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Microbial activity and carbon, nitrogen, and phosphorus content in a subtropical seagrass estuary (Florida Bay): evidence for limited bacterial use of seagrass production

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Abstract Bacterial abundance, production, and extracellular enzyme activity were determined in the shallow water column, in the epiphytic community of *Thalassia testudinum*, and at the sediment surface along with total carbon, nitrogen, and phosphorus in Florida Bay, a subtropical seagrass estuary. Data were statistically reduced by principle components analysis (PCA) and multidimensional scaling and related to *T. testudinum* leaf total phosphorus content and phytoplankton biomass. Each zone (i.e., pelagic, epiphytic, and surface sediment community) was significantly dissimilar to each other (Global $R = 0.65$). Pelagic aminopeptidase and sum of carbon hydrolytic enzyme (esterase, peptidase, and α- and β-glucosidase) activities ranged from 8 to 284 mg N m^{-2} day^{-1} and 113–1,671 mg C m^{-2} day^{-1}, respectively, and were 1–3 orders of magnitude higher than epiphytic and sediment surface activities. Due to the phosphorus-limited nature of Florida Bay, alkaline phosphatase activity was similar between pelagic (51–710 mg P m^{-2} day^{-1}) and sediment (77–224 mg P m^{-2} day^{-1}) zones but lower in the epiphytes (1.1–5.2 mg P m^{-2} day^{-1}). Total (and/or organic) C (111–311 g C m^{-2}), N (9.4–27.2 g N m^{-2}), and P (212–1,623 mg P m^{-2}) content were the highest in the sediment surface and typically the lowest in the seagrass epiphytes, ranging from 0.6 to 8.7 g C m^{-2}, 0.02–0.99 g N m^{-2}, and 0.5–43.5 mg P m^{-2}. Unlike nutrient content and enzyme activities, bacterial production was highest in the epiphytes (8.0–235.1 mg C m^{-2} day^{-1}) and sediment surface (11.5–233.2 mg C m^{-2} day^{-1}) and low in the water column (1.6–85.6 mg C m^{-2} day^{-1}). At an assumed 50% bacterial growth efficiency, for example, extracellular enzyme hydrolysis could supply 1.8 and 69% of epiphytic and sediment bacteria carbon demand, respectively, while pelagic bacteria could fulfill their carbon demand completely by enzyme-hydrolyzable organic matter. Similarly, previously measured *T. testudinum* extracellular photosynthetic carbon exudation rates could not satisfy epiphytic and sediment surface bacterial carbon demand, suggesting that epiphytic algae and microphytobenthos might provide usable substrates to support high benthic bacterial production rates. PCA revealed that *T. testudinum* nutrient content was related positively to epiphytic nutrient content and carbon hydrolase activity in the sediment, but unrelated to pelagic variables. Phytoplankton biomass correlated positively with all pelagic components and sediment aminopeptidase activity but negatively with epiphytic alkaline phosphatase activity. In conclusion, seagrass production and nutrient content was unrelated to pelagic bacteria activity, but did influence extracellular enzyme hydrolysis at the sediment surface and in the epiphytes. This study suggests that seagrass-derived organic matter is of secondary importance in Florida Bay and that bacteria rely primarily on algal/cyanobacteria production. Pelagic bacteria seem coupled to phytoplankton, while the benthic community appears supported by epiphytic and/or microphytobenthos production.

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Introduction

Seagrass estuaries are among the most productive marine ecosystems, where seagrass and its epiphytes are the dominant

primary producers of organic carbon (Penhale 1977; Pollard and Moriarty 1991). Heterotrophic bacteria are important decomposers of seagrass production, and are a necessary component of the detritus-based food web of seagrass ecosystems (Harrison 1989). In these ecosystems, bacteria inhabit pelagic, seagrass epiphytic, and sediment zones (used here in place of community to emphasis both abiotic and biotic components of each zone). The proximity and interconnectedness of these zones creates potential for benthic–pelagic coupling of heterotrophic and autotrophic processes (Ziegler and Benner 1999a, b). For example, pelagic bacterial production and water column respiration increased during the light period when seagrass primary production and organic carbon release peaked (Moriarty and Pollard 1982; Ziegler and Benner 1999a). However, not all seagrass ecosystems experience tight coupling between pelagic bacteria and seagrass production. In a few *Zostera marina* seagrass beds, sediment bacteria relied mostly on benthic micro- and macro-algae to fulfill their carbon demands (Boschker et al. 2000).

Heterotrophic bacteria, cyanobacteria, and algae produce extracellular enzymes to access much of the estuarine organic matter pool, whose molecules are too large to pass through microbial membranes without extracellular hydrolysis (Hoppe et al. 2002). Heterotrophic bacterial extracellular enzymes are used to gain carbon, nitrogen, and/or phosphorus from organic matter >600 Daltons (Weiss et al. 1991), while cyanobacterial and algal extracellular enzymes are likely used only to obtain inorganic nutrients (Hoppe et al. 2002). Analysis of extracellular enzyme activities in pelagic, seagrass epiphytic, and sediment surface zones of seagrass estuaries can provide useful information regarding the type of organic matter (i.e., amino acids, lipids, and carbohydrates) used by bacteria to fulfill their carbon demand and the importance of organic nutrients to the microbial (i.e., bacteria, cyanobacteria, and algae) community. The relative magnitude of extracellular enzyme activities between zones might allow assessment of the availability of or demand for inorganic nutrients and/or labile photosynthetic carbon exudates. For example, alkaline phosphate activity in algae is typically inhibited by phosphate additions and related negatively to ambient phosphorus concentration (Hoppe et al. 2002). Heterotrophic bacteria use preferentially labile photosynthetic carbon exudates over larger, chemically more complex organic carbon forms. Low extracellular enzyme activities for carbon hydrolase enzymes (i.e., glucosidases, esterases, and lipases among others) might, consequently, provide indirect evidence for bacterial use of seagrass and/or algal photosynthetic exudates. Conversely, high extracellular activity of carbon hydrolase enzymes relative to bacterial carbon demand would indicate extensive use of the enzyme-hydrolysable organic matter pool.

In this study, extracellular enzyme activity was linked to bacterial carbon demand in order to determine the potential importance of labile photosynthetic exudates and higher molecular weight extracellular enzyme-hydrolysable organic matter to bacterial use. In addition, the influence of seagrass nutrient content and phytoplankton biomass on organic matter use and bacterial production was assessed. Specifically, extracellular enzyme activity, bacteria abundance and production, and carbon, nitrogen, and phosphorus contents were measured in pelagic, seagrass epiphytic, and sediment surface zones. These data were examined by multidimensional scaling and principle components analysis.

Materials and methods

Station description and sampling plan

Florida Bay is a shallow, wedge shaped, subtropical estuary bounded in the north by the Everglades wetlands, the south and east by the Florida Keys, and the west by the Gulf of Mexico (Fig. 1). Florida Bay is compartmentalized into semi-isolated basins by biogenic carbonate mudbanks, which restrict water exchange and tidal amplitude throughout the eastern and central bay. The seagrass, *Thalassia testudinum*, and its epiphytes are the dominant primary producers (Zieman et al. 1989; Frankovich and Zieman 1994), though transient phytoplankton blooms can contribute to local productivity (Phlips et al. 1999). Macroalgae are not abundant in the study area (central and eastern Florida Bay, Zieman et al. 1989). At four locations across Florida Bay, microphytobenthos biomass (measured as benthic chlorophyll *a*) was typically tenfold greater than *T. testudinum* epiphytic chlorophyll *a* (Armitage et al. 2006), suggesting that microphytobenthos is also an important primary producing component.

