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DYNAMICS OF DISSOLVED ORGANIC CARBON, NITROGEN AND PHOSPHORUS IN A SEAGRASS MEADOW OF LAGUNA MADRE, TEXAS

Susan Ziegler, Edith Kaiser, and Ronald Benner

ABSTRACT

Seasonal and diel dynamics of dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) were investigated in a *Thalassia testudinum* Banks & Soland. ex Koenig dominated seagrass meadow. Heterotrophic utilization was an important determinant of the seasonal and diel changes in the C, N, and P content of DOM. Bacterial growth efficiency (BGE) was positively correlated with the C:N ratio of bulk DOM and negatively correlated with the C:N ratio of bioavailable DOM. Highest BGE and DOM bioavailability were associated with the highest concentrations of DON and DOP, suggesting N and P were major components of bioavailable DOM. The concentration of DOC accounted for only ~30% of the variation in DON and DOP concentrations, indicating a decoupling of DOC cycling from that of DON and DOP. Mean residence times (R_t) of DOC were always higher (12–16 d) than those of DON (3–6 d) and DOP (1.4–5.0 d), suggesting the following reactivity of these pools: DOP>DON>DOC. Differences in mean R_t were attributed to differences in sources and processes affecting each organic nutrient. Results from this study demonstrate the importance of DOM cycling to the nutrient dynamics of a productive seagrass ecosystem, and how changes in both microbial activity and DOM source impact the biogeochemical cycling of N and P.

Seagrass-dominated estuaries are among the most productive ecosystems, often comparable with tropical forests, coral reefs, swamps, and marshes (Odum et al., 1959; Odum, 1971; Duarte and Chiscano, 1999). Primary production and respiration are often in balance in seagrass-dominated ecosystems, suggesting that the import and export of organic matter are limited (Lindeboom and Sandee, 1989; Nienhuis et al., 1989; Ziegler and Benner, 1998). Productive yet balanced ecosystems such as these must have efficient nutrient cycling to support high rates of primary production. In fact, rates of nitrogen (N) mineralization have been determined to be quite high in seagrass-dominated sediments (Boon et al., 1986) and in the water column (Ziegler and Benner, 1999a). Mineralization and fixation of N in the benthos appear to be fueled by seagrass photosynthate released through the roots (Jorgensen et al., 1982; Moriarty et al., 1986; Blackburn et al., 1994; Stapel and Hemminga, 1997). Significant retention of N, however, has been found in a tropical seagrass meadow demonstrating that inorganic nutrients are efficiently recycled (Stapel et al., 2001; Gacia et al., 2002). Dissolved inorganic phosphorus (DIP) is rarely released from sediments in seagrass meadows, and in carbonate-rich sediments, phosphate is retained and dissolved organic phosphorus (DOP) is usually released to the water column (Jensen et al., 1998).

In both oligotrophic and eutrophic ecosystems, dissolved organic nitrogen (DON) and DOP have been found to represent an important reservoir of N and P participating in biogeochemical cycles (Seitzinger and Sanders, 1997; McGlathery et al., 2001). Most studies of N and P cycling in estuaries, however, have focused on the dynamics of inorganic nutrients. Few studies have investigated the cycling of organic nutrients in estuarine ecosystems (Dollar et al., 1991; Smith, 1991). Inorganic nutrients in the water column and sediments of seagrass-dominated ecosystems are largely supplied from rapidly cycling

organic matter (Boon et al., 1986; Blackburn et al., 1994; Ziegler and Benner, 1999a). Release, via exudation or leaching, and mineralization of seagrass photosynthate could be an important source of inorganic N and P in the overlying waters of these ecosystems. Dissolved organic carbon (DOC) release via seagrass exudation represents ~10% of benthic net primary production in southern Laguna Madre, an oligotrophic seagrass meadow (Ziegler and Benner, 1999b). The mineralization of this dissolved organic matter (DOM) was determined to be a more significant source of water column dissolved inorganic nitrogen (DIN) than release from the benthos (Ziegler and Benner, 1999a). DON is, in fact, a major form of N released from the benthos in some estuaries indicating the importance of organic nutrients to the nutrient budget of estuarine ecosystems (Moriarty et al., 1986; Middelboe et al., 1998).

The C, N, and P components of DOM may not cycle at the same rate nor be controlled by the same process. In some coastal ecosystems, sources and transformations of DOC and DON are decoupled and the bioavailability of these organic nutrient pools is variable (Stepanauskas et al., 2000). Benthic primary production and microbial activity in the water column of Laguna Madre are strongly coupled with water column respiration and bacterial production being fueled by DOC released from seagrasses (Ziegler and Benner, 1999b). It is likely that C-rich DOM is released directly from seagrasses with larger amounts of DON and DOP being supplied through leaching and other processes. Such variability in DOC, DON, and DOP sources could result in seasonal variation in available DON and DOP and consequently impact the nutrient cycling of seagrass ecosystems.

In the following investigation, seasonal and diel measurements of DON and DOP concentrations, as well as net water column and benthic flux measurements of DON and DOP, were conducted during three different seasons in the Laguna Madre. We hypothesize that cycling of DON and DOP are often uncoupled from DOC due to differences in source and the processes controlling C, N, and P cycling. Temporal measurements and fluxes were established to assess the role of DOM and its elemental composition in nutrient cycling of the southern Laguna Madre. This study was conducted as part of an 18-mo investigation of ecosystem metabolism, carbon, and nutrient cycling (see Ziegler and Benner, 1998; 1999a,b), enabling us to relate DON and DOP dynamics to DOC cycling as well as primary production, microbial activity, and nutrient dynamics.

