

The role of dissolved organic matter bioavailability in promoting phytoplankton blooms in Florida Bay

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Abstract

The clear, shallow, oligotrophic waters of Florida Bay are characterized by low phytoplankton biomass, yet periodic cyanobacteria and diatom blooms do occur. We hypothesized that allochthonous dissolved organic matter (DOM) was providing a subsidy to the system in the form of bound nutrients. Water from four bay sites was incubated under natural light and dark conditions with enrichments of either DOM (> 1 kD, 2×DOM) or inorganic nutrients (N+P). Samples were analyzed for bacterial numbers, bacterial production, phytoplankton biomass, phytoplankton community structure, and production, nutrients, and alkaline phosphatase (AP) activity. The influence of 2×DOM enrichment on phytoplankton biomass developed slowly during the incubations and was relatively small compared to nutrient additions. Inorganic nutrient additions resulted in an ephemeral bloom characterized initially as cyanobacterial and brown algae but which changed to dinoflagellate and/or brown algae by day six. The DIN:TP ratio decreased 10-fold in the N+P treatments as the system progressed towards N limitation. This ratio did not change significantly for 2×DOM treatments. In addition, these experiments indicated that both autotrophic and heterotrophic microbial populations in Florida Bay may fluctuate in their limitation by organic and inorganic nutrient availability. Both N+P and 2×DOM enrichments revealed significant and positive response in bioavailability of dissolved organic carbon (BDOC). Potential BDOC ranged from 1.1 to 35.5%, with the most labile forms occurring in Whipray Basin. BDOC at all sites was stimulated by the 2×DOM addition. Except for Duck Key, BDOC at all sites was also stimulated by the addition of N+P. BDOC was lower in the dry season than in the wet season (5.56% vs. 16.86%). This may be explained by the distinct chemical characteristics of the DOM produced at different times of year. Thus, both the heterotrophic and autotrophic microbial communities in Florida Bay are modulated by bioavailability of DOM. This has ramifications for the fate of DOM from the Everglades inputs, implicating DOM bioavailability as a contributing factor in regulating the onset, persistence, and composition of phytoplankton blooms.

Introduction

Florida Bay is a wedge-shaped lagoonal estuary separated from the Straits of Florida by the Florida Keys (Fig. 1). Carbonate mud banks compartmentalize Florida Bay into a network of shallow basins, restricting circulation amongst them. Tidal advection from the Gulf of Mexico is quickly attenuated by the western mud banks leaving most of central and northeastern Florida

Bay unaffected (Turney & Perkins, 1972; Holmquist et al., 1989). Freshwater inputs to Florida Bay are mostly from rainfall and flow from the southern Everglades through the Taylor Slough basin. Taylor Slough is significantly affected by water management activities, which can subsequently influence salinities in the eastern Bay (Boyer & Jones, 1999). Another potentially important source of freshwater to the Bay is overland flow along the north-central terrestrial

