $p < 0.05$). Bacterial abundances were also significantly higher in certain inorganic nutrient additions compared to the controls (e.g. NP and N treatments at BC during fall and winter, respectively; Table 3; $p < 0.05$).

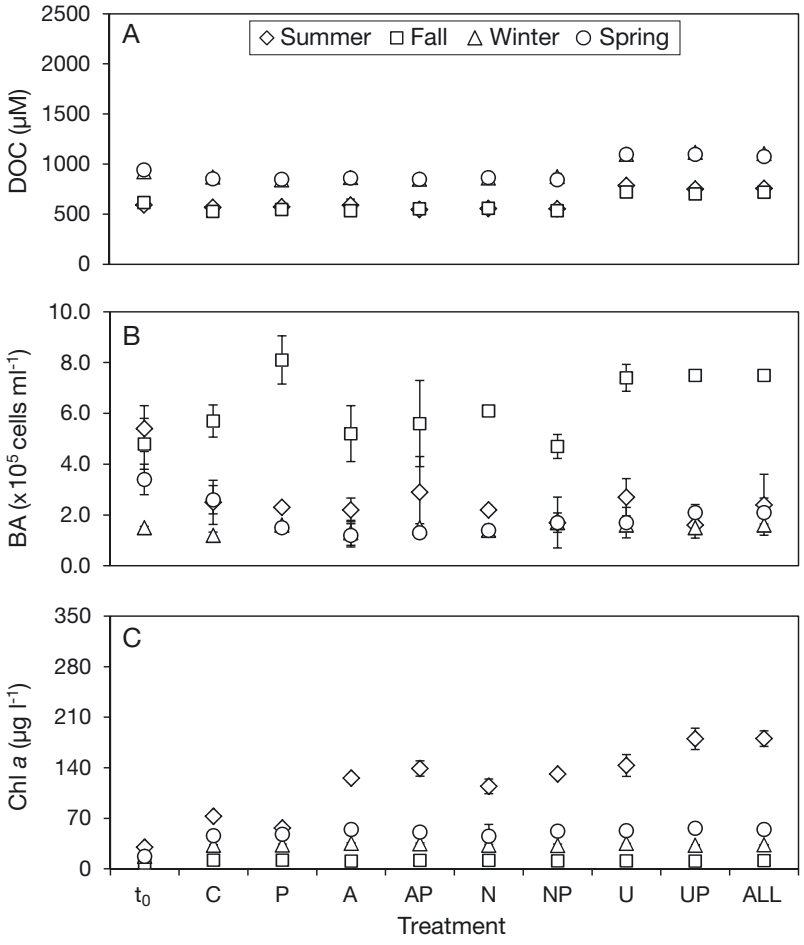


Fig. 3. Mean (n = 3) (± SD) (A) dissolved organic carbon (DOC) concentrations, (B) bacterial abundances (BA), and (C) chl *a* at Thousand Acre during each bioassay. Treatments are as defined in Table 3. Symbols obscure each other in some instances

Phytoplankton biomass. Final control chl *a* concentrations were higher than t_0 concentrations at TA, BC, and WC throughout the study (Figs. 3, 4 & 6). Chl *a* concentrations generally followed trends in DOC at BC and K075 (Figs. 4 & 5), with treatments containing urea having significantly higher chl *a* and DOC concentrations than other addition treatments as well as the controls (Table 3; $p < 0.05$), and this difference was often highly significant ($p < 0.001$). In particular, mean chl *a* at BC was highest during the summer, reaching 277.5 ± 45.3 µg l^{-1} in the ALL treatment compared to 34.3 ± 3.7 µg l^{-1} in the control, and mean chl *a* levels were significantly greater than the control in each N addition (Table 3; $p < 0.05$). At TA during the summer, chl *a* in each N addition treatment was significantly higher than the control (Table 3; $p < 0.001$), but concentrations were not significantly different during other seasons ($p > 0.05$). By comparison, at WC, chl *a* in the A treatment was significantly greater than the control during the spring (Table 3; $p < 0.05$), but not significantly different in any other treatment or season ($p > 0.05$). Detailed descriptions of the taxonomic composition of associated phytoplankton communities fall beyond the scope of the present study, but are provided in a companion study (Reed 2014).

DISCUSSION

We examined seasonal trends of DOC, bacterial abundances, and chl *a* in 4 coastal SC systems with differing land uses. Concentrations of DOC, bacteria, and chl *a* were not significantly correlated with each other. However, initial (t_0) DOC was highest overall at the least developed site (WC), lowest at TA, and was significantly and positively correlated with precipitation as well as negatively correlated with salinity. Although trends in DOC levels did not always coincide with bacterial abundances, they often followed chl *a* levels, which were further influenced by temperature, particularly in treatments containing urea. These findings indicate that temperature, salinity, and precipitation influenced microplankton abundances within SC coastal systems, and that elevated N inputs (primarily organic N) may increase phytoplankton biomass that may, in turn, contribute a greater proportion to total DOC.