Four sampling stations in Florida Bay were chosen based on existing benthic plant distribution and water quality data. Each station was expected to represent a distinct area of Florida Bay (Fig. 1). The station in the northeast, Little Madeira Bay [Florida Coastal Everglades (FCE)-LTER station TSPh 7], represents the mainland region of Florida Bay and is characterized by its influence from Everglades freshwater runoff and sparse to patchy seagrass beds (Zieman et al. 1989). Duck Key (FCE-LTER station TSPh 9), also in the northeastern bay, is characteristic of the eastern bay and has patchy seagrass beds and low phosphorus availability (Zieman et al. 1989; Boyer et al. 1997). Located in the south-central bay, Bob Allen Key (FCE-LTER station TSPh 10) is representative of the marine-influenced area. Bob Allen Key is a transitional area between the phosphorus-depleted eastern bay and the nitrogen-limited Gulf of Mexico and has patchy seagrass beds (Zieman et al. 1989;

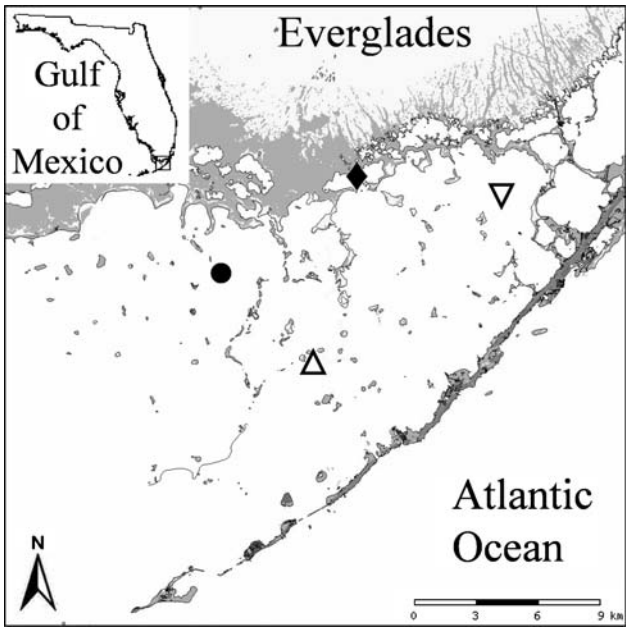

Fig. 1 Florida Bay map with sampling stations, Little Madeira Bay (*filled diamond*), Duck Key (*inverted open triangle*), Bob Allen Key (*open triangle*), Whirpay Basin (*filled circle*), indicated

Phlips et al. 1995; Boyer et al. 1999). Whipray Basin (Southeast Environmental Research Station 13) in north-central bay is representative of the core/interior bay with dense seagrass beds, patchy phytoplankton blooms, and elevated phosphorus concentrations (Zieman et al. 1989; Boyer et al. 1997; Phlips et al. 1999).

From January to December 2006, sampling stations were visited bi-monthly ($n = 6$) over a 1-week period. Overall water depth ranged from 0.6 to 2.1 m with an annual average depth of 0.8, 1.7, 1.5, and 1.3 m at Little Madeira Bay, Duck Key, Bob Allen Key, and Whipray Basin, respectively. During each sampling event, the pelagic zone was sampled on day 1, seagrass and its epiphytes on day 3, and the sediment surface on day 5.

For the pelagic zone, three replicate integrated water samples were collected using a modified three meter PVC pipe, which, in one pull, captured the complete water column vertically from the water surface to just above the seagrass canopy. Care was taken not to disturb the bottom or capture rooted seagrass leaves during water collection. Water samples were stored in 8-l amber polycarbonate or frosted Lowboy containers. Seagrass, *T. testudinum*, was collected by hand using snorkeling techniques. Whole seagrass short shoots (30–50) were collected randomly from ca. 50 m^2 around the research vessel. Short shoots were stored in frosted 4-l polycarbonate bottles filled with station water. Sediment samples were collected using a 5-ml syringe with the tip removed. At each station, 98 individual syringe cores were collected randomly from around the stern of the research vessel using snorkel techniques. From each core, the surface 1 cm (i.e., total volume of 1 ml) was retained in polyethylene scintillation vials or test tubes (depending on purpose). Samples were kept cool and dark and transported to the laboratory within 5–8 h after collection. In the laboratory, samples were further processed for analysis of total (TC) and organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), extracellular enzyme activity, bacterial abundance (BAC), and bacterial production (BP).

Laboratory sample processing and preparation

Sterile-filtered station water was prepared for subsequent epiphyte and sediment incubations. One liter of ambient water from each station was filtered through 0.2-μm 47-mm diameter polycarbonate filters. The filtrate was microwave-sterilized (high power until boil) and the volume adjusted with deionized water to correct for evaporation. Sterile-filtered sample water was stored in the dark at 4°C until 2 h before use when the water was warmed to room temperature in the dark.

Thalassia testudinum short shoot morphometrics (length, width, and number of leaves) were determined by hand. Only green leaves were used in this study; brown and black, decomposed leaves were discarded. For CNP and BAC analysis, seagrass short shoots were scraped with a razor blade to remove the epiphytes. For CNP analysis, *T. testudinum* and epiphyte samples were frozen separately, freeze-dried, and then stored at −20°C for later analysis. For BAC, the epiphytes were collected, fixed with ca. 3.7% v/v final concentration (f.c.) formaldehyde, flash frozen in liquid nitrogen, and stored at −20°C for later direct counts. In preparation for BP and extracellular enzyme analysis, an epiphyte mix was created, where seagrass leaves with the epiphytes attached were cut into 2 cm length pieces and individual pieces were placed in vials containing sterile-filtered station water.

For CNP and BAC, 5 ml of pooled and 1 ml of surface sediment was used, respectively, and processed like the epiphytic samples (see above). In preparation for BP and extracellular enzyme analysis, sediment slurries were created by adding 1 ml of sediment surface to 1 ml of sterile-filtered station water.

Analytic methods

Two replicate unfiltered water samples were analyzed for pelagic TOC and TN using a Shimadzu TOC-5000 and Shimadzu TOC-V/TNM-1 organic carbon/nitrogen analyzer, respectively. Two replicate cleaned seagrass, epiphyte, and sediment surface samples were analyzed separately for TC and TN using a CHN Fisons NA1500 analyzer. For sediment samples, TOC was determined by ashing in an oven at

500°C for 4 h. Ash was then measured for TC content and sediment TOC calculated by weighted difference between ash and sediment TC. TOC could not be determined for epiphytes due to sample size limitations. TP from replicate pelagic, seagrass, epiphytic, and sediment samples was determined by colorimetric analysis after sample dry ashing and acid hydrolysis (Fourqurean et al. 1992b; Boyer et al. 1999).