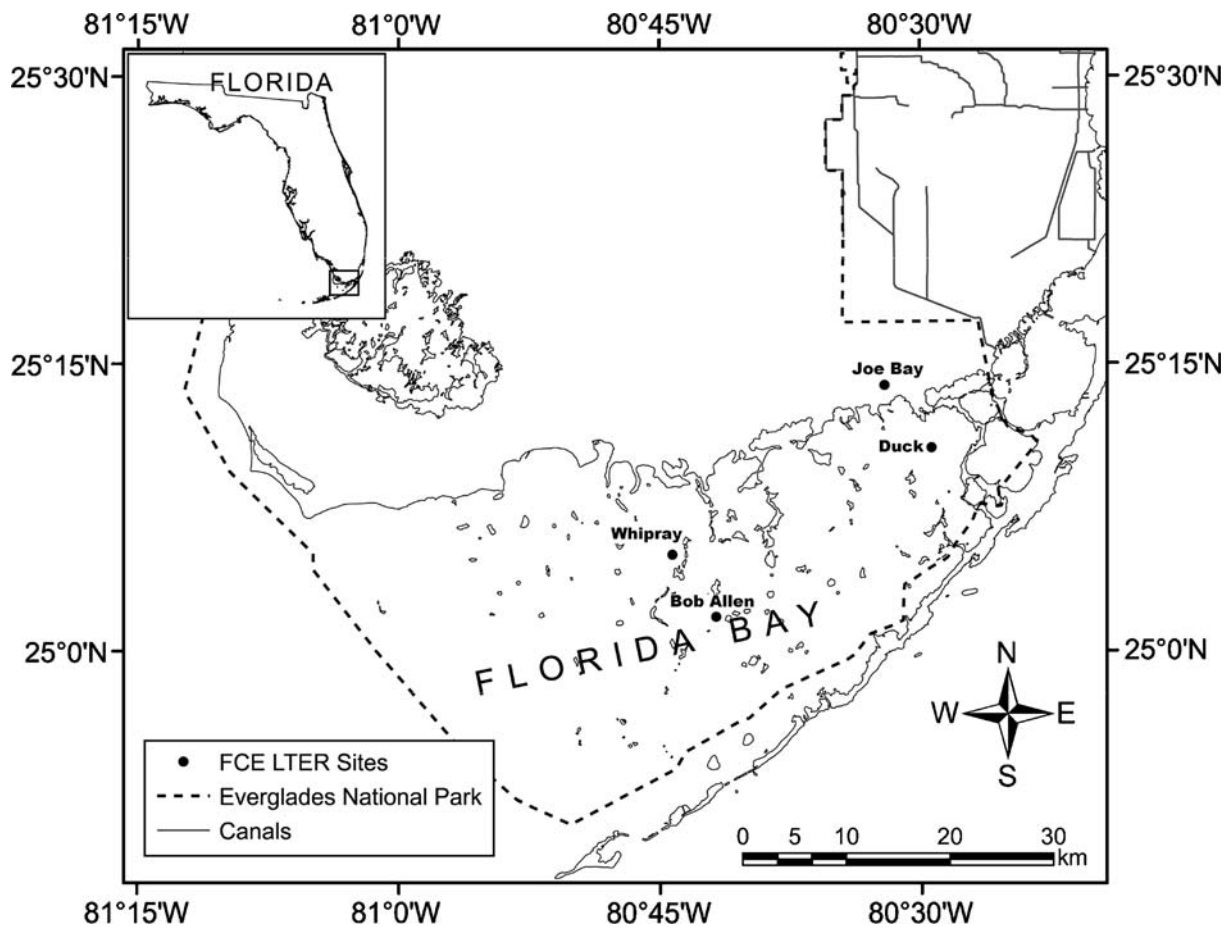


Figure 1. The four sites in Florida Bay which were selected for BDOC experiments: Joe Bay, Duck Key, Whipray Basin, and Bob Allen Key. Each site is representative of different water quality and hydrology conditions.

boundary, the Buttonwood Embankment, which is high in DOM (Fig. 2, Boyer, 2005).

Phytoplankton biomass in eastern Florida Bay is strongly limited by P (Fourqurean et al., 1993; Phlips & Badylak, 1996; Boyer et al., 1997; Lavrentyev et al., 1998), however other resources (e.g. light, N, Si) have been shown to partially control productivity in the central and western bay (Lavrentyev et al., 1998; Brand, 1999). Dissolved inorganic P concentrations (measured as soluble reactive P, SRP) are extremely low ($\sim 0.03 \mu\text{M}$; eastern bay), while dissolved inorganic nitrogen concentrations (DIN; mostly ammonium) can reach $100 \mu\text{M}$ in the central bay (Boyer et al., 1997, 1999).

The occurrence of extensive phytoplankton blooms in central Florida Bay has precipitated a

significant amount of public, political, and scientific concern. Interestingly, these blooms are often dominated by cyanobacteria (Phlips & Badylak, 1996; Steidinger et al., 1996; Phlips et al., 1999). The ability to fix atmospheric N_2 may give cyanobacteria a selective advantage in areas of both low N concentrations as well as low dissolved N:P ratios (Phlips et al., 1989). In the ocean gyres where N concentrations are very low, N_2 fixation by cyanobacteria may provide a significant source of N to the system. In freshwater lakes and rivers, where P concentrations may be high due to external loading, N_2 fixation fuels the massive floating mats of cyanobacteria. Neither of the above conditions applies to Florida Bay, necessitating a new conceptual model to explain the prevalence of cyanobacteria in the system.

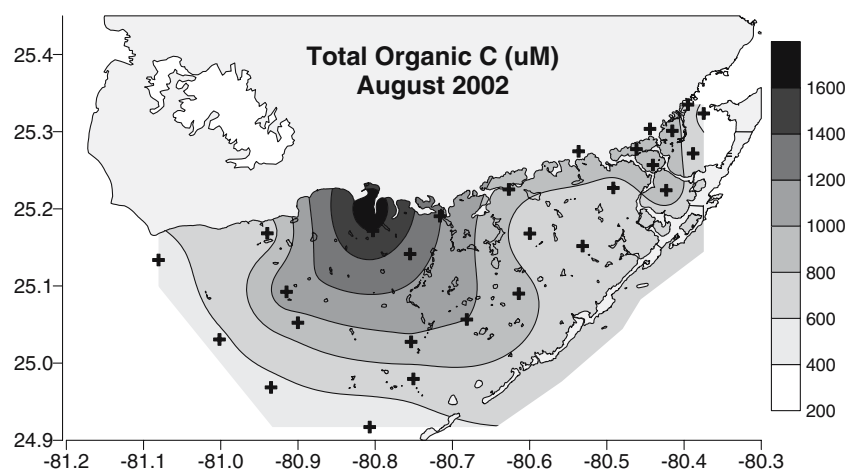


Figure 2. Distribution of total organic carbon (μM) across Florida Bay in August 2002, showing usual concentration of high levels in north-central bay (from Boyer, 2005).

The connection between phytoplankton productivity and nutrient loading inputs to estuaries has been repeatedly demonstrated (Boynton et al., 1982). However, data from eastern Florida Bay show that while nutrient loading increases with flow through the Taylor Slough, inflowing nutrient concentrations actually decline (Boyer & Jones, 1999; Rudnick et al., 1999). This pattern does not follow the typical eutrophication model because the limiting nutrient actually becomes more dilute as the input flows increase. In addition, the concentration of incoming P is slightly below the ambient concentrations of the bay, meaning that the concentration of P loading is at or below the kinetic threshold for phytoplankton uptake.

Most nutrient and phytoplankton studies to date have been primarily concerned with the amounts of dissolved inorganic N and P as these fractions are directly available for phytoplankton uptake. However, most of the N and P in the water column of Florida Bay is present as DOM (Boyer et al., 1997). The question we then ask is how much of the N and P in DOM is accessible to the phytoplankton? Specific components of DOM, such as urea, may be taken up directly by some phytoplankton (Waser et al., 1998; Lewitus et al., 2000; Glibert et al., 2004), but they are typically minor fractions of the pool. Therefore, the majority of the DOM pool must first be remineralized to the inorganic forms by bacteria prior to phytoplankton uptake.

The bioavailability of DOM depends upon both the chemical structure (Benner et al., 1986; Amon & Benner, 1996) and the C:N:P ratio of the source material (Tezuka, 1990). Bacterial utilization of DOM, and subsequent grazing on these bacteria by protists and microzooplankton (the microbial loop), is an important alternative pathway in many aquatic food webs (Azam et al., 1983). The microbial loop may also play a significant role in nutrient remineralization when C:N and C:P ratios are low. Conversely, when nutrient concentrations are limiting, bacteria compete with phytoplankton for inorganic nutrients (Caron, 1994). Therefore, the bioavailability of the DOM pool can determine whether the microbial loop is a source or sink for dissolved nutrients in the water column.