DOC concentrations reported herein were within the range of those previously reported for other southeastern US estuaries. As examples, DOC concentrations at WC were typically >1000 µM, similar to those found in the Ogeechee River estuary (located in northern Georgia), which typically has DOC concentrations of 12 mg l^{-1} (~1000 µM; Moran et al. 1999). The Ogeechee River receives inputs from the Piedmont and coastal plain regions (Moran et al. 1999), and the ACE Basin receives inputs solely from the coastal plain region (Marion 2008). DOC concentrations at TA measured in this study were similar to previously reported DOC levels at both TA (Buzzelli et al. 2004) and other sites within the lower portion of Winyah Bay (Goñi et al. 2003), but they were lower than concentrations measured in an isolated cypress-

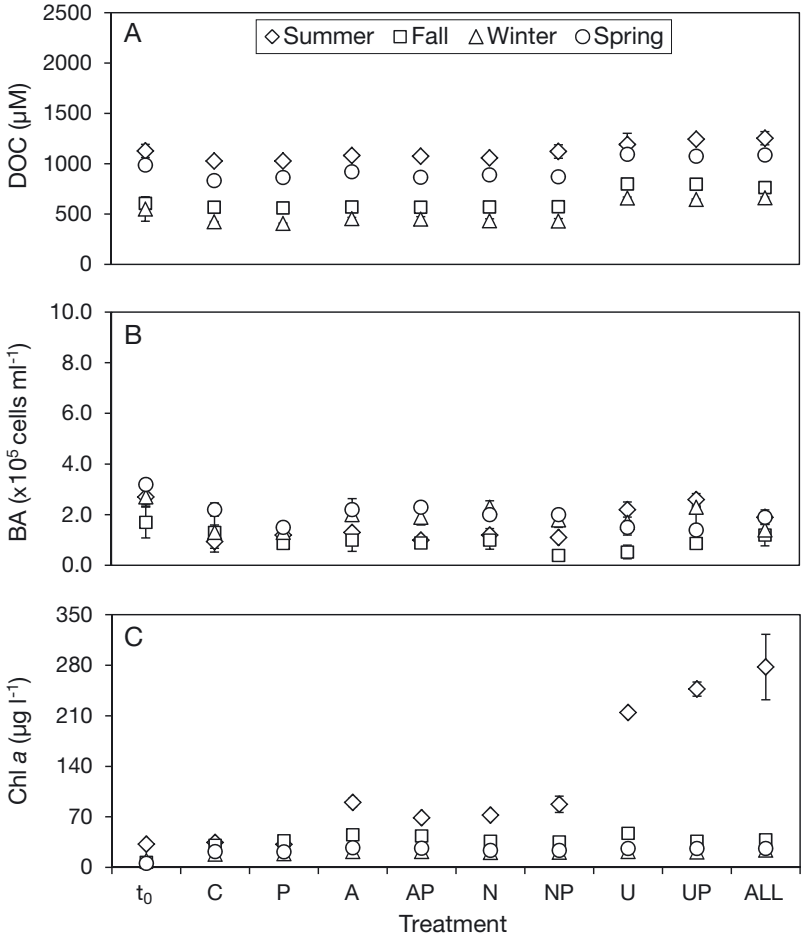


Fig. 4. Mean (n = 3) (±SD) (A) dissolved organic carbon (DOC) concentrations, (B) bacterial abundances (BA), and (C) chl *a* at Bull Creek during each bioassay

tupelo wetland (Crabhaul) located in northern Winyah Bay. The latter is not surprising because Crabhaul receives significant DOC inputs from surrounding leaf litter, with concentrations ranging from 42 to 55 mg l^{-1} (~3500 to 4580 µM) during peak litterfall months (Chow et al. 2013). The DOC concentrations observed during the summer and spring at K075 are consistent with DOC concentrations measured in 2 similar KI stormwater detention ponds (25 April and 5 July 2001), with mean concentrations in each pond of 1094 and 1484 µM, respectively (Lewitus et al. 2003). While there is no available literature on DOC levels at BC, concentrations were within the range of the levels measured at our other sites, albeit with different seasonal maxima than TA and WC.