The long-term average (3–5 year) *T. testudinum* short shoot densities at Little Madeira Bay (486 SS m^{-2}), Duck Key (592 SS m^{-2}), and Bob Allen Key (295 SS m^{-2}; J. W. Fourqurean, unpublished data) and the median density (750 SS m^{-2}) reported by Hall et al. (1999) were used to convert epiphytic and seagrass measurements to areal concentrations, abundances, or rates. Pelagic variables were converted from volumetric to areal units using the station water depth measured during each sampling event.

Extracellular enzyme activities for alkaline phosphatase (APA), aminopeptidase (AMA), esterase, α-glucosidase and β-glucosidase were determined using fluorogenic model substrate analogs corresponding to classes of chemical bonds found in natural substrates (Hoppe 1993; Williams and Jochem 2006). For the pelagic community, triplicate dark incubations (45–180 min) at 21°C were conducted in 96-well plates with 180 µl of unfiltered station water and 20 µl of model substrate. For each replicate, four model substrate concentrations of 1–200 µM f.c. (depending on substrate) were used to create a unique saturation curve. Fluorescence readings were recorded with a BioTek FLx800TB computer-controlled plate reader at T_0 and T_{final} and converted to activity rates using fluorescence end-product standard curves at the corresponding instrument settings. Maximum hydrolytic enzyme activity, V_{max}, was estimated by applying the resulting activities at each substrate concentration to the Michaelis–Menten kinetic model.

The pelagic enzyme method was modified to measure activity in the sediment and epiphytes, while still using the existing 96-well plate setup. For the epiphytes, triplicate epiphyte mixes were spiked with model substrate to the pelagic f.c., which produced 12 discrete incubations per station per enzyme. After model substrate additions, incubations were immediately vortexed and 200 µl from each were pipetted rapidly from the incubations into the 96-welled plate. Once all replicates were transferred, the plate was measured as above and this measurement was used as T_0. Epiphyte mixes were then allowed to incubate in the dark on a mixing table for 45–180 min. (depending on substrate). At T_{final}, 200 µl were again removed from each mix, transferred to the 96-welled plate, and measured. V_{max} was determined as above after activities were corrected for incubation volume. The sediment slurry was treated similar to the epiphytes with one addition. Prior to collecting the 200 µl measurement subsamples, sediment slurries were centrifuged for 2 min at 906–1,698 g, depending on sample location in rotor, to prevent particles from masking fluorescence.

For comparison of zones and other variables, extracellular enzyme activities (µmol product m^{-2} day^{-1}) were converted to mg CNP (depending on enzyme) m^{-2} day^{-1} using the non-fluorescent hydrolytic product of each fluorogenic model substrate. Model substrates were 3-*o*-methylfluorescein phosphate, L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferon (MUF) heptanoate, and 4-MUF-α- and β-D-glucopyranoside for APA, AMA, esterase, and α- and β-glucosidase, respectively. In addition, activities for substrates that contained carbon as a product (i.e., esterase, aminopeptidase, and α- and β-glucosidase) were summed to provide an estimate of maximum total Carbon HydrolyASE activity (CHASE; mg C m^{-2} day^{-1}). This might underestimate the potential total carbon gain from extracellular enzymes because APA was not included in CHASE. In nature, APA can provide carbon in addition to phosphorus for bacteria use (Hoppe et al. 2002; Williams and Jochem 2006), but hydrolysis of 3-*o*-methylfluorescein phosphate does not produce a usable product containing carbon.

Chlorophyll *a* (phytoplankton biomass; mg Chl m^{-2}) was measured in pelagic samples only from particles collected on GF/F filters from 0.05–1.0 l integrated water samples. Filters were homogenized in ice-cold 90% acetone, allowed to extract at −20°C for 2 h, and measured using a spectrophotometrically calibrated Turner 111 fluorometer.

Pelagic bacteria were counted in triplicate on a FACSort flow cytometer after staining with SYBR Green I and pretreatment with RNAse (Marie et al. 1997; Jochem 2001). Epiphytic and sediment BAC were determined in triplicate by direct counts (Schallenberg et al. 1989). Samples were treated with pyrophosphate, sonicated, and diluted from 1:20 to 1:1,000, depending on bacterial abundance and masking. Subsamples from well-mixed slurry were stained with DAPI, filtered onto black 0.2-µm polycarbonate filters, and bacteria enumerated on a Zeiss Axioskop epifluorescence microscope (Yoon and Rosson 1990; Kirchman 1993).

BP was measured by ^{3}H-leucine incorporation. For pelagic bacteria, triplicate 10-ml samples and one formaldehyde-fixed control were incubated with 90.9-nM ^{3}H-leucine for 2 h at 21°C. Incubations were stopped by ca. 1% f.c. formaldehyde addition, filtered onto 0.2-µm polycarbonate filters, and rinsed three times with 2 ml of ice-cold 5% trichloroacetic acid (TCA, Kirchman 2001). Epiphyte mixes and sediment slurries were processed following a modification of Thomaz and Wetzel (1995) and Thomaz and Esteves (1997). Triplicate live and duplicate ca. 3.7% f.c. formaldehyde-fixed samples were incubated with 400-nM ^{3}H-leucine for 1 h. Incubations were stopped by adding

TCA to 5% f.c. and heating for 30 min at 95°C. Incubation contents and leaf material (epiphytes) or well-mixed subsamples (sediment) were filtered onto 0.2-μm polycarbonate filters and rinsed three times with 2 ml of ice-cold 5% TCA. All samples were analyzed by liquid scintillation counting (Kirchman 2001).

Bacterial carbon budget

A simplified bacterial carbon budget was created for pelagic, epiphytic, and sediment surface bacterial communities using BP and CHASE data (Table 2) and previous estimates of *T. testudinum* and its epiphytes productivity and photosynthetic carbon exudation rates (Jones 1968; Brylinsky 1977; Zieman et al. 1989). The purpose of the bacterial carbon budget was to estimate the amount of daily bacterial carbon demand in each zone that could be supported by CHASE and *T. testudinum* photosynthetic carbon exudation. In addition, the amount of daily *T. testudinum* and *T. testudinum* epiphytic production needed to fulfill bacterial carbon demand was estimated. Bacterial carbon demand was calculated from BP using low (10%) and high (50%) estimates of bacterial growth efficiency (BGE), which fit within the range observed in aquatic ecosystems (4–55%; Biddanda et al. 1994, 2001). Unfortunately, phytoplankton and microphytobenthos productivity have not been measured in Florida Bay. Benthic macroalgae were not considered because macroalgal production represents an insignificant amount of total autotrophic production in eastern and central Florida Bay (L. Collado-Vides and J. W. Fourqurean, unpublished data).

Previous measurements of productivity were used to construct the carbon budget and assumed to be representative of the metabolic activity of current primary producers. *T. testudinum* production rates measured by Zieman et al. (1989) are similar to more recent estimates of *T. testudinum* production based on the leaf mark method (J. W. Fourqurean, unpublished data). Due to methodological differences, epiphyte production rates reported by Jones (1968) could not be directly related to more recent epiphyte production rates determined by Frankovich and Zieman (1994). Given these uncertainties, primary production and exudation rates were modified to reflect current biomass/density of seagrass and epiphytes in Florida Bay.