Little is known about the microbial loop in Florida Bay. Data on bacterial production, community structure, and grazing activity of heterotrophic nanoflagellates and ciliates are virtually absent. Bugden et al. (1998) measured heterotrophic potential (^{14}C -glucose uptake) and direct counts of bacteria and found the central Bay to have highest activity and biomass, followed by the western bay. They also found significant correlations between DOC, heterotrophic activity and bacterial counts. Differences in biogeochemical nutrient cycles and processing of DOM appear to influence the productivity of sub-regions of Florida Bay (e.g. the presence vs. lack of phytoplankton blooms; Philips et al., 1999) and these differences were linked to the structure and activity of the

microbial food web (Lavrentyev et al., 1998). The little available information reveals that dissolved nutrient concentrations increased during the wet season and towards the river mouths and heterotrophic bacterioplankton growth was less nutrient limited at sites closer to Everglades runoff and during periods of high rain (Cotner et al., 2000).

In Florida Bay, 88% of the total phosphorus pool (TP) is in the form of dissolved organic phosphorus (DOP; Boyer et al., 1997). This is a result of biological uptake, the extremely low SRP loading concentrations (from uptake within the Everglades) and because of abiotic binding of PO_4^{3-} to carbonate sediments (Kitano et al., 1978). This results in the emergence of DOP as a potentially important P source, for those organisms which can access it. Alkaline phosphatase (AP) is an inducible ectoenzyme produced in response to low SRP concentrations by bacteria, cyanobacteria, and some algae, which hydrolyzes DOP to PO_4^{3-} (Chrost, 1990). AP activity is usually inversely related to (Smith & Kalff, 1981) and down-regulated by, high SRP concentrations (Ammerman & Azam, 1991). We have measured AP activity at 28 sites in Florida Bay for the past 15 years (Boyer, 2005) and found that AP activity is positively correlated with DOC concentrations, not with SRP or algal biomass as expected (Boyer et al., 1997). Both AP activity and DOC concentrations are three times higher in the central bay than the eastern bay. We believe that the microbial community in the eastern Bay is co-limited by P and C as it serves no purpose for microorganisms to express AP when there is no substrate present. Unfortunately, neither DOP bioavailability nor its contribution to primary production is known for this ecosystem.

Our understanding of bacterial and algal coupling dynamics and nutrient mineralization is incomplete, largely because no one experiment addresses DOM uptake by both trophic constituents. In addition, the quality/labability of DOM is a function of its chemical characteristics, molecular weight, elemental ratio (C:N:P:other) and age. Stimulation of AP activity and algal biomass as chlorophyll *a* (CHLA) by the addition of DOM has been demonstrated in estuarine microcosms (Carlsson & Graneli, 1993). Previous studies have also demonstrated the importance of DOP in phytoplankton metabolism (see review by Cem-

bella et al., 1984) but kinetic experiments by Bentzen et al. (1992) showed that the bacterial fraction dominated P uptake (as ATP), while small nanoplankton (1–12 μm) obtained some P and phytoplankton $>12 \mu\text{m}$ took up very little. These results lead us to believe that AP activity is the main pathway by which both bacteria and phytoplankton in Florida Bay obtain P for growth and reproduction.

In addition to AP activity, DOM bioavailability may also help to explain the prevalence of cyanobacteria over eukaryotic algae in blooms in the central Bay. Cyanobacteria have much higher cell-specific AP production than do eukaryotic algae (Garaudet et al., 1997). This means that when P is limiting, cyanobacteria have an enhanced ability to hydrolyze DOM for the P contained therein. We also proposed that the bioavailability of DOM provides a labile N source that may be important in promoting bacterial and/or cyanobacterial production. The observation that the cyanobacteria blooms usually begin in the central Bay where episodic DOM inputs occur adds support to this idea. Therefore we propose that spatial patterns in primary production of bacteria and phytoplankton in Florida Bay are a product of dissolved inorganic and dissolved organic nutrient availability and that, under P limited conditions, cyanobacteria are favored over eukaryotic algae by their greater cell-specific production of AP.

We tested these hypotheses in Florida Bay by incubating water samples using a combination four treatments: ambient light incubation, dark incubation, DIN and SRP enrichment, and DOM amendments. From these treatments we expected to quantify (1) the effect of inorganic nutrients vs. DOM on phytoplankton production and community structure; (2) the bacterial contribution to ambient community production; and (3) the effect of bioavailability of ambient and amended DOM on phytoplankton production and community structure.

Methods

DOM bioavailability experiments

We sampled four sites distributed throughout Florida Bay for routine BDOC experiments

(Fig. 1). These sites, Joe Bay, Duck Key, Bob Allen Key and Whipray Basin, were representative of different water quality zones existing in the bay (Boyer et al., 1997). All 4 sites have higher median AP activity and CHLA concentration relative to the other 24 sites in Florida Bay (Boyer, 2005). Thus we expected to see the greatest response of microbial activity to increased substrate availability from these sites. We conducted a total of five bioavailability incubation experiments from July 2001 to July 2002. We also collected field and nutrient data from all sites monthly during this period in conjunction with our Coastal Water Quality Monitoring Network (Boyer, 2005). Duplicate samples were collected in pre acid-washed, autoclaved distilled deionized (ADDI) water rinsed, 2.5 l polycarbonate bottles. Bottles were rinsed with sample water three times immediately prior to sample collection. Water was collected from a ~10 cm depth and bottles were kept in the dark and on ice for transport.