Our findings suggest that DOC concentrations may have been influenced by a number of factors, including surrounding land cover. The highest DOC concentrations were observed at the least developed site (WC), consistent with prior studies reporting inverse relationships between DOC concentrations and extent of surrounding land development (Wahl et al. 1997, Mallin et al. 2009). WC is primarily surrounded by marsh grass and forested wetlands (Reed 2014), and this vegetation was likely a major source of organic matter to the coast. The influence of freshwater inflow for transporting terrigenous matter, including DOC, has been reported elsewhere within the ACE Basin (Johnson et al. 2006). Contrary to our expectations, sites BC and K075, characterized by relatively greater development, had the second and third highest mean concentrations of DOC, respectively, with TA having the lowest DOC concentrations. The comparatively lower DOC concentrations at TA were primarily driven by summer and fall levels and coincided with the highest bacterial abundances, suggesting that DOC was likely a primary resource for bacterial growth and metabolism during these seasons. Precipitation was also a key factor influencing DOC levels, as the generally significant and positive correlations, combined with the negative correlations between DOC and salinity, suggest that rainfall, not surprisingly, reduced salinity levels and mobilized DOC delivery from the land to the receiving study sites. For example, WC received 65.3 and 34.3 mm of precipitation 5 d prior to the winter and spring 2013 deployments, respectively, contributing to the significant positive correlation between DOC and precipitation. These findings support prior studies in which precipitation was shown to affect estuarine organic matter levels and cycling (Mallin et al. 2009, Chow et al. 2013). The exception was BC, where DOC and precipitation were weakly and negatively correlated, despite negative correlations between DOC and salinity. Possible explanations could be that either other freshwater sources (such as urban runoff) contributed to DOC inputs or that development reduced inputs of terrestrially derived (e.g. leaf litter, marsh grass, etc.) DOC to this system.

Since bacterial abundances were also generally negatively correlated with salinity and positively correlated with precipitation, mobilization of terrestrially derived DOC into the study systems by precipita-

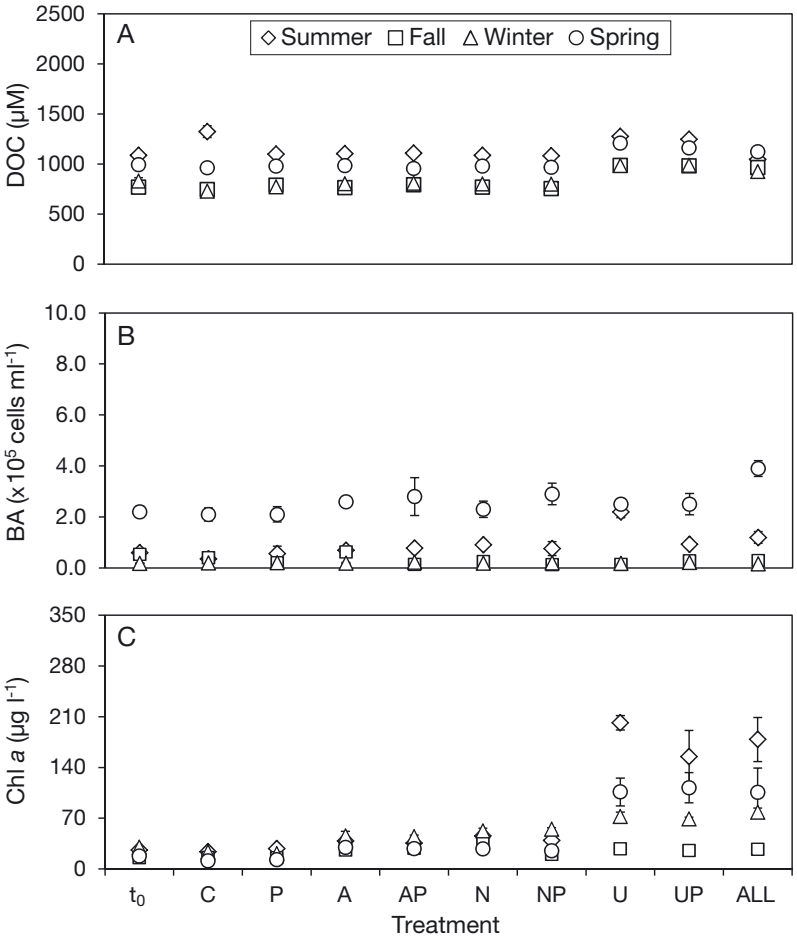


Fig. 5. Mean (n = 3) (±SD) (A) dissolved organic carbon (DOC) concentrations, (B) bacterial abundances (BA), and (C) chl *a* at Kiawah Island Pond number 075 during each bioassay

tion and enhanced freshwater flow likely fueled bacterial production and numbers, although we did not measure flow rates. This idea is supported by previous studies showing that salinity gradients influence both estuarine bacterioplankton community composition (Bouvier & del Giorgio 2002, del Giorgio & Bouvier 2002) and regulate trophic status, such as within the Chesapeake Bay (Kemp et al. 1997). Similarly, the generally positive correlations between t_0 bacterial abundances and DOC at all sites but TA underscored the importance of DOC supply for microbial growth. However, trends in bacterial abundances measured during bioassays did not always follow DOC concentrations, possibly due to the relatively short experimental duration or the bioavailability of DOC to bacterial communities. DOM composition and source (including DOC) have been shown to affect bacterial community composition (Wear et al. 2014) and regulate nutrient cycling (Foreman & Covert 2003, Carlson et al. 2007, Nagata 2008) in other blackwater estuaries. The availability of organic substrates for bacterial consumption is influenced by a wide variety of factors including, but not limited to, chemical composition (Sun et al. 1997), the ratio of C:N (Hunt et al. 2000), and irradiance (Moran & Covert 2003, Smith & Benner 2005).