Using ^{14}C uptake measurements, *T. testudinum* leaf productivity was 51 ± 1 mg C (g dw)$^{-1}$ day^{-1} (mean ± SE) across Florida Bay (Zieman et al. 1989). For the carbon budget, areal leaf productivity was back-calculated using the current station-specific leaf gram dry weight per short shoot (data not shown) and the seagrass short shoot areal density (SS m^{-2}; see Sect. "Analytic methods"). Net photosynthetic carbon exudation rates for *T. testudinum* leaves and its epiphytes in the lower Florida Keys were 19.3 and 6.1 μg C (g dw)$^{-1}$ day^{-1} under light and dark incubations, respectively (Brylinsky 1977). These rates represented the net release of dissolved organic carbon from seagrass and it epiphytes into the water column and, thus, may underestimate the total carbon exuded from seagrass to epiphytic bacteria. Carbon exudation rates were converted to daily rates assuming a 12:12 light:dark cycle and converted to areal rates as above. Using an O_2 method, *T. testudinum* epiphyte productivity averaged 1.5 and 0.8 g C m^2 day^{-1} during the summer and winter, respectively, across coastal areas of south Florida (Biscayne Bay, the Florida Keys, and Florida Bay; Jones 1968). These productivities represented 33–42% of gross *T. testudinum* leaf production. Areal epiphytic productivities were scaled to reflect the areal rates of *T. testudinum* leaf productivity modified from Zieman et al. (1989). These modifications adjusted previous primary productivity and carbon exudation estimates to the same areal density used to convert epiphytic bacterial production and CHASE activity and allowed seagrass and epiphytic production rates to be compared on the same relative scale with bacterial carbon demand (mg C m^{-2} day^{-1}; Table 3).

Statistical analysis

Multivariate analyses were used to determine benthic–pelagic linkages and spatial relationships for total carbon, nitrogen, and phosphorus content, bacterial abundance and production, and alkaline phosphatase, aminopeptidase, and carbon hydrolase activity. Pelagic, sediment surface, and epiphytic variables were treated as independent. To allow comparison, data were Z-score transformed prior to analysis. Analysis of similarity (ANOSIM) determined dissimilarity between zones and stations. Multidimensional scaling (MDS) visualized the similarity matrix. Principle components analysis (PCA) was used following the procedure of Boyer et al. (1997) with VARIMAX rotation of the PCA solution. *T. testudinum* leaf nutrient content and phytoplankton biomass were not included in PCA and MDS analyses. Instead, linear regression was used to find relationships between principle component (PC) scores or MDS axes and *T. testudinum* leaf nutrient content and log-transformed phytoplankton biomass.

Results

Variable description

Epiphytic TC (range; 0.6–8.7 g C m^{-2}), TN (0.02–0.99 g N m^{-2}), and TP (0.5–43.5 mg P m^{-2}) concentrations were consistently lower than in the other zones (Table 1). Pelagic and *T. testudinum* TOC or TC, TN, and TP concentrations were similar and ranged from 7.2 to 26.7 g C m^{-2}, 0.29 to

Table 1 Range and median value ($n = 6$) of total organic carbon (TOC), nitrogen (TN), and phosphorus (TP) and molar TN:TP ratio for estuarine zones

	TOC[a] (g C m^{-2})		TN (g N m^{-2})		TP (mg P m^{-2})		TN:TP	
	Range	Median	Range	Median	Range	Median	Range	Median
Pelagic								
Little Madeira Bay	7.3–11.6	10.1	0.29–0.46	0.38	11.7–26.7	18.7	48–86	72
Duck Key	11.9–21.4	17.6	0.66–0.97	0.82	25.0–61.9	37.1	43–111	65
Bob Allen Key	17.1–26.7	18.0	0.60–1.14	0.78	32.6–49.7	39.3	37–74	60
Whipray Basin	8.5–21.7	11.0	0.46–1.10	0.55	27.0–46.1	32.1	33–59	42
Epiphytic								
Little Madeira Bay	0.6–3.5	1.0	0.02–0.12	0.06	0.5–3.2	1.7	62–141	93
Duck Key	0.8–3.9	1.9	0.04–0.19	0.07	0.8–4.5	1.3	60–157	118
Bob Allen Key	0.6–2.8	1.7	0.05–0.12	0.08	0.8–4.7	2.0	35–133	108
Whipray Basin	2.0–8.7	3.2	0.10–0.99	0.30	4.0–43.5	14.3	45–58	49
Sediment								
Little Madeira Bay	216–311	240	12.6–16.6	14.3	407–570	467	65–69	68
Duck Key	111–230	171	9.4–14.4	12.8	212–418	349	73–98	82
Bob Allen Key	148–241	210	13.0–17.4	15.6	287–475	369	81–105	94
Whipray Basin	145–321	199	11.8–27.2	16.0	651–1623	1033	28–41	39
Thalassia testudinum								
Little Madeira Bay	7.2–22.9	9.2	0.44–1.49	0.64	13.3–41.5	17.5	63–84	78
Duck Key	8.0–14.3	10.2	0.55–0.83	0.68	12.7–28.7	17.8	67–116	90
Bob Allen Key	7.5–16.0	8.9	0.55–1.14	0.68	13.9–43.9	17.3	67–104	80
Whipray Basin	24.5–93.3	45.8	1.84–6.54	3.50	74–276	125.7	48–86	72

Median values are reported because data were not normally distributed

[a] Epiphytic and *T. testudinum* reported as total carbon (TC); sample size limitations did not permit TOC measurement

1.49 g N m^{-2}, and 11.7 to 61.9 mg P m^{-2}, respectively. *T. testudinum* areal concentrations at Whipray Basin were not included in above ranges because TC (24.5–93.3 g C m^{-2}), TN (1.84–6.54 g N m^{-2}), and TP (74–276 mg P m^{-2}) concentrations were much higher than at the other stations. Sediment surface TOC, TN, and TP concentrations were higher than in the other zones and ranged from 111 to 321 g C m^{-2}, 9.4 to 27.2 g N m^{-2}, and 651 to 1,623 mg P m^{-2}, respectively. Molar TN:TP ratios were similar among pelagic, epiphytes, sediment, and *T. testudinum* (28–157; Table 1).

Across zones, combined carbon hydrolase activity for esterase, peptidase, and α- and β-glucosidase (CHASE) was controlled by esterase and peptidase activities. α- and β-glucosidase activities were commonly 2–3 orders of magnitude lower than esterase and peptidase activities. Epiphytic and sediment surface glucosidase activities were significantly lower than pelagic activities (data not shown). Extracellular enzyme activities for the epiphytes were lower than pelagic and sediment activities (Table 2). Epiphytic CHASE, AMA, and APA ranged from 0.4 to 3.7 mg C m^{-2} day^{-1}, 0.01 to 0.62 mg N m^{-2} day^{-1}, and 1.1 to 5.2 mg P m^{-2} day^{-1}, respectively. Pelagic CHASE (113–1,671 mg C m^{-2} day^{-1}) and AMA (8–284 mg N m^{-2} day^{-1}) were higher than sediment CHASE (28.0–61.5 mg C m^{-2} day^{-1}) and AMA (0.7–3.3 mg N m^{-2} day^{-1}). APA was similar between pelagic and sediment zones and ranged from 51 to 710 mg P m^{-2} day^{-1}.