Enrichments of dissolved inorganic nutrients (N+P), DOM, and a control were applied under both the light and dark conditions. Raw water from each site was filtered through a 0.5 mm Nitex screen prior to use to remove any large particles or zooplankton. Nutrient treatments were conducted by adding both NH_4NO_3 and KH_2PO_4 to a final added N+P concentration of 10 μM and 1 μM , respectively. DOM additions were conducted by concentrating water using tangential flow fractionation filtration (TFF) with a Millipore Pellican-2 system with a 1 kD pore diameter membrane. Using this method, particles smaller than the membrane pore size are discarded in the wastewater and only particles larger than 1 kD are retained. DOM concentrate was obtained from Taylor Slough (Florida Coastal Everglades (FCE) LTER site TS/Ph 6 or Argyle Henry). During the TFF process we concentrated the DOM to a final concentration 8–12 times the original. We dispensed the DOM concentrate to a final concentration of ca. twice the original ($2\times\text{DOM}$) to a total volume of 2 l. Control bottles were set up same as the treatments, but otherwise left unaltered.

Incubations were conducted in 2.5 l polycarbonate bottles. Clear bottles were used for light treatments; dark bottles were wrapped with aluminum foil tape. Bottles were incubated on a floating line in the FIU campus lake in order to

expose them to ambient light, temperature, and wave action (mixing).

Sub-samples were collected from the incubation bottles on 0, 1, 3, 5 or 6, and 10-day intervals. The sampling procedure consisted of collecting water from the incubation bottles using sterile, amber HDPE bottles for transport. Incubation bottles were homogenized by gentle inversion three times and the transport bottles were rinsed with sample water three times prior to filling.

Nutrient analyses

Unfiltered water samples were analyzed for total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and alkaline phosphatase (AP) activity. TOC was measured by direct injection onto hot platinum catalyst in a Shimadzu TOC-5000 after first acidifying to $\text{pH} < 2$ and purging with CO_2 -free air. TN was measured using an ANTEK 7000N Nitrogen Analyzer using O_2 as carrier gas instead of argon to promote complete recovery of the nitrogen in the water samples (Frankovich & Jones, 1998). TP was determined using a dry ashing, acid hydrolysis technique (Solorzano & Sharp, 1980). The AP assay measures the activity of alkaline phosphatase, an enzyme used by microorganisms to mineralize phosphate from organic compounds (Hashimoto et al., 1985). This assay is performed by adding a known concentration of an organic phosphate compound (*o*-methylfluorescein phosphate) to an unfiltered water sample. AP in the water sample cleaves the phosphate, leaving *o*-methylfluorescein, a highly fluorescent compound. The fluorescence of initial and 2 h incubations were measured using a Gilford Fluoro IV spectrofluorometer (excitation = 430 nm, emission = 507 nm) and subtracted to give AP activity ($\mu\text{M h}^{-1}$).

Filtrates (Whatman GF/F) were analyzed for soluble reactive phosphorus (SRP), nitrate + nitrite (NO_x^-), nitrite (NO_2^-), ammonium (NH_4^+), and silicate ($\text{Si}(\text{OH})_4$) by flow injection analysis (Alpkem RFA 300). Filters for CHLA content ($\mu\text{g l}^{-1}$) were allowed to extract for a minimum of 2 days at -20°C before analysis. Extracts were analyzed using a Gilford Fluoro IV Spectrofluorometer (excitation = 435 nm, emission = 667 nm) and compared to a standard curve of pure CHLA (Sigma).

Some parameters were not measured directly, but were calculated by difference. Nitrate (NO_3^-) was calculated as $\text{NO}_x^- - \text{NO}_2^-$. Dissolved inorganic nitrogen (DIN) was calculated as $\text{NO}_x^- + \text{NH}_4^+$. Total organic nitrogen (TON) was defined as $\text{TN} - \text{DIN}$. Concentrations for all of these water quality variables are reported in μM , except where noted.

Microbial analyses

Pulse amplitude modulated fluorometry (Walz PhytoPAM) was used to quantify the phytoplankton community structure and photosynthetic quantum yield (QY) or maximum efficiency of photosystem II (PSII). QY was expressed as the ratio of variable fluorescence to maximum fluorescence (F_v/F_m). Sample water was allowed to sit at 4 °C overnight prior to analysis to ensure complete reduction of PSII. A 3 ml aliquot was first run in the PAM fluorometer to determine the range of fluorescence for the individual sample and gain was adjusted accordingly. Next a 3 ml aliquot was filtered through a 0.2 μm filter and used to determine the background fluorescence at each site. Finally, 3 ml of whole water was analyzed again for total CHLA, CHLA concentrations for cyanobacteria fraction (CHLA_{CY}), green (CHLA_{GR} , which also included dinoflagellates), and brown algal groups (CHLA_{BR} – mostly diatoms). The sample was then subjected to an actinic light saturation pulse regime in order to measure QY.

Bacterial counts (BACT) were determined by epifluorescence microscopy using DAPI staining technique (Coleman, 1980; Porter & Feig, 1980). Sample water was collected and fixed with buffered formalin solution to a final concentration of 2%. Samples were incubated at a final concentration of 25 $\mu\text{g ml}^{-1}$ DAPI (Molecular probes[®]) in a dark filtration tower for 20 min prior to filtration onto a 0.2 μm black polycarbonate filter. The filter was mounted onto a slide with low fluorescent immersion oil and examined under a 100 W Hg bulb by counting 10 sampling fields of a known size per slide, with a minimum of 300 cells per slide counted. A final value of cells ml^{-1} was obtained with a formula using the sample volume counted and the percentage of effective filter area counted.

Bacteria production (BP) was determined using ^3H -thymidine incorporation incubations (Bell,

1993). Triplicates of each sample were analyzed along with a 4% formalin blank sample for specific activity determination. Disintegrations per minute (dpm) from the liquid scintillation counter were converted using then following equation:

$$\mu\text{g C l}^{-1} \text{ h}^{-1} = (\text{moles thymidine l}^{-1} \text{ h}^{-1}) \\ * (\text{cells mole}^{-1}) * (\text{carbon cell}^{-1})$$

where picomoles of thymidine incorporated were calculated through using the actual activity of the ^3H thymidine (dpm) versus the bacterial activity from live-killed (dpm).