Although bacterial metabolic rates were not evaluated in this study, positive correlations between bacterial abundances and temperature at TA and K075 support prior research showing that heterotrophic bacterial physiological processes are often facilitated at higher temperatures (Hoch & Kirchman 1993, Shiah & Ducklow 1997, Pomeroy et al. 2000, reviewed by Apple et al. 2008). Since temperature and bacterial abundances were negatively correlated at BC and WC, it is possible that microzooplankton grazing within treatment bottles prevented the bacterial community from attaining higher abundances. As the goal of this study was to evaluate net microbial numbers and responses, bioassay water was not filtered so grazing may have been a factor. Alternatively, resource supply has been shown to influence bacterial metabolism more than temperature at higher overall temperatures (Felip et al. 1996, Apple et al. 2006, 2008). Therefore, additional resources (other forms of C, N, and P) may have also controlled bacterial processes during warmer months at these sites.

Elevated chl *a* concentrations in N addition treatments often coincided with higher DOC levels, particularly during the summer, in treatments containing urea, and at BC, K075, and TA. Previous studies have described the importance of N for phytoplankton assemblages and production in southeastern coastal systems (Mallin et al. 2004, Piehler et al. 2004), including the stimulatory effects of urea in both estuaries (Glibert et al. 2004, Reed 2014) and stormwater detention ponds (Siegel et al. 2011, Reed 2014). DOC concentrations in treatments containing urea were almost always higher than control and t_0 concentrations throughout the study, coincident with chl *a* levels often being significantly greater in treatments containing urea than control and t_0 concentrations. Thus, N additions (especially urea) not only