Phytoplankton biomass ranged from 0.3 to 31.5 mg Chl m^{-2} with a median concentration of 1.1 mg Chl m^{-2} across Florida Bay and did not follow a normal distribution (data not shown). Phytoplankton biomass was consistently low at Little Madeira Bay and at the other sampling stations during more typical non-bloom conditions, ranging from 0.3 to 2.1 mg Chl m^{-2}. Five phytoplankton blooms occurred over the study period. Bloom biomass ranged from 8.9 mg Chl m^{-2} at Duck Key to 31.5 mg Chl m^{-2} at Whipray Basin.

BAC was typically highest in the sediment (7.8–25.9 × 10^{12} cells m^{-2}), intermediate in the water column (0.8–21.8 × 10^{12} cells m^{-2}), and two orders of magnitude lower in the epiphytes (1.2–23.2 × 10^{10} cells m^{-2}; note units are different; Table 2). Abundances were similar to reported BAC in multiple seagrass ecosystems (Kirchman et al. 1984; Pollard and Kogure 1993; Törnblom and Søndergaard 1999). BP was more variable and displayed different patterns. Pelagic BP was lower (1.6–85.6 mg C m^{-2} day^{-1}) than epiphytic and sediment BP, which were similar (8.0–235.1 mg C m^{-2} day^{-1}; Table 2).

Bacterial carbon budget

Pelagic bacteria could satisfy their carbon demand by relying solely on extracellular enzyme-hydrolysable organic substrates (Table 3). Sediment surface bacteria

Table 2 Range and median value ($n = 6$) for extracellular enzymes [sum of α- and β-glucosidase, esterase, and peptidase activities (CHASE), aminopeptidase activity (AMA), and alkaline phosphatase activity (APA)], bacterial abundance (BAC) and bacterial production (BP) for estuarine zones

	CHASE (mg C m^{-2} day^{-1})		AMA (mg N m^{-2} day^{-1})		APA (mg P m^{-2} day^{-1})		BAC ($\times 10^{a,b}$ cells m^{-2})		BP (mg C m^{-2} day^{-1})	
	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median
Pelagic										
Little Madeira Bay	113–295	176	8–44	14	51–98	63	1.1–2.0	1.3	1.6–4.8	3.3
Duck Key	176–476	245	8–67	22	76–239	87	1.2–5.6	2.1	3.8–12.8	5.4
Bob Allen Key	311–1671	436	25–284	47	124–710	198	1.7–10.4	2.5	6.4–62.2	8.9
Whipray Basin	245–739	324	14–122	37	52–544	105	0.8–21.8	4.1	4.6–85.6	12.9
Epiphytic										
Little Madeira Bay	0.7–1.3	0.9	0.02–0.08	0.05	1.7–2.8	2.3	1.4–4.6	2.3	22.9–44.9	35.9
Duck Key	0.9–1.7	1.2	0.03–0.18	0.09	3.9–5.2	4.2	2.0–4.8	2.2	34.6–80.8	44.1
Bob Allen Key	0.4–0.9	0.4	0.01–0.10	0.02	1.1–1.6	1.2	1.2–5.1	2.2	8.0–61.0	13.9
Whipray Basin	0.9–3.7	1.8	0.06–0.62	0.09	1.7–2.8	2.6	6.6–23.2	12.1	48.3–235.1	101.2
Sediment										
Little Madeira Bay	35.7–43.0	38.9	0.7–1.6	0.9	91–150	126	12.8–25.9	16.3	11.5–31.6	25.3
Duck Key	28.0–34.5	31.3	0.9–2.0	1.6	77–110	83	8.1–20.6	15	44.4–123.8	60.7
Bob Allen Key	37.2–54.5	41.3	1.6–2.9	2.2	89–138	100	7.8–16.3	11.3	51.2–233.2	76.1
Whipray Basin	41.9–61.5	61.5	1.9–3.3	2.0	137–224	191	12.0–24.4	19.1	14.7–173.3	46.8

Median values are reported because data were not normally distributed

[a] Pelagic and benthic bacterial abundance as $\times 10^{12}$ cells m^{-2}

[b] Epiphytic bacterial abundance as $\times 10^{10}$ cells m^{-2}

Table 3 Bacterial carbon budget calculated at 10 and 50% bacterial growth efficiency (BGE)

Bacterial zone	Percentage of bacterial carbon demand fulfilled by				Percentage of PP needed to fulfill bacterial carbon demand			
	CHASE		EOC[b]		*T. testudinum*[c]		Epiphyte[d]	
	10% BGE[a]	50% BGE	10% BGE	50% BGE	10% BGE	50% BGE	10% BGE	50% BGE
Pelagic	>100	>100	26 ± 5	>100	6.2 ± 1.9	1.2 ± 0.4	17 ± 5	3.3 ± 1.0
Epiphytic	0.4 ± 0.1	1.8 ± 0.3	3.8 ± 0.6	19 ± 3	23 ± 3	4.6 ± 0.6	51 ± 8	10 ± 2
Sediment	14 ± 4	69 ± 18	5.3 ± 1.7	27 ± 9	38 ± 10	7.7 ± 1.9	86 ± 23	17 ± 5

Mean (%) and standard error ($n = 6$) at each BGE are estimated for % of bacterial carbon demand fulfilled by extracellular carbon hydrolytic enzyme activity (CHASE) and extracellular organic carbon exudation (EOC) from *Thalassia testudinum* and its epiphytes. In addition, the % of *T. testudinum* and the % of seagrass epiphytic primary production (PP) needed to fulfill bacterial carbon demand was estimated

[a] 10 and 50% were used as estimates of low and high growth efficiency, respectively (Biddanda et al. 1994, 2001)

[b] EOC rate obtained from Brylinsky (1977) and scaled to current *T. testudinum* density used for areal calculations in this study

[c] *Thalassia testudinum* primary production rate obtained from Zieman et al. (1989) and scaled to current *T. testudinum* density

[d] *Thalassia testudinum* epiphytic production rate obtained from Jones (1968) and scaled to current *T. testudinum* density

required a mixture of small (<600 Da) and enzyme-hydrolysable substrates, while CHASE activities suggested that epiphytic bacteria relied almost exclusively on carbon sources that did not require extracellular hydrolysis. Carbon exudates from *T. testudinum* and its epiphytes accounted for 25 to >100% of pelagic bacteria carbon demand. However, 50–90% of *T. testudinum* exudates that reach the water column would likely require extracellular enzyme hydrolysis before bacterial uptake (Wetzel and Penhale 1979). In Florida Bay, seagrass and its epiphytes' exudates could only account for 3.8–27% of bacterial carbon demand at 50 and 10% BGE in the sediment and seagrass epiphytes. Across zones at 50 and 10% BGE, 1.2–38% of *T. testudinum* production and 3.3–86% of seagrass epiphyte production was needed to fulfill bacterial carbon demand.