Methods

Site Description.—Southern Laguna Madre is a seagrass-dominated shallow estuary located on the Texas coast and separated from the Gulf of Mexico by Padre Island. Our study was conducted in a *Thalassia testudinum* Banks & Soland. ex Koenig dominated seagrass meadow located in the southernmost portion of Laguna Madre where seagrasses cover 70% of the bottom (Quammen and Onuf, 1993). This meadow has been the subject of some extensive seagrass production studies in southern Laguna Madre (Herzka and Dunton, 1996; Lee and Dunton, 1996; Kaldy et al., 2002). The study site was located (26°10′N, 97°12′W) east of the Gulf Intracoastal Waterway (for map see Herzka and Dunton, 1996). Beds of *Syringodium filiforme* Kützing and *Halodule wrightii* Ascherson as well as drift algal species such as *Digenia simplex* (Wulfen) C. Agardh and *Laurencia poitei* (Lamouroux) Howe also occur in lower Laguna Madre (Humm and Hildebrand, 1962; Onuf, 1996). Sediments in lower Laguna Madre are primarily silty sand deposits from the Rio Grande with more recent inputs of quartz from the surrounding dunes.

Samples were collected during three 5 d trips to the study site in November 1996, March 1997, and June 1997. These sampling dates were chosen to represent periods of seagrass senescence,

initial and maximal growth, respectively (Lee and Dunton, 1996; Ziegler and Benner, 1998). The DOC data presented in this paper are taken from Ziegler and Benner (1999b). We present data for a direct comparison to the DON and DOP data, which are from the same samples and are presented here for the first time.

Water Column Sampling.—Comparisons of filtered (muffled GF/F) versus unfiltered samples collected at our site in November 1996 and June 1997 indicated that particulate organic carbon represented a small fraction (1–6 %) of total organic carbon. Therefore, samples for dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) analysis were not filtered. However, samples collected in March 1997 were filtered through muffled GF/F filters due to the occurrence of a phytoplankton bloom. Average DOC, DON, and DOP concentrations were determined from 12–24 samples collected over a 24 hr period using an autosampler (see Ziegler and Benner, 1998). Southern Laguna Madre has relatively weak tides, and the use of neutrally buoyant floats during each incubation experiment verified that sampling points retained the same water mass during each sampling period. Concentration changes detected over each 24 hr sampling period, therefore, represented primarily internal reactions rather than the result of mixing with other water masses. Samples were collected under vacuum into 45 ml glass bulbs (acid washed and muffled) at 60, 70, or 120 min intervals from ~0.6 m depth. Sample bulbs were kept in the dark and collected from the sampler around sunrise and sunset. The 35 ml samples for DON and DOP analysis were transferred from the bulbs into muffled 50 ml glass vials with teflon lined caps and immediately placed on ice. Samples were frozen within 4 hrs of collection. The remaining 10 ml was collected in muffled 20 ml glass vials, acidified (pH 2.5) with H_3PO_4, sealed with teflon-lined caps, and stored upright on ice until returned to the laboratory where samples were refrigerated for up to 1 wk before DOC analysis.

Water Column and Benthic Fluxes of DON and DOP.—Net fluxes of DOC, DON, and DOP in the water column were measured using in situ light and dark bottle incubations. Water was collected around dawn and incubated in light ($n \geq 3$) and dark ($n \geq 3$) bottles for ~12 hrs during the day to estimate the net fluxes of DOM in the water column. Another water sample was collected around sunset and incubated overnight in dark ($n \geq 3$) bottles to estimate nutrient fluxes at night. Water samples were dispensed into clean (acid washed) 300 ml biological oxygen-demand bottles (dark) or 90 ml quartz bottles (light). Dark bottles were wrapped in aluminum foil and all bottles were incubated in situ on racks set at mid-depth (~0.5 m) in the water column. Initial and final samples for DON and DOP were dispensed into muffled 50 ml glass vials and preserved as described for the diel water column samples. Rates of net DOC, DON, and DOP production or consumption were determined from the slope of the least-square linear regression analysis of concentrations versus time. These volume-based rates were multiplied by the average water depth measured during the incubation to calculate the water column fluxes per m^2. The daily water column net flux occurring in the light was based on the number of daylight hours. Benthic primary production accounts for ~96% of community primary production in southern Laguna Madre (Ziegler and Benner, 1999b). Therefore, the number of daylight hours was based on the number of hours of saturating PAR light for seagrasses (~300 $\mu E\ m^{-2}\ s^{-1}$; Herzka and Dunton, 1996), determined from underwater continuous PAR light data collected close to the study site (K. Dunton, J. Kaldy, and J. Kowalski, unpubl. data). The daily net flux occurring in the dark was calculated from the number of hours below saturating light levels. Positive fluxes indicated the regeneration of DOM and negative fluxes indicated the uptake of DOM. Water column demand for DON and DOP was estimated from net water column DIN and DIP fluxes (Ziegler and Benner, 1999a), bacterial production (Ziegler and Benner, 2000), and assuming a bacterial C:N:P ratio of 43:10:1.

Net benthic fluxes of DOC, DON, and DOP were determined by measuring changes in concentrations in light and dark chambers. Chambers were constructed from 20 L polycarbonate carboys by removing the bottoms and adding a sampling port at the shoulder. Caps of the chambers were fitted with a current-driven stirring mechanism to mimic in situ water movement (see Ziegler and Benner, 1998). Covers for the chambers were constructed of dark gray plastic and used for the dark incubations. Four chambers were placed carefully ~8 cm into the sediment at three

seagrass-dominated sites (primarily *T. testudinum*) and one unvegetated area (void of all macrophytes) adjacent to the other chambers. An average of the four chambers was used to estimate net benthic DOM fluxes. This sampling design was based on estimates of the aerial coverage of vegetated (75%) and unvegetated (25%) sediments in the lower Laguna Madre (Quammen and Onuf, 1993). Aerial coverage of vegetated sediments at the study site was greater than that estimated for the lower Laguna Madre. Dark incubations were conducted early in the morning and late in the afternoon to avoid dramatic shifts in light levels. Multiple light incubations were conducted throughout a single day with each incubation lasting for 1.5–4 hrs depending upon level of activity. Benthic flux measurements were made on two consecutive days in June when light levels varied dramatically due to a storm. This comparison was conducted to determine the influence of light levels on net benthic fluxes during the most productive time of the year. Daily net fluxes were based on the number of hours of saturating PAR light for seagrasses (~300 μE m^{-2} s^{-1}; Herzka and Dunton, 1996) as described above. Positive flux values indicated a flux from the sediments to the water column and negative fluxes indicated a flux from the water column to the sediments.