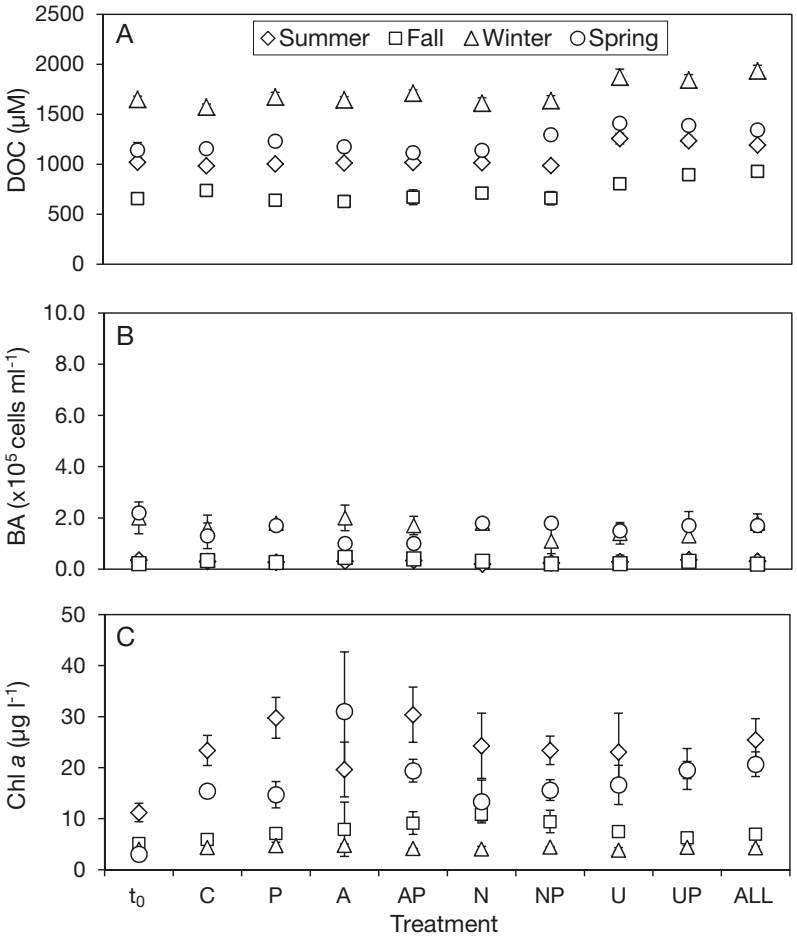


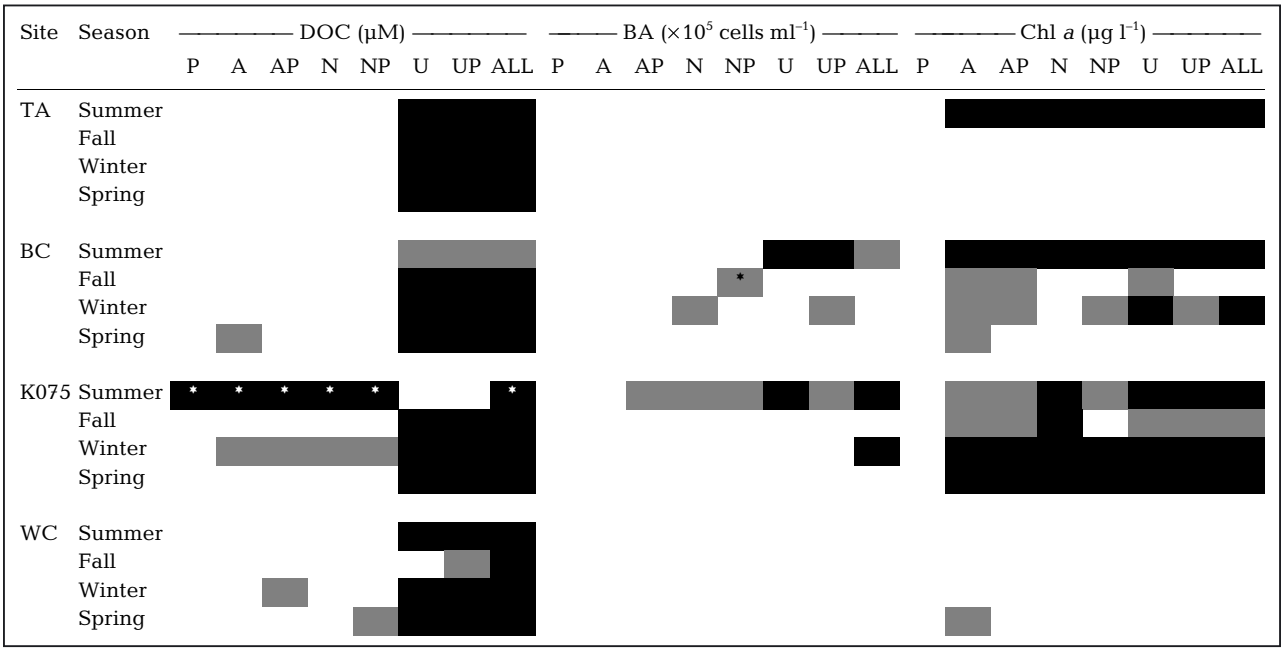
Fig. 6. Mean (n = 3) (±SD) (A) dissolved organic carbon (DOC) concentrations, (B) bacterial abundances (BA), and (C) chl *a* at Wimbee Creek during each bioassay

resulted in increased phytoplankton biomass, but presumably a greater contribution by phytoplankton to the total DOC pool. One implication of these findings is that with continued land development and N loadings to coastal systems, phytoplankton biomass and bloom incidences are likely to increase and contribute a greater proportion to the total DOC pool. This could affect the biogeochemical cycling of C within these systems. Even though bacterial abundances and chl *a* were not significantly correlated, increased phytoplankton production may provide additional DOC for bacterial uptake, leading to greater coupling between heterotrophic bacteria and phytoplankton assemblages. This process has been observed across a wide range of estuarine systems (e.g. Fuhrman et al. 1980, Cole et al. 1988, Rooney-Varga et al. 2005, Lonsdale et al. 2006, Apple et al. 2008).

Future studies are needed to further elucidate interactions between DOC, bacterial abundances, and chl *a*. For example, since bioavailability of DOC is influenced by numerous factors, it is possible that some DOC was simply not bioavailable to bacteria. Thus, studies are needed to determine DOC source (such as the relative contribution of phytoplankton vs. allochthonous terrestrial matter to the total DOC pool) and composition within our study sites. Furthermore, future studies should consider sampling along a salinity gradient to examine DOC transport and fate. Additionally, since we reported overall bacterial abundances, we could not distinguish heterotrophic vs. autotrophic production. Measurements of specific C uptake rates during long-term incubations may elucidate how DOC is incorporated within microbial growth and metabolic processes. Similarly, accessory pigments are known to affect algal chl *a* concentrations (e.g. Marra et al. 2007 and others) as well as the C:chlorophyll ratio (Cloern et al. 1995). Diatoms have been shown to be the primary taxon contributing to total chl *a* across sites considered here, although biomass increases in a wide range of phytoplankton taxa also responded to N additions (Reed 2014). Since DOM release rates differ among algal growth stages and species (e.g. Wetz & Wheeler 2007), future research could explore species-specific DOC production in sites considered here. Finally, studies should evaluate the potential role of grazing on bacteria and phytoplankton concentrations because the lack of significant correlations between bacterial abundances and chl *a* may have been related to different selective grazing rates on bacteria vs. phytoplankton.