Multivariate analyses

All zones were significantly dissimilar from one another (ANOSIM, Global $R = 0.65$, $P = 0.001$). Pairwise comparison suggested that epiphytic and pelagic zones were more similar to each other than to the sediment surface. The combination of distinctly different extracellular enzyme activities, total carbon and nutrient concentrations, and bacterial abundances in pelagic, epiphytic, and sediment zones drove these dissimilarities. All stations were significantly dissimilar to each other (ANOSIM, Global $R = 0.54$, $P = 0.001$; Fig. 2), which was not unexpected, given that each station was selected based on prior known differences.

Principle components analysis produced six PCs, which explained 87.3% of the variance in the original data with *T. testudinum* nutrient content and phytoplankton biomass excluded. The PCs formed two benthic–pelagic ($PC_{I,VI}$), two benthic ($PC_{II,IV}$), one pelagic (PC_{III}), and one sediment (PC_V) groups, where benthic–pelagic and benthic PCs explained 35.4 and 33.6%, respectively, of the combined variance (See Table 4 for specific variables included in each PC).

Regression analysis was used to determine the relationships between PCs (Table 4) or MDS axes (Fig. 2) and *T. testudinum* leaf nutrient content and phytoplankton biomass. *T. testudinum* leaf TP was significantly related to leaf TN and TC (data not shown) and, thus, leaf TP and TN:TP ratios were used in this analysis. In Florida Bay, *T. testudinum* leaf TP content is a good indicator of spatial changes in P availability and seagrass productivity (Fourqurean et al. 1992a, b). *T. testudinum* leaf TN:TP ratios explained 28% of the variation on the MDS *x*-axis ($F_{1,22} = 8.6$, $P = 0.008$) but did not relate to PCs. Areal leaf TP concentration explained 49% of the variation on the MDS *x*-axis ($F_{1,22} = 20.8$, $P < 0.001$), indicating that the MDS *x*-axis represented spatial patterns in phosphorus availability (Fig. 2). Areal leaf TP concentration explained 77% of the variation in PC_{II} ($F_{1,22} = 72.2$, $P < 0.001$). These results suggest that seagrass and epiphytic carbon and nutrient pools are linked and that seagrass contribute organic matter for extracellular carbon hydrolysis in the sediment surface. However, pelagic and sediment surface nutrient pools, BP, and BAC and pelagic extracellular enzyme activity (i.e., PC_I, PC_{III}, and PC_V) were independent of seagrass nutrient content.

Phytoplankton biomass (mg Chl m^{-2}) was related significantly to the MDS *y*-axis ($R^2 = 0.74$, $F_{1,22} = 63.0$, $P < 0.001$; Fig. 2). Phytoplankton biomass was related positively to PC_I ($R^2 = 0.40$, $F_{1,22} = 14.4$, $P < 0.001$). This relationship suggests that phytoplankton influenced significantly extracellular enzyme activity in the water column, but phytoplankton were also associated with, possibly through sedimentation of phytoplankton detritus (i.e., Evrard et al. 2005), enhanced bacterial production and organic nitrogen cycling in the sediment surface. In addition, phytoplankton biomass was related significantly to PC_{III} ($R^2 = 0.34$, $F_{1,22} = 11.4$, $P = 0.003$), suggesting coupling between pelagic bacteria and phytoplankton and a positive association with water column nutrients and TOC. Phytoplankton biomass was unrelated to seagrass epiphyte (PC_{II}) and sediment nutrient content (PC_V).

Discussion

Water quality, sediment, seagrass, and seagrass epiphyte characteristics have been studied for nearly two decades in Florida Bay. As expected, Little Madeira Bay, Duck Key, Bob Allen Key, and Whipray Basin grouped independently of each other based on microbial pelagic, epiphytic, and sediment surface processes (Fig. 2). The ecosystem drivers behind this spatial patterns have been discussed previously

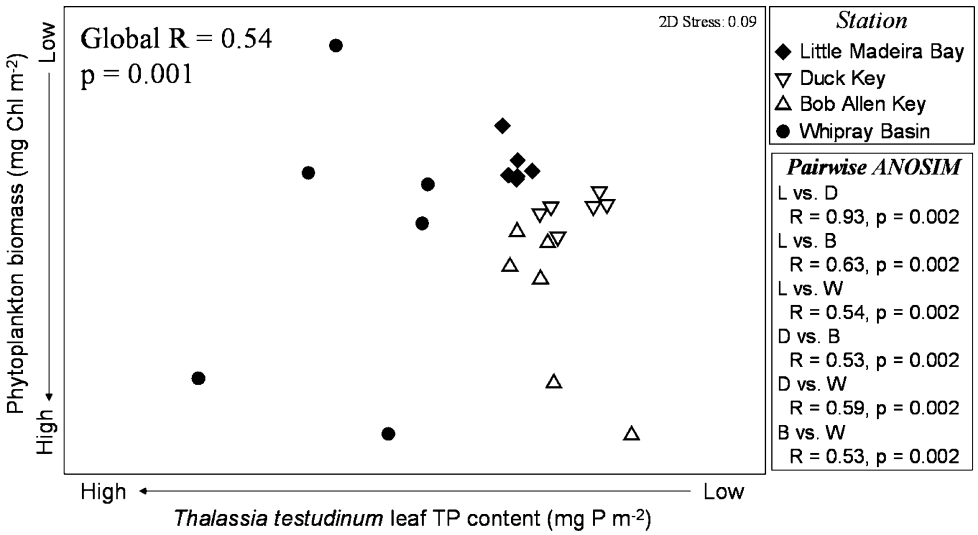

Fig. 2 Multidimensional scaling (MDS) plot of zone specific variables (see Table 4) within each station for bi-monthly replicates ($n = 6$). Pairwise analysis of similarity (ANOSIM) *R* and *P* values indicate which stations differed significantly. The MDS *x*-axis generally reflected *Thalassia testudinum* leaf phosphorus content ($R^2 = 0.49$, $F_{1,22} = 8.6$, $P = 0.008$) while the *y*-axis related positively with phytoplankton biomass ($R^2 = 0.74$, $F_{1,22} = 63.0$, $P < 0.001$)

Table 4 Results of principle component (PC) analysis that included pelagic, epiphytic, and sediment total organic carbon (TOC) or total carbon (TC), total nitrogen (TN), total phosphorus (TP), carbon hydrolase activity (CHASE), aminopeptidase activity (AMA), alkaline phosphatase activity (APA), bacterial abundance (BAC), and bacterial production (BP) as independent variables grouped by stations