Cells mole^{-1} was determined by multiplying the thymidine conversion factor of 2×10^{18} cells mole^{-1} by the moles of thymidine $\text{l}^{-1} \text{ h}^{-1}$. For the amount of carbon per cell we used the 20 fg conversion rate used in coastal waters. Our observations of water from these Florida Bay sites over the past two years have shown bacteria numbers that are consistently lower than those found in estuaries or other coastal systems but can be highly variable across sampling months. Thus we used a mid-value conversion factor to multiply the number of cells by to estimate the change in C content (Bell, 1993).

DOM fluorescence analyses

Fluorescence emission and synchronous fluorescence were measured using a Perkin Elmer LS50B spectrofluorometer with a 150 W Xenon light source to analyse 3 ml aliquots of sample. 20 ml of water was filtered through a Whatman GF/F prior to fluorescence analyses with all samples processed within 7 days of collection. Samples were first run for UV-visible data that were used to correct the data we collected for maximum fluorescence intensity (F_{max}), maximum wavelength of fluorescence (λ_{max}), fluorescence index (FI as $f_{450/500}$), and relative percent of Peak 1 (at 285 nm, Jaffe et al., 2003).

Statistical analyses

Data from incubation experiments were fitted to a one-phase exponential decay equation where X is time, and Y is DOC concentration.

$$Y = \text{Span} e^{-K*t} + \text{Plateau}$$

Y starts out equal to $\text{Span} + \text{Plateau}$ and decreases to Plateau at a rate of K (d^{-1}). The percent of bioavailable DOC (BDOC) in the pool was calculated as $\text{Span}/(\text{Plateau} + \text{Span}) * 100$. If the equation could not be resolved because of variability, the linear difference between beginning and end was used.

Variables were tested between groups using the nonparametric Wilcoxon Ranked Sign test (comparable to the t -test) and among groups by the Kruskal–Wallis test (comparable to ANOVA) with significance set at $p \leq 0.05$. Groups that were significantly different were tested by Mann–Whitney U test in order to say how the groups differed by comparing medians of all paired cluster groups among all physical site parameters and/ or taxa at a level of $p < 0.05$ (2-tailed test). In addition, variables by station were visualized using box-and-whiskers plots, where the center horizontal line of the box is the median, the top and bottom of the box are the 25th and 75th percentiles (quartiles), and the ends of the whiskers are the 5th and 95th

percentiles. Outliers were suppressed to reduce figure compression.

Results and discussion

Site characteristics

The sites we chose for this study were selected as being representative of four different zones of Florida Bay. Joe Bay receives seasonally driven, freshwater inputs from fringing mangrove forest. Duck Key is located in shallow estuarine lagoonal area of Florida Bay, which receives minimal tidal energy and is vegetated with sparse seagrass. The Bob Allen site is influenced most by the Atlantic Ocean mixing and is considered the most “oceanic” site. Whipray Basin is hydraulically isolated from the rest of the Bay and only receives episodic terrestrial runoff. It is the shallowest area of the Bay and experiences frequent hypersalinity events.

We hypothesized that difference in physical hydrography would result in dissimilarity of DOM source; ergo, its bioavailability and effect on the microbial loop. Other factors affecting phyto-

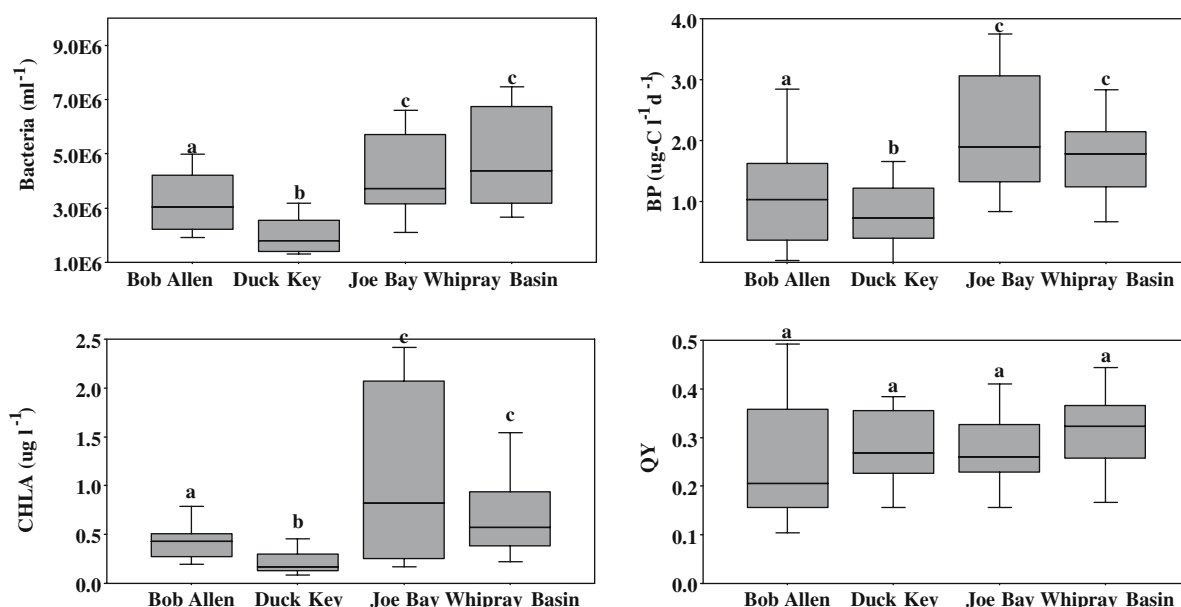


Figure 3. Box and whisker plots of bacterial counts (BACT in ml^{-1}), bacterial production (BP as $\mu\text{g-C l}^{-1} \text{d}^{-1}$), chlorophyll a (CHLA in $\mu\text{g l}^{-1}$), and phytoplankton quantum yield (QY). The center horizontal line of the box is the median of the data, the top and bottom of the box are the 25th and 75th percentiles (quartiles), and the ends of the whiskers are the 5th and 95th percentiles. Outliers were suppressed to reduce figure compression. Statistical differences among sites are shown as having different letter labels.