The limit of quantification for DOP flux measurements was determined to be 0.15 mmol P m^{-2} d^{-1} for light incubations and 0.30 mmol P m^{-2} d^{-1} for dark incubations. This was based on the 0.07 μM detection limit and 1% precision for the DOP analysis (see section on DOP analysis below), average chamber volume (17 L), chamber basal area (638 cm^2), and the average daylight period of saturating light.

The mean residence times (R_t) for DOC, DON, and DOP were calculated from water column respiration (Ziegler and Benner, 1999b), water column DON and DOP demand, net benthic fluxes of DOC, DON, and DOP, and the net water column DIN and DIP fluxes (Ziegler and Benner, 1999a).

Seagrass Carbon, Nitrogen, and Phosphorus.—Whole live plants were collected using a 15 cm diameter corer. The cores were sieved with water to remove live plants from the sediment. The plants were rinsed with seawater and then kept on ice until they were brought back to the laboratory. Within 2 d of collection the tissues were rinsed again with distilled water and separated into aboveground blades (shoots) and belowground roots and rhizome tissues (roots). Once separated, the tissues were placed in a drying oven at 45°C for 48 hrs. The dried above and belowground tissues were each milled separately with roots and rhizomes combined in a single sample. Carbon and nitrogen content of the seagrass tissues were determined on a Carlo Erba EA 1108 elemental analyzer (acetanilide standard). Organic phosphorus content of seagrass tissues was determined according to method of Solanzaro and Sharp (1980).

DOC, DON, and DOP Measurements.—Dissolved organic carbon was determined by high-temperature oxidation using a Shimadzu TOC-5000 analyzer (Benner and Strom, 1993). Total dissolved nitrogen (TDN) and phosphorus (TDP) were measured using the UV photooxidation method and procedures of Walsh (1989). Dissolved organic nitrogen and phosphorus were determined as the difference between DIN (ammonium, nitrate, nitrite) and TDN, and soluble reactive phosphate (SRP) and TDP, respectively. Detection limits and precision for DON and DOP measurements were based upon the analyses for both NH_4^+ and NO_3^-, and SRP respectively. Concentrations of NH_4^+ were determined using the phenol-hypochlorite method (Solorzano, 1969). The detection limit was 0.30 μM at a confidence level ≥ 90% (Skoog and Leary, 1992). The precision of the NH_4^+ analysis at 0.3 μM was 15%. Concentrations of NO_3^- + NO_2^- were determined using an Antek Model 745 Nitrate/Nitrite Reduction Assembly and Antek Model 7020 nitric oxide chemiluminescence detector (Braman and Hendrix, 1989). The precision for a concentration of 0.001 μM NO_3^- + NO_2^- was < 6%. The detection limit for NO_3^- was <6% for 1 pM or <1% for 0.01–1 μM. The detection limit for DON measurements in this study was 0.3 μM and the precision for all measurements above this limit was 16% (0.05 μM). The detection limits and precision of DOP was based upon the measurement of soluble reactive phosphate, using the standard colormetric method (Strickland and Parsons, 1972). The detection limit for DOP was 0.07 μM and the precision for concentrations ≥ 0.07 μM was 1%.

Statistical Analyses.—Two tailed t-tests ($\alpha = 0.05$) were performed to determine if concentrations, ratios, or rates were significantly different between pairs of data. An ANOVA ($\alpha =$

0.05) was performed to determine if the elemental composition of DOM was significantly different among the seasons sampled. Regression analyses were used to determine if a significant ($P < 0.05$) relationship existed between DOC and DON or DOP.

Results

Seasonal Variations in C, N, and P Composition of DOM.—Highest concentrations of DOC, DON, and DOP were measured during March when a phytoplankton bloom ("brown tide") occurred. DOC concentrations ranged from 167–225 μM, while DON ranged from 10.5–12.1 μM, and DOP ranged from 0.22–0.30 μM (Table 1). DON and DOP represented the dominant fractions of total dissolved nitrogen (96–98%) and total dissolved phosphorus (58–100%), respectively (Table 1).

Seasonal measurements of the C:N:P ratio of DOM indicated an enrichment in C in March, when phytoplankton production was highest, and some depletion in P during the summer when seagrasses were most productive (Table 1). The molar C:N ratio of DOM ranged from 16–19 and was highest during March. The C:P ratio, ranged from 700–821, and was highest during June. The C:N ratio in March and C:P ratio in June were significantly higher then the respective DOM ratios in November (t-test; $\alpha = 0.05$).These elemental ratios were based upon the average DOC, DON, and DOP concentrations measured throughout a diel period during each season. Substantial changes in the concentrations of DOC, DON, and DOP were measured throughout each diel period (Figs. 1,2). Plotting the concentration of DON versus the corresponding DOC concentration for each season indicates a significant (least squares regression; $P < 0.05$) relationship between DOC and DON during March ($P = 0.03$) and June ($P = 0.01$), but not during November ($P = 0.10$).

Seasonal changes in elemental ratios of DOM were primarily attributed to the change in DOC concentrations. Statistically significant differences in the concentration of DOC (ANOVA, $\alpha = 0.05$, $P < 0.0001$, $F = 153.72$, $df = 60$) and DOP (ANOVA, $\alpha = 0.05$, $P = 0.002$, $F = 7.88$, $df = 35$) were found among the three seasons sampled. The DON concentrations, however, were not significantly different among the seasons (ANOVA, $\alpha = 0.05$, $P = 0.09$, $F = 2.66$, $df = 35$). These comparisons were based on all diel samples collected for each season. Combining diel data for the three sampling seasons, it appears that only ~30% of the change in either DON or DOP concentration can be explained by changes in DOC concentration ($r^2 = 0.30$ for DON and $r^2 = 0.28$ for DOP).