In conclusion, this study showed that temperature, salinity, and precipitation were the primary environmental factors influencing DOC concentrations, bacterial abundances, and chl *a* levels across the 4 study sites. DOC concentrations were likely further influenced by surrounding land use, with the least developed site generally exhibiting the highest DOC concentrations. Higher DOC concentrations in treatments containing urea often corresponded with elevated chl *a*, suggesting that phytoplankton biomass was stimulated by organic N additions and likely

Table 3. Summary of univariate ANOVAs followed by Tukey HSD tests showing significantly greater dissolved organic carbon (DOC), bacterial abundances (BA), and chl *a* concentrations within bioassay treatments relative to control concentrations. Shaded boxes depict significant values of $p < 0.001$ (black), $p < 0.05$ (gray), and $p > 0.05$ (unshaded). *Indicates values that were significantly lower than controls; shaded values were significantly higher than controls elsewhere. Treatments are represented as P (PO_4^{3-}), A (NH_4^+), AP ($NH_4^+ + PO_4^{3-}$), N (NO_3^-), NP ($NO_3^- + PO_4^{3-}$), U (urea), UP (urea + PO_4^{3-}), and ALL ($NO_3^- + NH_4^+ +$ urea + PO_4^{3-}). Study sites are shown in Fig. 1



contributed a greater proportion to total DOC. Finally, this study has broader management implications, as continued alteration of the natural SC coastal landscape will likely affect the inputs and sources of DOM (including DOC) to coastal systems. Consequently, allochthonous sources of DOC from terrestrial matter will probably decrease in tandem with coastal development, but N inputs associated with development and runoff will increase. Since certain N-forms (especially organic N) can stimulate phytoplankton growth and blooms, this elevated phytoplankton production may increase DOC inputs from phytoplankton.

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LITERATURE CITED

Aitkenhead-Peterson JA, McDowell WH, Neff JC (2003) Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter, Academic Press, San Diego, CA, p 25–70

Allen J, Lu K (2003) Modeling and prediction of future urban growth in the Charleston region of South Carolina: a GIS-based integrated approach. Conserv Ecol 8:2

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM and others (2008) Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. Harmful Algae 8:39–53

Apple JK, del Giorgio PA, Kemp WM (2006) Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquat Microb Ecol 43:243–254

Apple JK, Smith EK, Boyd TJ (2008) Temperature, salinity, nutrients, and the covariation of bacterial production and chlorophyll-*a* in estuarine ecosystems. J Coast Res 55(Spec Issue):59–75

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Baines SB, Pace ML (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol Oceanogr 36:1078–1090

Bertilsson S, Jones JB (2003) Supply of dissolved organic matter to aquatic ecosystems: autochthonous sources. In:

Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego, CA, p 3–24

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47: 453–470

Brock LM (2006) Water quality, nutrient dynamics, phytoplankton ecology and land uses within defined watersheds surrounding six detention ponds on Kiawah Island, South Carolina. MS thesis, College of Charleston, Charleston, SC

Buzzelli C, Akman O, Buck T, Koepfler E, Morris J, Lewitus A (2004) Relationships among water-quality parameters from the North Inlet-Winyah Bay National Estuarine Research Reserve, South Carolina. J Coast Res 45(Spec Issue):59–74

Carlson CA, del Giorgio PA, Herndl GJ (2007) Microbes and the dissipation of energy and respiration: from cells to ecosystems. Oceanography 20:89–100

Cauwet G (2002) DOM in the coastal zone. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, San Diego, CA, p 579–609

Chow AT, Dai J, Conner WH, Hitchcock DR, Wang JJ (2013) Dissolved organic matter and nutrient dynamics of a coastal freshwater forested wetland in Winyah Bay, South Carolina. Biogeochemistry 112:571–587

Cloern JE, Grenz C, Vidergar-Lucas L (1995) An empirical model of the phytoplankton chlorophyll:carbon ratio: the conversion factor between productivity and growth rate. Limnol Oceanogr 40:1313–1321

Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar Ecol Prog Ser 43:1–10

Davis SE, Childers DL, Noe GB (2006) The contribution of leaching to the rapid release of nutrients and carbon in the early decay of wetland vegetation. Hydrobiologia 569:87–97

del Giorgio PA, Bouvier TC (2002) Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnol Oceanogr 47:471–486

DiDonato GT, Stewart JR, Sanger DM, Robinson BJ, Thompson BC, Holland AF, Van Dolah RF (2009) Effects of changing land use on the microbial water quality of tidal creeks. Mar Pollut Bull 58:97–106

Drescher S, Messersmith M, Davis B, Sanger D (2007) State of the knowledge report: stormwater ponds in the coastal zone. South Carolina Department of Health and Environmental Control, Office of Ocean and Coastal Resource Management, Columbia, SC

Felip M, Pace ML, Cole JJ (1996) Regulation of planktonic bacterial growth rates: the effects of temperature and resources. Microb Ecol 31:15–28

Flynn AM (2008) Organic matter and nutrient cycling in a coastal plain estuary: carbon, nitrogen, and phosphorus distributions, budgets, and fluxes. J Coast Res 55(Spec Issue):76–94