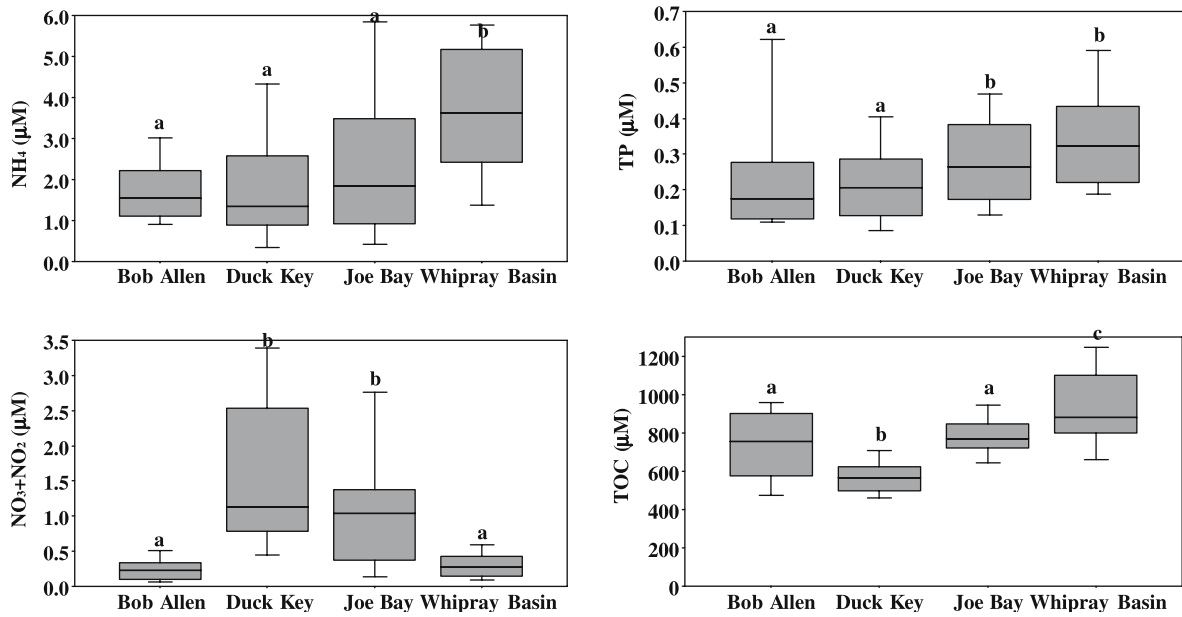


Figure 4. Box and whisker plots of NH_4^+ (μM), TP (μM), $\text{NO}_3^- + \text{NO}_2^-$ (μM), and TOC (μM) showing similarity in distribution of NH_4^+ and TP and inverse relationship between $\text{NO}_3^- + \text{NO}_2^-$ and TOC.

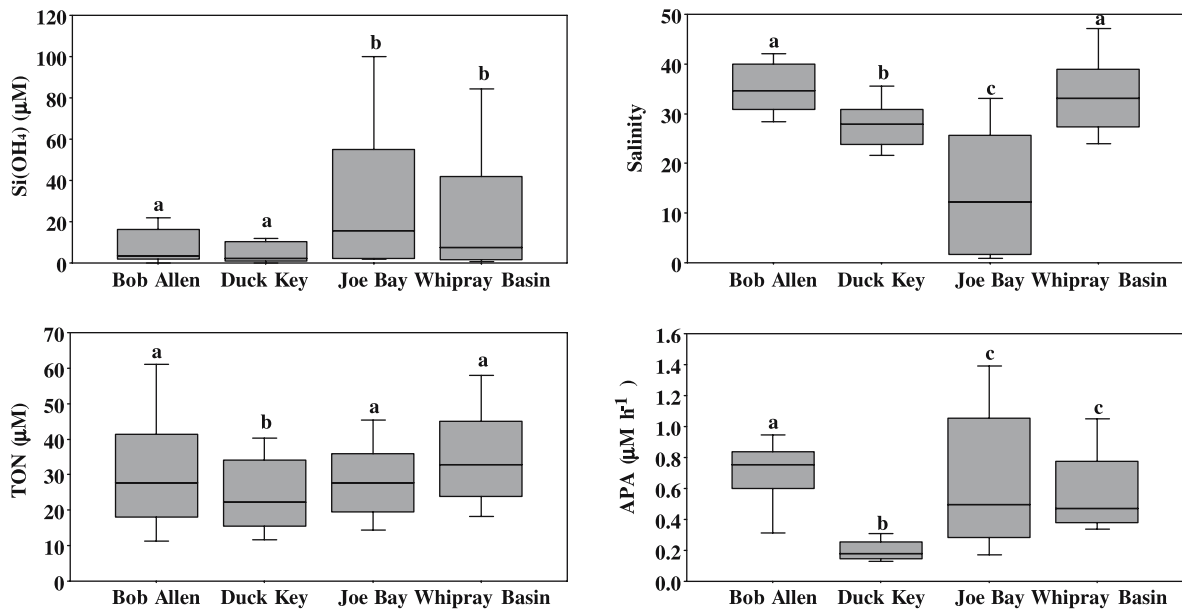


Figure 5. Box and whisker plots of $\text{Si}(\text{OH})_4$ (μM), salinity, TON (μM), and AP activity ($\mu\text{M h}^{-1}$).

plankton and bacteria are the concentration and relative proportion of different nutrient species in the total pool. There were significant differences in all measured variables among sites, except for TON and QY (Figs. 3–6). Joe Bay exhibited lowest salinity, FI, and Peak1 but was high in BACT,