Diel Variations in C, N, and P Composition of DOM.—Diel changes in concentrations of DOC, DON, and DOP greatly exceeded the relatively small changes measured among the three seasons. Diel changes in the concentrations of DON ranged from 9.8–15.7 μM in March, 7.8–12.9 μM in June, and 8.1–12.2 μM in November (Fig. 1). The largest diel change in DOP concentration was found in March when concentrations ranged from 0.17–0.42 μM (Fig. 2). During each of the three seasons the C:N ratio of DOM increased during the day. This diel change was most dramatic in June (data not shown). The diel change in C:P ratio of DOM during each season was more variable and tended to decrease throughout the day during both March and June (data not shown). In November no measurable change in C:P ratio of DOM occurred during the daylight hours (data not shown).

Water Column DOM Fluxes.—Water column fluxes measured in June indicated net uptake of DON and DOP during night and daytime incubations (Table 2). Difference

Table 1. Average water temperature reported as °C. Concentrations of dissolved organic carbon (DOC; Ziegler and Benner 1999b), dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP) in lower Laguna Madre. Average molar ratios (C:N; C:P; N:P) of DOM are given. Dissolved inorganic nitrogen (DIN) and soluble reactive phosphate (SRP) are taken from Ziegler and Benner (1999b). All concentrations are reported in μM units, and each concentration and ratio is given as the average ± 1 standard deviation (SD) of the number (n) of samples taken throughout a 24 hr period.

Date	n	Water temp.	DOC	DON	DOP	DIN	SRP	C:N	C:P	N:P
March	11	22.1	225 ± 20	12.1 ± 2.0	0.30 ± 0.08	0.55 ± 0.22	0.22 ± 0.14	18.5 ± 0.2	796 ± 0.3	43 ± 0.3
June	13	30.5	172 ± 6	10.5 ± 2.1	0.22 ± 0.04	0.43 ± 0.17	0.12 ± 0.03	16.8 ± 0.2	821 ± 0.2	49 ± 0.3
November	12	25.1	167 ± 10	10.9 ± 1.1	0.25 ± 0.03	0.37 ± 0.08	< 0.07	15.6 ± 0.1	700 ± 0.3	45 ± 0.2

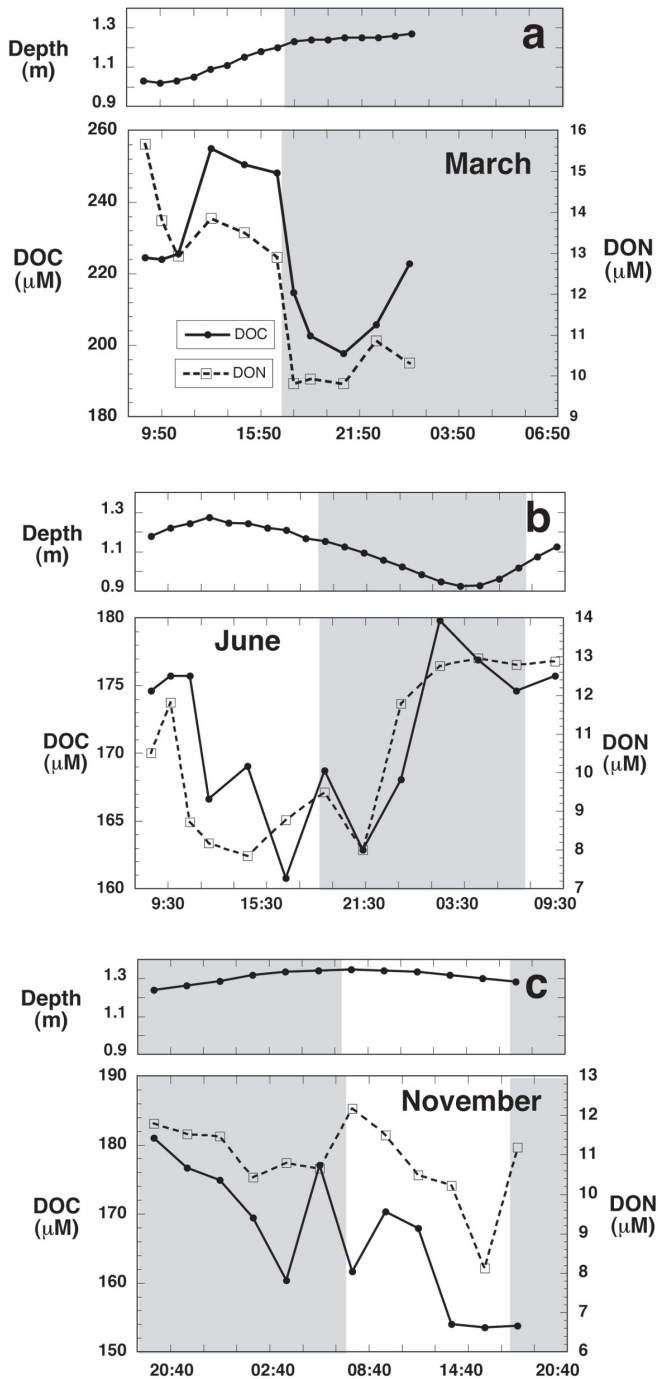

Figure 1. Concentrations of dissolved organic carbon (DOC; solid line) and dissolved organic nitrogen (DON; dashed line) for samples collected over 24 hr periods on March 7–8, 1997, June 24–25, 1997, and November 5–6, 1996. Upper plot associated with each diel plot is the recorded water depth for that sampling period.