	PC_I	PC_{II}	PC_{III}	PC_{IV}	PC_V	PC_{VI}
Pelagic						
TOC	0.29	−0.18	**0.87**	−0.12	0.03	−0.14
TN	0.14	−0.12	**0.88**	−0.04	−0.12	0.03
TP	0.16	0.02	**0.69**	0.27	−0.40	−0.14
CHASE	**0.87**	−0.01	0.34	0.05	−0.04	0.00
AMA	**0.86**	−0.03	0.32	0.06	0.02	0.03
APA	**0.79**	−0.04	**0.50**	−0.03	0.02	0.19
BAC	0.24	−0.01	**0.63**	0.10	0.29	**0.65**
BP	0.43	0.00	**0.63**	0.11	0.27	**0.52**
Epiphytic						
TC	−0.01	**0.91**	−0.17	0.15	−0.12	0.04
TN	−0.05	**0.96**	0.00	0.08	0.05	0.13
TP	0.00	**0.96**	0.00	0.12	0.07	0.16
CHASE	−0.22	0.15	−0.06	**0.90**	0.13	0.21
AMA	0.00	0.04	0.07	**0.95**	0.21	0.12
APA	**−0.73**	−0.04	0.21	0.28	−0.31	−0.22
BAC	0.14	0.40	−0.18	**0.69**	−0.14	**0.48**
BP	−0.09	0.41	−0.13	0.34	−0.42	**0.64**
Sediment						
TOC	0.11	−0.06	−0.16	0.21	**0.85**	0.00
TN	0.33	0.16	0.24	0.39	**0.68**	0.26
TP	0.07	0.35	0.12	**0.51**	**0.49**	**0.51**
CHASE	0.43	**0.60**	−0.17	0.28	0.26	0.26
AMA	**0.47**	0.31	0.33	**0.57**	0.00	−0.02
APA	0.13	0.29	−0.10	0.29	0.17	**0.83**
BAC	−0.42	0.45	−0.13	−0.03	**0.53**	0.11
BP	**0.65**	0.05	0.09	0.05	**−0.64**	−0.02
% Total variance	30.3	24.2	11.6	9.4	6.8	5.1

Correlations between the first six PC factor loadings are shown after VARIMAX rotation. Variables included in each PC are in bold-face type

in much detail with respect to Florida Bay and other aquatic systems (Fourqurean et al. 1992a, b; Frankovich and Zieman 1994; Boyer et al. 1997, 1999; Cotner et al. 2000; Williams and Jochem 2006). Rather than reinforcing existing principles, this discussion will focus on advances in the understanding of organic matter use and benthic–pelagic interactions in seagrass ecosystems. The results of bacterial carbon budget and multivariate analysis indicate: (1) pelagic bacteria production and organic matter use (enzyme hydrolysis) were linked to phytoplankton biomass, (2) pelagic carbon cycles were independent of benthic seagrass production (leaf nutrient content), (3) benthic algae/cyanobacteria (epiphytes and/or microphytobenthos) production was likely the primary source of carbon to benthic bacteria, and (4) benthic–pelagic nutrient cycles were connected.

To understand better the sources of organic matter for bacterial use, the benthic and pelagic autotrophic communities of Florida Bay are here defined. In 1984 and 1994 Florida Bay benthic surveys, *T. testudinum* was present at 93–95% of the 105 stations visited (Zieman et al. 1989; Hall et al. 1999). Two additional seagrass species, *Halodule wrightii* and *Syringodium filiforme*, are present in Florida Bay, but their bay-wide standing crop was one to two orders of magnitude lower than that of *T. testudinum* (Zieman et al. 1989; Hall et al. 1999). *T. testudinum* epiphytic algae are dominated by calcarious red algae and diatoms, and calcarious epiphyte production is an important source of carbonate to the sediment (Frankovich and Zieman 1994; Armitage et al. 2006). Benthic macroalgae are present mostly as calcareous green and drift red algae but are not abundant in eastern and central Florida Bay (L. Collado-Vides and J. W. Fourqurean, unpublished data). At Duck Key and Bob Allen Key, microphytobenthos biomass (benthic cholorophyll *a*) ranged from ca. 30 to 55 mg Chl m^{-2}, which was 6–10× higher than epiphytic chlorophyll *a* (ca. <5 mg Chl m^{-2}; Armitage et al. 2006). Heterotrophic bacteria are a relatively minor component of the overall epiphytic and sediment surface community biomass. In terms of biomass and autotrophy-associated organic matter, *T. testudinum* carbon biomass is greater than that of microphytobenthos, which is greater than that of seagrass epiphytes.

Phytoplankton biomass is dominated by pico- and nanoplankton and cyanobacteria are the main bloom-forming group in the bay (Phlips et al. 1995, 1999; Lavrentyev et al. 1998). In the current study, phytoplankton biomass ranged from 0.3 to 32.4 mg Chl m^{-2}. Much of the pelagic organic matter is not associated with living microbial (bacteria and algae) biomass but is detritus that originated from seagrass and algae (Maie et al. 2005). In coastal areas, the detritus pool is further augmented by periodic influx of organic matter from the Everglades. Phytoplankton biomass fluctuates greatly and is similar to microphytobenthos during bloom conditions, but lower than epiphytic biomass during non-bloom conditions.

Similar to other calcium carbonate-rich aquatic systems, Florida Bay is considered a phosphorus-limited ecosystem (Fourqurean et al. 1992a; Boyer et al. 1999), though nitrogen has been suggested to limit or co-limit ecosystem production in the central and western regions of the bay (Lavrentyev et al. 1998; Armitage et al. 2006). In the current study, the MDS *x*-axis of Fig. 2 generally followed geographic patterns in phosphorus concentration (Table 1; Figs. 1, 2; Armitage et al. 2006). Within benthic and pelagic zones, nutrients and organic matter are concentrated

in the sediment pore-water relative to the nutrient-poor overlying water column (Pollard and Moriarty 1991; Fourqurean et al. 1992b; Erftemeijer and Middelburg 1995; Table 1). These nutrient and organic matter gradients impose restrictions on bacterial production and organic matter use in seagrass ecosystems. In Florida Bay, phosphorus availability was suggested to limit microbial degradation of organic matter in the water column (Maie et al. 2005), which implies bacterial organic matter use should increase in the relatively phosphorus-rich sediment.

Extracellular enzyme activities differed between benthic and pelagic zones, though in an unexpected way. Enzyme activities tended toward low, intermediate, and high rates in epiphytic, sediment, and pelagic zones, respectively (Table 2). Pelagic activities in this study were similar to those reported previously for Florida Bay (Williams and Jochem 2006) and a broad range of oligo- to meso-trophic ecosystems (Hoppe et al. 2002 for review). Extracellular enzymes are measured less frequently in sediment and epiphytic zones, and direct comparison between studies is hampered by the absence of standardized units and methodologies. APA measured from *Zostera noltii*, which was scraped free of loosely attached epiphytes, was on average higher than activities reported here, but median values of epiphytic APA in Florida Bay were within the lower range of activities for *Z. noltii* (Hernández et al. 1994). Periphyton α-glucosidase activity on decomposing *Typha* litter was an order of magnitude higher than activities observed here for the seagrass epiphytes, but seagrass epiphytic β-glucosidase activity, AMA, and APA were within the range reported for *Typha* litter periphyton (Francoeur et al. 2006).

Florida Bay sediment surface α- and β-glucosidase activity and AMA were comparable to activities measured in beach sand of the NW Mediterranean (Misic and Fabiano 2005; Misic and Harriague 2007), but were an order of magnitude lower than sediment activities reported over a eutrophication gradient in the coastal Baltic Sea (Köster et al. 1997). In the surface 5 cm of sediment from a NW Mediterranean *Posidonia oceanica* bed, α-glucosidase activity and AMA were one to two orders of magnitude higher than activities recorded for Florida Bay sediments and more similar to pelagic activities in Florida Bay, though the relative difference between AMA and α-glucosidase activity remained similar to that found in Florida Bay and Mediterranean beach sand (Table 2; López et al. 1995; Misic and Fabiano 2005; Misic and Harriague 2007). However, sediment surface APA in Florida Bay was 2–100 times higher than APA reported in the above environments, concomitant with the predominant P limitation of Florida Bay, compared to predominantly N limitation in the other systems.