Foreman CM, Covert JS (2003) Linkages between dissolved organic matter composition and bacterial community structure. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego, CA, p 343–362

Fuhrman JA, Ammerman JW, Azam F (1980) Bacterioplankton in the coastal euphotic zone: distribution, activity, and possible relationships with phytoplankton. Mar Biol 60:201–207

Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar Ecol Prog Ser 280: 73–83

Goñi MA, Thomas KA (2000) Sources and transformations of organic matter in surface soils and sediments from a tidal estuary (North Inlet, South Carolina, USA). Estuaries 23: 548–564

Goñi MA, Teixeira MJ, Perkey DW (2003) Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). Estuar Coast Shelf Sci 57: 1023–1048

Goñi MA, Voulgaris G, Kim YH (2009) Composition and fluxes of particulate organic matter in a temperate estuary (Winyah Bay, South Carolina, USA) under contrasting physical forcings. Estuar Coast Shelf Sci 85:273–291

Greenfield DI, Duquette A, Goodson A, Keppler CJ and others (2014) The effects of three chemical algaecides on cell numbers and toxin content of the cyanobacteria *Microcystis aeruginosa* and *Anabaenopsis* sp. Environ Manag 54:1110–1120

Heisler J, Glibert PM, Burkholder JM, Anderson DM and others (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13

Hitchcock JN, Mitrovic SM, Kobayashi T, Westhorpe DP (2010) Responses of estuarine bacterioplankton, phytoplankton and zooplankton to dissolved organic carbon (DOC) and inorganic nutrient additions. Estuaries Coasts 33:78–91

Hoch MP, Kirchman DL (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Mar Ecol Prog Ser 98:283–295

Holland AF, Sanger DM, Gawle CP, Lerberg SB and others (2004) Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. J Exp Mar Biol Ecol 298:151–178

Hunt AP, Parry JD, Hamilton-Taylor J (2000) Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. Limnol Oceanogr 45:237–241

Hutchins PR, Smith EM, Koepfler ET, Viso RF, Peterson RN (2014) Metabolic responses of estuarine microbial communities to discharge of surface runoff and groundwater from contrasting landscapes. Estuaries Coasts 37: 736–750

Johnson W, Lewitus AJ, Fletcher M (2006) Linking bacterioplankton community structures to environmental state variables and phytoplankton assemblages in two South Carolina salt marsh estuaries. Aquat Microb Ecol 45: 129–145

Kemp WM, Smith EM, Marvin-DiPasquale M, Boynton WR (1997) Organic carbon balance and net ecosystem metabolism in Chesapeake Bay. Mar Ecol Prog Ser 150: 229–248

Leff LG, Meyer JL (1991) Biological availability of dissolved organic carbon along the Ogeechee River. Limnol Oceanogr 36:315–323

Lewitus AJ, Holland AF (2003) Initial results from a multi-institutional collaboration to monitor harmful algal blooms in South Carolina. Environ Monit Assess 81: 361–371

Lewitus AJ, Schmidt LB, Mason LJ, Kempton JW and others (2003) Harmful algal blooms in South Carolina residential and golf course ponds. Popul Environ 24:387–413

Lewitus AJ, Hayes KC, Kempton JW, Mason LJ, Wilde SB, Williams BJ, Wolney JL (2004) Prevalence of raphidophyte blooms in South Carolina brackish ponds associated with housing and golf courses. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA (eds) Harmful algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and the Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL, p 350–352

Lewitus AJ, Brock LM, Burke MK, DeMattio KA, Wilde SB (2008) Lagoonal stormwater detention ponds as promoters of harmful algal blooms and eutrophication along the South Carolina coast. Harmful Algae 8:60–65

Lonsdale DJ, Greenfield DI, Hillebrand EM, Nuzzi R, Taylor GT (2006) Contrasting microplanktonic composition and food web structure in two coastal embayments (Long Island, NY, USA). J Plankton Res 28:891–905

Malinsky-Rushansky NZ, Legrand C (1996) Excretion of dissolved organic carbon by phytoplankton of different sizes and subsequent bacterial uptake. Mar Ecol Prog Ser 132:249–255

Mallin MA, Parsons DC, Johnson VL, McIver MR, CoVan HA (2004) Nutrient limitation and algal blooms in urbanizing tidal creeks. J Exp Mar Biol Ecol 298:211–231

Mallin MA, Johnson VL, Ensign SH (2009) Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. Environ Monit Assess 159:475–491

Marie D, Partensky F, Jacquet S, Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microbiol 63: 186–193

Marie D, Partensky F, Vaulot D, Brussaard C (1999) Enumeration of phytoplankton, bacteria, and viruses in marine samples. In: Robinson JP (ed) Current protocols in cytometry, Suppl 10. John Wiley & Sons, New York, NY, p 11.11.1–11.11.15

Marion C (2008) The effects of land use on sedimentation, inorganic substrate, organic substrate, and fish assemblages in South Carolina's coastal plain streams. MS thesis, Clemson University, Clemson, SC