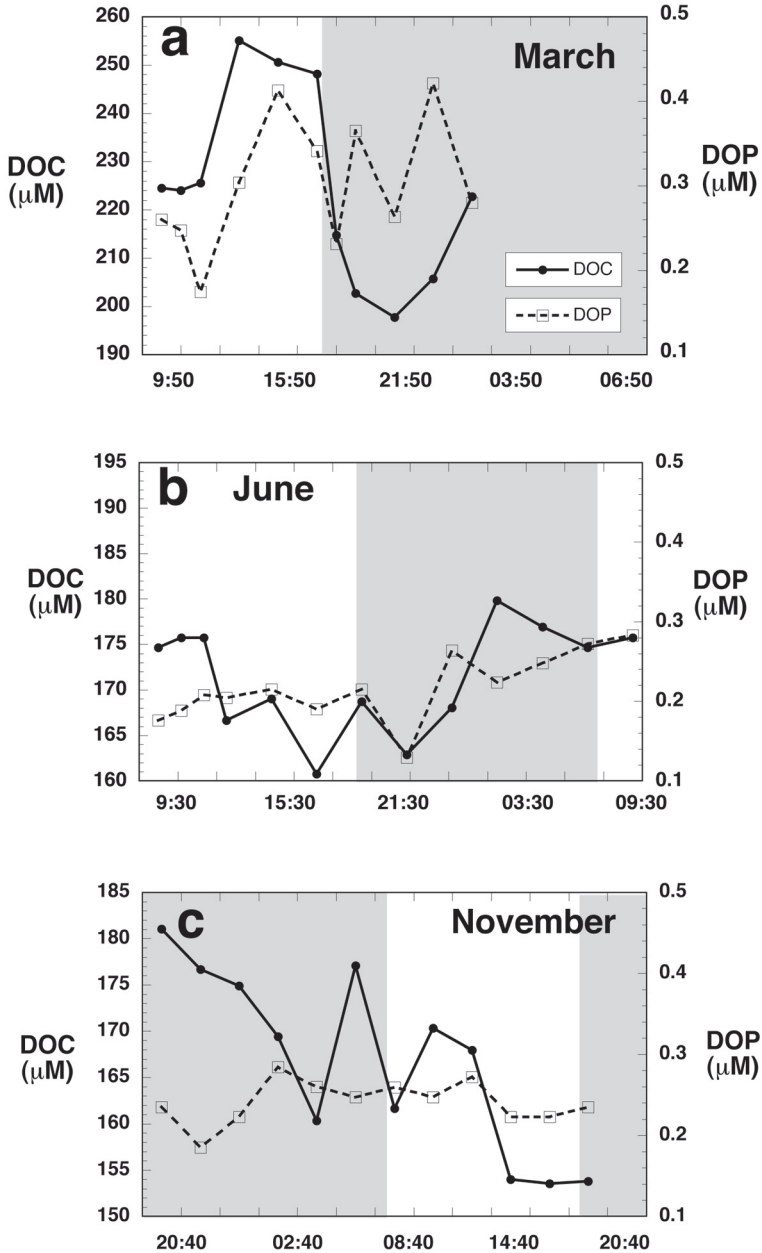

Figure 2. Concentrations of dissolved organic carbon (DOC; solid line) and dissolved organic phosphorous (DOP; dashed line) for samples collected over 24 hr periods on March 7–8, 1997, June 24–25, 1997, and November 5–6, 1996.

Table 2. Net water column fluxes measured on June 24–25, 1997. Net water column fluxes are reported as the slope of the least squares regression ± 1 SD of the slope (n = 8). Units are reported in mmol C, N, or P m^{-2} d^{-1} based on the number of hours between sunset and sunrise for "night" incubations, and the number of hours between sunrise and sunset for the "day dark" and "day light" incubations. Those fluxes determined to be significantly different from zero ($P < 0.05$) are denoted with " * ".

Incubation	DON	DOP
Night	−0.50 ± 0.26*	−0.04 ± 0.01*
Day dark	−1.08 ± 0.54*	−0.01 ± 0.02
Day light	−1.59 ± 0.56*	−0.03 ± 0.01*

in DON and DOP concentrations between the time points were significant in all but one case (DOP daytime dark incubation; Table 2). The DON fluxes ranged from -1.6 to -0.5 mmol N m^{-2} d^{-1} with the largest net uptake of DON during light incubations. The DOP fluxes did not vary as greatly among the three different incubations and ranged from -0.04 to -0.01 mmol P m^{-2} d^{-1}. There was no discernable difference in the net flux of DOP between light and dark incubations in the water column.

Net water column fluxes suggest water column demand for DON was ~10-fold higher than that of DOP in all seasons, ranging from 0.47 mmol N m^{-2} d^{-1} in November to 2.62 mmol N m^{-2} d^{-1} in March (Table 3). The water column demand for DOP followed the same seasonal pattern as DON with highest net uptake rates measured in March (0.21 mmol P m^{-2} d^{-1}) and lowest net uptake in November (0.05 mmol P m^{-2} d^{-1}).

Benthic Fluxes of DOM.—Net benthic fluxes of DOC were large (13–14 mmol C m^{-2} d^{-1}) and directed out of the benthos in the light incubations of vegetated chambers during March and June. Only those from June, however, were significantly different from zero (Table 4). Mean net benthic fluxes of DON and DOP were relatively small, not significantly different from zero, and did not appear to be related to light-mediated processes as found with DOC fluxes. The large variation in net benthic fluxes detected is typical of shallow benthic ecosystems (Dollar et al., 1991) and in most instances only enabled us to assess the general trends in the movement of organic nutrients between the benthos and water column. The net benthic fluxes of DOP ranged from -0.02–0.07 mmol P m^{-2} d^{-1} and did not appear to follow any pattern as far as light, season, or sediment type (vegetated versus unvegetated). The variability inherent in these data, however, precludes any conclusions regarding the relationship between benthic DON or DOP flux and light, season, or sediment type. However, net benthic fluxes on June 25th (PAR = 34 E m^{-2} d^{-1}), a sunny day relative to June 24th (PAR = 14 E m^{-2} d^{-1}), suggest a greater net uptake of DON and DOP in the vegetated sediments during periods of high light availability (Fig. 3). These data suggest a link between benthic primary production, which was significantly higher on June 25th (Ziegler and Benner, 1999b), and net fluxes of DOC, DON, and DOP flux. Benthic primary production also appeared to have an impact on the resulting C:N of DOM. Significantly higher ratios were measured at the end of the light chamber incubations on June 25th relative to the previous cloudy day (Fig. 4).