Benthic extracellular carbon hydrolytic enzyme activities were surprisingly low for an organic matter-rich seagrass ecosystem. Still, these observations corroborate previous studies that observed consistent slow rates of seagrass decomposition (Harrison 1989 for review) but deviate from expectations based on ecosystem organic matter and nutrient concentrations. Extracellular enzymes for carbon hydrolysis tend to increase in activity relative to sediment organic matter content (López et al. 1995), which was not observed in Florida Bay. Instead, CHASE in the sediment surface was positively related to *T. testudinum* leaf nutrient content. In all zones, extracellular enzymes that gained nitrogen and phosphorus (i.e., AMA and APA) showed high activities, suggesting that organic nutrients are cycled rapidly by bacteria, algae, and/or seagrass in Florida Bay.

Adding further complexity to the stark differences between benthic and pelagic organic matter use, bacteria production and carbon demand were highest in the epiphytes and sediment surface, where CHASE activity was lowest (Tables 2, 3). In multiple seagrass ecosystems, high BP rates are observed in the sediment and epiphytic zones while low to intermediate BP rates are found in the water column (Table 2; Moriarty et al. 1986, 1990; Pollard and Kogure 1993; Törnblom and Søndergaard 1999), though Moriarty and Pollard (1982) observed similar BP rates across benthic and pelagic zones. In the current study, benthic and pelagic BP was within the range reported for oligo- to meso-trophic seagrass ecosystems, but median production rates observed here were generally low for the sediment surface (Moriarty and Pollard 1982; Moriarty et al. 1986, 1990; Cotner et al. 2000). Given the low rates of benthic CHASE but high BP, epiphytic and sediment surface bacteria likely relied on labile photosynthetic carbon exudates from seagrass, epiphytes, and/or microphytobenthos to meet their carbon demand (Kirchman et al. 1984; Goto et al. 1999; Ziegler and Benner 1999a, b). CHASE activity was only sufficient to meet daily bacterial carbon demand in the pelagic zone (Table 3).

Across multiple seagrass beds, bacteria required 6–90% of daily seagrass production to fulfill their carbon demand at 50% BGE (Moriarty et al. 1986, 1990; Pollard and Kogure 1993). In Florida Bay, pelagic, epiphytic, and sediment surface bacterial carbon demands required <10% of *T. testudinum* daily primary production at 50% BGE (Table 3). In epiphyte-free *T. testudinum*, however, <5% of daily production was released as organic exudates by leaves into the water column, of which 40% could be used by bacteria without extracellular enzyme hydrolysis, and slightly more organic matter was released by root-rhizomes into the sediment (Wetzel and Penhale 1979). In Florida Bay, net carbon exudation by *T. testudinum* leaves was only sufficient to meet pelagic bacterial carbon demand at 50% BGE, supporting at most 19 ± 3 and 27 ± 9% of epiphytic and sediment surface bacterial carbon demand, respectively (Table 3). *T. testudinum* leaf

nutrient content, a corollary with seagrass primary production in Florida Bay (Fourqurean et al. 1992b), related positively with epiphytic nutrient content and sediment CHASE (PC_{II}, Table 4), suggesting that some detrital seagrass was utilized by the sediment surface bacteria and that seagrass and epiphytic nutrient and carbon stores were connected. Although the carbon budget indicated that seagrass production could satisfy pelagic bacteria carbon demand, pelagic organic matter concentrations, bacteria production, and extracellular enzyme activities were independent of *T. testudinum* leaf nutrient content but positively related to phytoplankton biomass.

In seagrass ecosystems, algae are increasingly recognized as important sources of carbon to benthic and pelagic bacteria. In a *Syringodium isoetifolium* bed, 4–6% of daily microalgal primary production was needed to meet bacterial carbon demand in the sediment surface (Pollard and Kogure 1993). In Florida Bay, each bacterial zone required <20% of *T. testudinum* epiphytic daily primary production to meet their carbon demand at a 50% BGE (Table 3). In general, phytoplankton and microphytobenthos release from 1.5 to 22% and 42 to 72%, respectively, of daily primary production as extracellular organic carbon, where an average of $3.4 \pm 1.1\%$ of phytoplankton and $\leq 12\%$ of microphytobenthos exudates were in a dissolved, non-colloidal form (Goto et al. 1999). In Florida Bay, seagrass epiphytic organic exudation rates are likely similar to microphytobenthos exudation rates, given the importance of diatoms in both algal communities. Under this assumption, *T. testudinum* epiphytic organic exudation could account for epiphytic bacterial carbon demand. Similarly, given that microphytobenthos biomass is up to ten times higher than epiphytic algal biomass (Armitage et al. 2006), organic carbon exudation from microphytobenthos could account for sediment surface bacterial carbon demand. Although speculative, the distribution of CHASE activity across zones is consistent with this interpretation. High CHASE activity occurs in the pelagic zone, where phytoplankton release a low percentage of dissolved organic matter directly, while low CHASE activity occurs in the benthic zones, where benthic algae release a relatively higher amount of dissolved organic carbon.

Linear regression analysis between phytoplankton biomass and PC scores indicated strong coupling between phytoplankton and pelagic bacteria (PC_I and PC_{III}; Table 4). In addition, the relationship between PC_I and phytoplankton suggests a link between benthic nitrogen cycles and phytoplankton. Phytoplankton biomass correlated positively with AMA and BP in the sediment surface. This relationship might be indicative of nitrogen remineralization through aminopeptidase by sediment bacteria from phytoplankton detritus (Evrard et al. 2005). Similarly, increases in sediment surface TP and APA were positively associated with epiphytic and pelagic BAC and BP (PC_{VI}). In combination with the relationship between seagrass and epiphytic nutrient content, these results suggest that nutrients are cycled between benthic and pelagic zones in Florida Bay, which in turn are associated with enhanced bacterial production in each zone. Benthic and pelagic carbon cycles, however, were not linked strongly in PCA. Pelagic bacteria responded to changes in phytoplankton, but benthic organic matter content and use did not influence pelagic carbon cycling (i.e., extracellular enzyme activity and bacterial production).

Although multiple forms of organic matter are available in seagrass ecosystems, this study suggests that bacteria select organic matter that is readily usable and more similar to themselves in terms of carbon, nitrogen, and phosphorus content. Pelagic bacteria were tightly coupled to phytoplankton biomass and spent the most extracellular enzyme effort to meet their carbon demand. Epiphytic bacteria exerted little CHASE effort, but seemed to benefit from their direct association with seagrass and its epiphytic autotrophs. These bacteria supported high production rates without the metabolic cost of synthesizing large quantities of extracellular enzymes. Sediment surface bacteria could supply about half of their carbon demand through the use of extracellular carbon hydrolytic enzymes but also required readily usable seagrass and microalgal photosynthetic exudates to fully meet their carbon demand. We hypothesize that seagrass-derived organic matter is of secondary importance in Florida Bay and that bacteria rely primarily on algal/cyanobacterial production. Pelagic bacteria seemed coupled to phytoplankton while benthic bacteria were supported primarily by epiphytic and/or microphytobenthic primary production.

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