Marra J, Trees CC, O'Reilly JE (2007) Phytoplankton pigment absorption: a strong predictor of primary productivity in the surface ocean. Deep-Sea Res I 54:155–163

Moran MA, Covert JS (2003) Photochemically mediated linkages between dissolved organic matter and bacterioplankton. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego, CA, p 243–262

Moran MA, Sheldon WM Jr, Sheldon JE (1999) Biodegradation of riverine organic carbon in five estuaries of the southeastern United States. Estuaries 22:55–64

Nagata T (2008) Organic matter–bacteria interactions in seawater. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley, Hoboken, NJ, p 207–241

Noble PA, Tymowski RG, Fletcher M, Morris JT, Lewitus AJ (2003) Contrasting patterns of phytoplankton community pigment composition in two salt marsh estuaries in southeastern United States. Appl Environ Microbiol 69: 4129–4143

Piehler MF, Twomey LJ, Hall NS, Paerl HW (2004) Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. Estuar Coast Shelf Sci 61:197–209

Pinckney JL, Paerl HW, Tester P, Richardson TL (2001) The role of nutrient loading and eutrophication in estuarine ecology. Environ Health Perspect 109:699–706

Pomeroy LR, Sheldon JE, Sheldon WM, Blanton JO, Amft J, Peters F (2000) Seasonal changes in microbial processes in estuarine and continental shelf waters of the southeastern U.S.A. Estuar Coast Shelf Sci 51:415–428

Raymond PA, Bauer JE (2000) Bacterial consumption of DOC during transport through a temperate estuary. Aquat Microb Ecol 22:1–12

Raymond PA, Bauer JE (2001) DOC cycling in a temperate estuary: a mass balance approach using natural ^{14}C and ^{13}C isotopes. Limnol Oceanogr 46:655–667

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221

Reed ML (2014) The influence of macronutrient form on the spatial and seasonal variability of phytoplankton assembles and bacterial abundances in four coastal South Carolina systems. MS thesis, College of Charleston, Charleston, SC

Rooney-Varga JN, Giewat MW, Savin MC, Sood S, LeGresley M, Martin JL (2005) Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb Ecol 49:163–175

Sanger D, Blair A, DiDonato G, Washburn T and others (2015) Impacts of coastal development on the ecology of tidal creek ecosystems of the US southeast including consequences to humans. Estuaries Coasts 38(Suppl 1):49–66

Shiah FK, Ducklow HW (1997) Bacterioplankton growth responses to temperature and chlorophyll variations in estuaries measured by thymidine:leucine incorporation ratio. Aquat Microb Ecol 13:151–159

Siegel A, Cotti-Rausch B, Greenfield DI, Pinckney JL (2011) Nutrient controls of planktonic cyanobacteria biomass in coastal stormwater detention ponds. Mar Ecol Prog Ser 434:15–27

Smith EM, Benner R (2005) Photochemical transformations of riverine dissolved organic matter: effects on estuarine bacterial metabolism and nutrient demand. Aquat Microb Ecol 40:37–50

South Carolina Department of Health and Environmental Control (2005) Watershed water quality assessment — Santee River basin. Tech Rep 003-003. South Carolina Department of Health and Environmental Control, Columbia, SC

South Carolina Department of Natural Resources (2003) Ashley Scenic River Management Plan, Report 25. South Carolina Department of Natural Resources, Columbia, SC

Steelink C (1985) Implications of elemental characteristics of humic substances. In: Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (eds) Humic substances in soil, sediment, and water. John Wiley & Sons, New York, NY, p 457–492

Sun L, Perdue EM, Meyer JL, Weis J (1997) Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol Oceanogr 42: 714–721

Teira E, Nieto-Cid M, Álvarez-Salgado XA (2009) Bacterial community composition and colored dissolved organic matter in a coastal upwelling ecosystem. Aquat Microb Ecol 55:131–142

Tufford DL, Samarghitan CL, McKellar HN, Porter DE, Hussey JR (2003) Impacts of urbanization on nutrient concentrations in small southeastern coastal streams. J Am Water Resour Assoc 39:301–312

Wahl MH, McKellar HN, Williams TN (1997) Patterns of nutrient loading in forested and urbanized coastal streams. J Exp Mar Biol Ecol 213:111–131

Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP (2005) The urban stream syndrome: current knowledge and the search for a cure. J N Am Benthol Soc 24:706–723

Wear EK, Koepfler ET, Smith EM (2014) Spatiotemporal variability in dissolved organic matter composition is more strongly related to bacterioplankton community composition than to metabolic capability in a blackwater estuarine system. Estuaries Coasts 37:119–133

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol Oceanogr 39:1985–1992

Wetz MS, Wheeler PA (2007) Release of dissolved organic matter by coastal diatoms. Limnol Oceanogr 52: 798–807

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