Dissolved Organic Matter Mean Residence Times.—The mean R_t of DOC, DON, and DOP suggest DOP was the most reactive, having mean R_t ranging from 1.4–5.0 d (Table 3). The mean R_t of DOC was shortest (12 d) in summer and longest (16 d) in spring, while DON was longest (6 d) in summer and shortest (2 d) in fall. The mean R_t of DOP was longest in fall (5.0 d) and shortest (1.4 d) in spring.

C, N, and P Composition of Seagrass Tissues.—Above and belowground seagrass tissues varied seasonally in C, N, and P content. The molar C:N ratios of leaf tissues

Table 3. Average bacterioplankton production (BP; Ziegler and Benner, 1999a), water column respiration (WCR; Ziegler and Benner 1999a) and bacterial growth efficiencies reported as a percent (BGE; determined from BP and WCR). Benthic and water column (WC) gross primary production (GPP) rates (Ziegler and Benner, 1999a), for the same dates bioavailability data were collected. Estimated water column demand for DON and DOP is based upon net water column dissolved inorganic nitrogen and phosphorus fluxes from Ziegler and Benner (1999b), bacterial production, and assuming a bacterial C:N:P ratio of 43:10:1. The mean residence times (R_t) for DOC, DON, DOP is based upon WCR, WC N, and P demand, net benthic fluxes of DOM, and net WC DIN and DIP fluxes (Ziegler and Benner 1999b). The relative bioavailability (BA) of dissolved organic matter is reported as the average bacterial production ($\mu M\ C\ h^{-1}$) divided by the concentration of dissolved organic carbon (DOC; mM) and is reported as $\mu MCh^{-1}\ mMC^{-1}$. All rates are reported as mmol C or N $m^{-2}\ d^{-1}$. All errors for average values are reported as ± one standard deviation of the mean.

	BP	WCR	BGE	Benthic	WC	WC demand		Mean R_t			
Month			%	GPP	GPP	DON	DOP	DOC	DON	DOP	BA
March	9.1 ± 1.6	17.0	35	123 ± 60	9.2 ± 3.0	2.62	0.21	16	3	1.4	1.37
June	5.9 ± 2.2	14.9	28	141 ± 72	5.7 ± 2.3	1.75	0.14	12	6	1.6	1.60
November	1.8 ± 1.0	6.6	21	93 ± 40	1.0 ± 0.7	0.47	0.05	14	2	5.0	0.36

Table 4. Net benthic dissolved organic matter fluxes (mmol C, N, or P $m^{-2}\ d^{-1}$) measured in light and dark incubations. Daily rates for light and dark incubations were calculated from the number of hours of daylight or night, respectively. Values for vegetated chambers are an average of three replicate vegetated chambers (n = 3). Benthos refers to the average flux (n = 4) of the three vegetated and one unvegetated chambers on the site which is approximately 75% vegetated and 25% unvegetated. All values are provided as the mean ± 1SD. Those fluxes which were determined to be significantly different ($\alpha = 0.05$) from zero are denoted with *.

Vegetated

	DOC		DON		DOP	
Date	Light	Dark	Light	Dark	Light	Dark
March	13.7 ± 8.2	0.5 ± 3.5	−1.2 ± 0.8	−0.84 ± 3.1	0.002 ± 0.009	−0.010 ± 0.01
June	13.3 ± 2.6*	−7.7 ± 13.5	0.1 ± 0.3	0.5 ± 1.9	0.004 ± 0.006	0.050 ± 0.03
November	8.4 ± 18.3	−4.8 ± 12.7	−0.9 ± 0.9	−4.0 ± 3.5	0.070 ± 0.110	−0.020 ± 0.09

Benthos

Date	DOC		DON		DOP	
	Light	Dark	Light	Dark	Light	Dark
March	10.4 ± 9.4	0.4 ± 2.8	−0.7 ± 1.1	−1.3 ± 2.6	0.00 ± 0.01	0.01 ± 0.03
June	10.2 ± 6.5*	−10.3 ± 12.1	−0.1 ± 0.4	0.3 ± 1.6	0.00 ± 0.01	0.02 ± 0.04
November	−0.2 ± 18.3	−8.8 ± 12.7	−0.9 ± 0.8	−4.0 ± 3.5	0.07 ± 0.11	0.01 ± 0.09

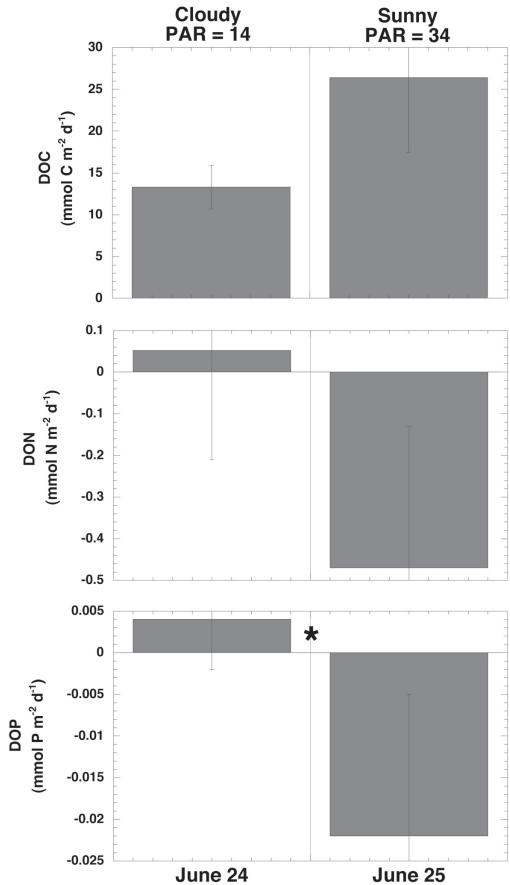

Figure 3. Average net benthic fluxes of dissolved organic carbon, nitrogen, and phosphorus (DOC, DON, and DOP) measured over seagrass-dominated sediments on June 24, 1997 (a cloudy day) and June 25, 1997 (a clear day). Negative values indicate a flux into the sediment and positive values indicate a flux out of the sediment. Error bars represent 1 standard deviation of the mean (n = 3). Photosynthetically active radiation (PAR) is given as E m^{-2} d^{-1}. The "*" denotes a significant difference between the DOM flux measured on the cloudy versus sunny day ($P < 0.05$).