BP, TP, CHLA, AP activity, TOC, $\text{Si}(\text{OH})_4$, λ_{max} , and F_{max} . The terrestrial DOM subsidy (high TOC, λ_{max} , and F_{max} with low FI) increased organic P levels (only 4% of TP is PO_4^{3-} , Boyer et al., 1997) along with a corresponding increase in bacterial biomass and production as

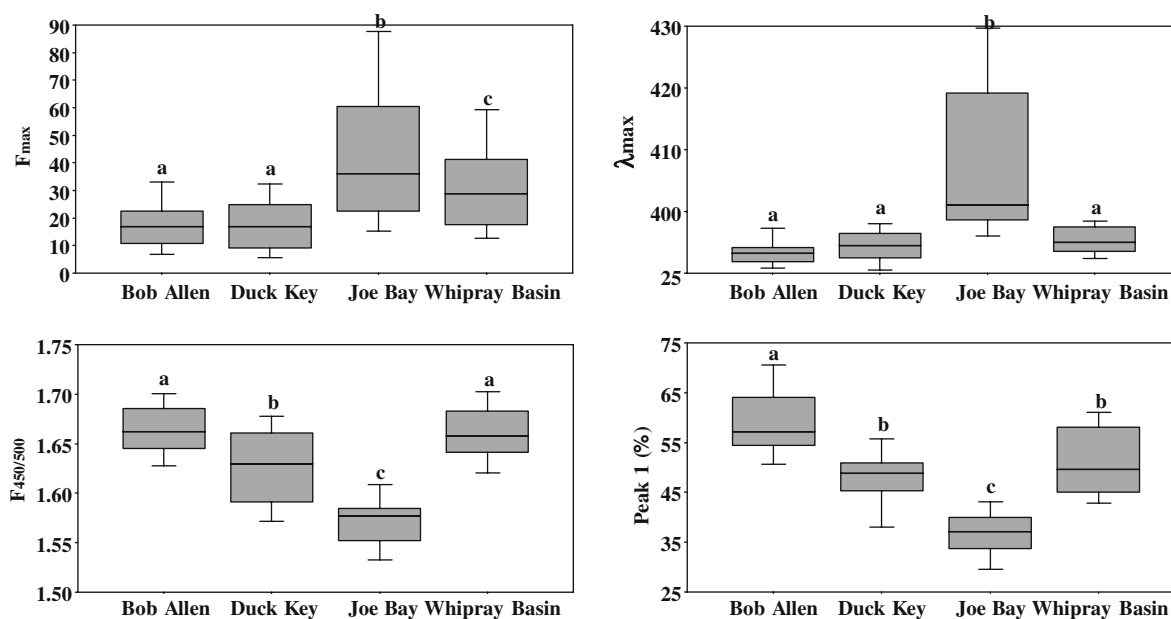


Figure 6. Box and whisker plots of F_{\max} (QSU), λ_{\max} (nm), FI ($f_{450/500}$), and Peak1 (%) showing similarity in distribution of F_{\max} and λ_{\max} and inverse relationship between these variable and $f_{450/500}$ and Peak1.

well as phytoplankton biomass. The fluorescence characterization of DOM (Fig. 6) showed very clearly the inverse relationship between terrestrial DOM (high F_{\max} and λ_{\max}) and those sites having significant microbial and/or seagrass DOM (high FI and Peak1).

Duck Key had low levels of BACT, BP, TP, AP activity, CHLA, TOC, $\text{Si}(\text{OH})_4$ and F_{\max} (Figs 3–6) but was highest in NO_x^- . This area achieves the greatest extent and duration of P limitation conditions seen in the Bay, which results in a buildup of excess NO_x^- and low biological biomass and activity. Bob Allen was very low in NO_x^- , TP, λ_{\max} , and F_{\max} but high in AP activity, salinity, FI and Peak1. We believe the area around Bob Allen is subsidized by seagrass DOM production (Jaffé pers. comm.) which helps explain the high AP activity, FI, and Peak1 (protein). Whipray Basin was low in NO_x^- , but high in BACT, NH_4^+ , TP, TOC, salinity, and FI. The high levels of these variables indicate a relatively closed system driven by remineralization and benthic flux.

N + P vs. DOM stimulation of autotrophic community (light incubations)

In this part of the experiment we hypothesized that N + P additions would have the greatest effect on

primary production and phytoplankton community structure at those sites which had high DOM and low nutrient concentrations – Joe Bay and Whipray Basin. This hypothesis was clearly validated as BACT, BP, CHLA, CHLA_{GR} , and CHLA_{BR} were all significantly higher after ten days of incubation, relative to control (Table 1). Interestingly, nutrient additions did not stimulate CHLA_{CY} in Joe Bay, where CHLA_{CY} increased after 3 days but then declined by the end of the incubation. In addition to those sites, we found that BACT, BP, CHLA, CHLA_{GR} , and CHLA_{BR} at both Duck Key and Bob Allen were also stimulated by N + P additions. CHLA_{CY} was stimulated by N + P additions at Duck Key but not Bob Allen. AP activity was strongly inhibited by N + P additions as expected.

We saw no measurable difference in QY across treatments or sites. This was due in part to the very low levels of CHLA present, which were at or near the limits of detection for the assay. The only changes we observed in DOM fluorescence variables was an increase in Peak1 (protein/polyphe- nolic) at Duck Key and Whipray Basin over time, but it was not statistically significant. This implies that a qualitative change in DOM occurred at these sites as a result of increased phytoplankton production.