ranged from 13–25 over the three seasons and highest ratios were found during June when benthic primary production was highest (Ziegler and Benner, 1998). A similar seasonal pattern was found for the molar C:P ratio of leaf tissues, which ranged from 250–650. The seasonal change in the N:P ratios was not as dramatic but followed the same seasonal pattern and ranged from 18–24. The C:N:P ratios of the seagrass root and rhizome tissues were generally higher and varied seasonally (Table 5). The molar C:N and C:P ratios for root and rhizome tissues ranged from 26–60 and 580–1050, respectively, with highest ratios in June and lowest ratios in November. The N:P ratios were lowest in June (16) and highest in March (26).

Discussion

Microbial activity influences seasonal variability in DOM in wetlands and coastal ecosystems (Stepanauskas et al., 1999, 2000), and heterotrophic activity was an important factor shaping the composition of DOM in Laguna Madre. Heterotrophic utilization

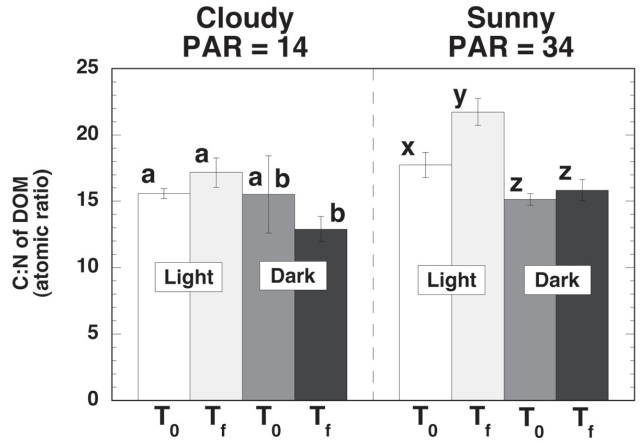

Figure 4. The molar carbon to nitrogen (C:N) ratios for DOM collected from both light and dark benthic chambers deployed over seagrass-dominated sediments during June 24 (cloudy day) and June 25 (clear day). Values are reported for the initial samples collected immediately before the start of each experiment (T_o) and the final samples collected at the end of the incubation (T_f). Error bars represent the cumulative standard deviation of the ratio of the mean DOC to DON concentrations (n = 3). Photosynthetically active radiation (PAR) is given as E m^{-2} d^{-1}. Significant differences are reported for C:N ratios within each date reported. Those ratios, which are significantly (t-test; $\alpha = 0.05$) different are labeled with a different letter (June 24th: a or b; June 25th x, y, or z).

of DOC, DON, and to a lesser extent DOP, may be largely responsible for seasonal and diel changes in the C, N, and P composition of DOM in Laguna Madre. The impact of heterotrophic activity on DOM composition was greatest in June when rapid microbial utilization of DOC coincided with net water column consumption of DON and DOP. Microbial utilization of DOC and DON in June, measured as net diel changes in water column concentrations, were 15 μM C and 3.5 μM N, and the ratio (4.3) of this utilization was similar to the C:N ratio of bacteria. DOP had a short mean R_t (1.6 d) in summer, and diel patterns in DOP concentrations were not apparent. Tidal influences in June are another potential factor responsible for the observed diel change in DOM concentrations. This, however, is unlikely given the strong linkage between water column respiration and benthic primary production in summer and the small tidal range in Laguna Madre (Ziegler and Benner, 1999b).

In fall, water column heterotrophic activity had less impact on DOM composition. Microbial activity was lower in November, and the absence of a distinct diel pattern in the DOC concentrations appeared to be a consequence of lower rates of heterotrophic activity. The lowest bacterial growth efficiency (BGE) and DOM bioavailability was measured in November, indicating a decrease in DOM quality relative to June. One key difference in DOM composition in fall was the lower C, relative to N or P, content of DOM as indicated by significantly lower C:N and C:P ratios relative to spring and summer, respectively. The decline in C:N and C:P ratios and bioavailable DOM in the fall was probably related to the minimal release of carbon-rich, seagrass-derived DOM during this period of low primary productivity (Ziegler and Benner, 1998).

The relationship between BGE and the elemental composition of DOM is further evidence that heterotrophs influence DOM composition. Bacterial growth efficiency ranged from 21% in November to 35% in March (Ziegler and Benner, 1999a) and was positively

Table 5. Carbon (C), nitrogen (N), and phosphorous (P) content of *Thalassia testudinum* shoot and root tissues collected at the study site. Values are given in weight percent, and provided with the corresponding molar ratios.

Date	Tissue	C	N	P	C:N	C:P	N:P
March	Shoot	34.3	3.1	0.36	13	243	19
	Root	31.2	1.1	0.10	32	845	26
June	Shoot	35.7	1.7	0.14	25	646	26
	Root	36.4	0.7	0.09	60	1,045	17
November	Shoot	32.5	2.0	0.20	19	420	23
	Root	30.8	1.4	0.14	26	581	22

correlated to the C:N ratio of bulk DOM ($P < 0.04$, $r^2 = 0.99$; Fig. 5A). This is contrary to what might be expected (Goldman et al., 1987), however, GE was negatively correlated with the C:N ratio of bioavailable DOM, calculated from water column respiration and DIN regeneration (Fig. 5B). The difference in these relationships indicates that bulk DOM composition is not necessarily indicative of the bioavailable DOM utilized by microorganisms. Relative bioavailability of DOM, based on the ratio of bacterial production to DOC concentration, supports the seasonal trend observed in BGE. The relative bioavailability of DOM was highest in summer (1.60 $\mu MC\ h^{-1}\ mMC^{-1}$) and lowest in fall (0.36 $\mu MC\ h^{-1}\ mMC^{-1}$). The trends in mean R_t of DON and DOP, however, more closely follow the decrease in BGE from spring through fall as compared to that of DOC suggesting that DON and DOP concentrations may be more indicative of bioavailable DOM.

Bioavailability of DOC and DON can be quite different in coastal ecosystems, suggesting an uncoupling of organic nutrients due to differences in source and extent of diagenetic alteration (Stepanauskas et al., 2000). Although linear correlations between the concentration of DOC and DON or DOP were significant ($P < 0.01$), they suggest variation in the concentration of DOC and can only explain about 30% of the variation in DON or DOP concentration in Laguna Madre. The mean R_t for DOC was much higher than that of DON or DOP throughout the year, indicating the following reactivity of these pools: DOP > DON > DOC. The rapid turnover of DON and DOP relative to DOC indicated that N and P-rich components of DOM were preferentially utilized.

The mean R_t of DON and DOP increased from spring to fall as opposed to DOC, which exhibited the shortest mean R_t in summer. Elevated DOC concentrations during more productive seasons have been linked to seagrass productivity and the release of DOC at this study site (Ziegler and Benner, 1999b). However, seagrass exudation may not be an important source of DON and DOP. Benthic fluxes of DON or DOP were quite variable and could not be related to seagrass production or any light-mediated process as DOC fluxes have been (Ziegler and Benner, 1999b). In summer, a significant increase in the C:N ratio of DOM at midday in the water column and within light chamber incubations provide further evidence for the flux of C-rich DOM derived from benthic primary production. Exudates are likely to be carbohydrate rich, representing a highly bioavailable form of DOC. The cycling of DON and DOP may not be influenced by this source of DOC, but instead by seasonal variation in the heterotrophic utilization of DOM as well as more N and P-rich sources of DOM.

A highly bioavailable source of DON and DOP likely contributed to the shorter mean R_t of DON and DOP in spring. The lower C:N ratio (26) for the DOM mineralized in the water column in spring relative to summer (54) and fall (55) in Laguna Madre (Ziegler

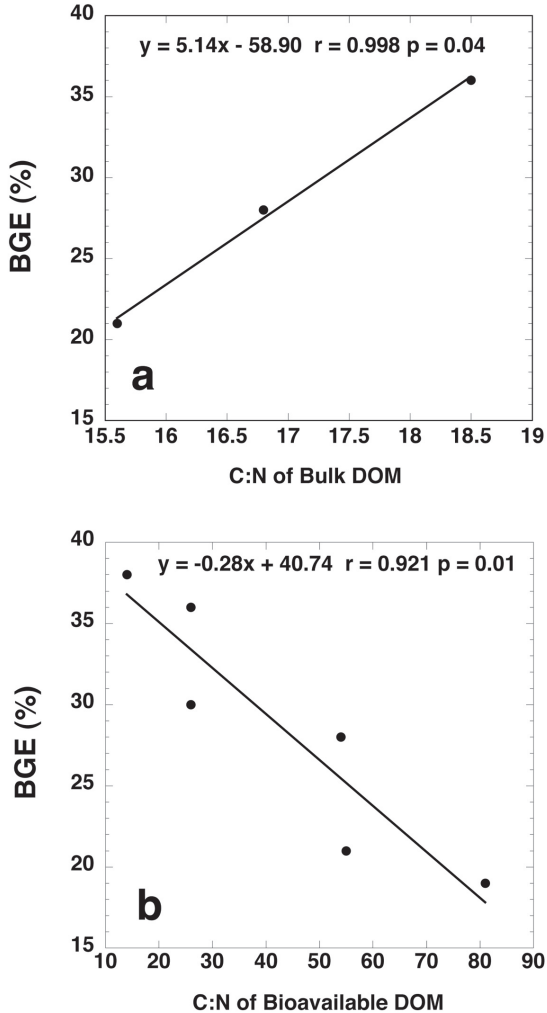

Figure 5. Linear regression of the average molar C:N ratio of (a) DOM during March, June, and November and (b) bioavailable DOM during July, September, and November 1996 and January, March, and June 1997 (Ziegler and Benner, 1999a) versus the corresponding calculated bacterial growth efficiency based on average bacterial production (Ziegler and Benner, 2000) and water column respiration (Ziegler and Benner, 1999b). Statistics are provided for each regression.

and Benner, 1999a) suggests a source of some N-rich component of DOM in spring. The early spring phytoplankton bloom ("brown tide") may have been an important source of both DON and DOP in March. Brown tides in other ecosystems have been correlated with C and P-rich and N-poor DOM (Lomas et al., 2001). The role of phytoplankton as a source of DOM in spring, however, is supported by the relationship between DON and nitrate plus nitrite concentrations (Ziegler and Benner, 1999a) in March, the only time of year such a relationship was significant ($r^2 = 0.60$; $P < 0.01$).

Mean R_t for DOC, DON, and DOP were all highest in fall as a result of the high levels of activity in summer which likely resulted in less bioavailable DOM in fall, when benthic primary production was low and exudation of C-rich DOM became less important. Neither the diel pattern of DOM concentrations, nor net benthic fluxes indicated any

release of C-rich DOM in November. Reactivity of DOP was less than that of DON as evidenced by the longer mean R_t of DOP. The decrease in concentration and increase in mean R_t of DON and DOP suggests that utilization of the most bioavailable components of these pools occurred through summer, leaving more refractory pools of DON and DOP in fall when productivity was lowest (Ziegler and Benner, 1998). Microbial utilization and cycling of DOM during the most productive times of year appear to be dominant forces controlling the composition and cycling DOM in Laguna Madre. Results of this study demonstrate the important role DOM cycling plays in estuarine nutrient dynamics and how changes in DOM source and microbial activity can impact the biogeochemical cycling of N and P.

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