Table 1. Matrix of measured variable response to treatment relative to control. Direction of response is positive or negative with statistical significance set at $p < 0.05$

Condition	Site	Treatment	BACT	BP	APA	CHLA	CHLA _{CY}	CHLA _{GR}	CHLA _{BR}	λ_{\max}	F _{max}	FI	Peak1	BDOC
Light	Duck Key	DOM	+	++	++		++		+					
		N+P	++	+++	--	+++	+++	+++	+++					
	Joe Bay	DOM	+	++			+		+					
		N+P	+++	+++		+++		+++	+++					
	Whipray Basin	DOM	++	+	++	++	+			++				
		N+P	++	+++	-	++	+	++	+++					
Bob Allen	DOM	++	+	++	++	+			++					
	N+P	+++	+++	-	++		+	+						
Dark	Duck Key	DOM	+	+	+					-	-	+	+	+
		N+P		++	--						-	-	-	-
	Joe Bay	DOM	+	+	+					-	-		+	+
		N+P			--								+	-
	Whipray Basin	DOM	+	+						+			-	++
		N+P		++	--									+
Bob Allen	DOM	+	+	+					-	-	+	+	++	
	N+P		++	--								+	-	+

The number of + or - was related to the magnitude of response (+ was up to a doubling in response, ++ was up to a tripling in response, etc.).

The second part of the light experiment assessed the effect of a doubling of DOM concentration on the phytoplankton community. Because the added DOM was isolated from an upstream Taylor Slough marsh/mangrove site, the net effect was to simulate the potential impact of terrestrial DOM loading to these sites. Contrary to N + P treatment, we hypothesized that the effect of the 2×DOM addition would be greatest at those sites having the lowest DOM concentrations, Duck Key and Bob Allen. DOM subsidy did indeed have a significant effect on the microbial community, but results were not exactly as predicted. Not only were BACT and BP at Duck Key and Bob Allen stimulated, but these variables increased at all sites, regardless of DOM concentration (Table 1). The increases were not as high as those produced by N + P additions. The 2×DOM subsidy did not enhance CHLA levels at Duck Key or Joe Bay but did affect CHLA at Bob Allen and Whipray Basin, however this occurred later in the incubation (day 10). The 2×DOM addition significantly altered the phytoplankton community as well; all sites showed increased CHLA_{CY} and CHLA_{BR}; no increase was noted in CHLA_{GR}.

One of the most significant effects of 2×DOM addition was the large stimulation in AP activity at

all sites but Joe Bay. This implies that AP activity was induced by addition of organic substrate, not just by decline in inorganic P. Nausch & Nausch (2004) have shown that AP is another method by which cells can access organic C along with P. Confounding this result is the fact that the added DOM was from a different source (freshwater marsh) and therefore might be of different quality than that found in the Bay. This might help explain why 2×DOM stimulated the phytoplankton community in Whipray Basin, an area of high DOM. We are wary about using the fluorescence information as new organic C was produced during the light incubations, which might confound the interpretation.

The results of the light experiments show that the bacterial community responded to 2×DOM addition in a similar manner to N + P addition, but at a lower response level. The phytoplankton community responded in a differential manner. Cyanobacterial biomass was stimulated at all sites with DOM additions, while N + P addition stimulated CHLA_{CY} only Duck Key and Whipray Basin. Both N + P and 2×DOM additions increased diatom biomass but for different reasons: N + P were taken up directly by the cells while DOM required the intermediate step of AP

induction before the P was available. Apparently, diatoms and cyanobacteria in Florida Bay are capable of producing AP whereas the green algae are not.

N + P vs. DOM stimulation of heterotrophic microbial community (dark incubations)

Under dark conditions, N + P addition did not have any effect on BACT but did increase BP at all but Whipray Basin (Table 1). In contrast, 2×DOM additions stimulated both BACT and BP at all sites. We did not expect to see significant increases in BP at the high DOM sites, Joe Bay and Whipray Basin, but once again, the terrestrial DOM proved to be more usable than the *in situ* DOM. Stimulation of BP was greater in N + P than 2×DOM treatments. These results are consistent with others (Ducklow & Carlson, 1992; del Giorgio & Cole, 1998) who have shown that the bacterial response to nutrients is to increase their bacterial growth efficiency (BGE) while the general response to C is to increase growth and reproduction.

As with the light treatment, a dramatic difference in AP activity between nutrient treatments was observed. N+P additions had a strong negative effect on AP activity while 2×DOM increased AP activity in all but Whipray Basin. It seems that AP activity was already high in Whipray and no further stimulation was possible.

Fluorescent properties λ_{\max} and F_{\max} declined with time in DOM addition treatments (except for λ_{\max} in Whipray Basin), implying that there was a qualitative shift from terrestrially derived to microbially produced DOM (Table 1). This interpretation was reinforced by the overall increase in FI at the same sites. Peak1 declined at Whipray Basin but increased at the other three sites. A decline in Peak1 is interpreted as a loss of the protein/polyphenol fraction through photo- or biodegradation (Scully et al., 2004). We expected to see declines in Peak1 across all sites as the more labile proteins were consumed, but this was not the case.

N + P additions had a negative effect on λ_{\max} and F_{\max} only at Duck Key. This implied that conversion of high molecular weight DOM by bacterial activity was significant only at this site. Oddly, FI also declined at Duck Key but increased at Joe Bay and Bob Allen as the microbial contri-

bution to the DOM pool became more pronounced. Peak1 decreased at all but Whipray Basin, indicating microbial use of these components.

Bioavailability of dissolved organic carbon (BDOC)

We used degradation of DOC as a proxy for overall breakdown of DOM. Batch incubations, such as those employed in this study, typically result in a declining loss of labile DOC where the less reactive fraction becomes a larger component of the total pool over time. In many cases a power function is used to fit the data in an effort to discriminate among dissimilar pools of DOC having different reactivities (del Giorgio & Davis, 2003). The single phase exponential decay gave us the ability to discriminate between labile and recalcitrant DOC pools without having to assume a constantly decreasing reactivity of a single pool. The single phase exponential decay constant (K), therefore, tells only one part of the story; that of how fast the labile fraction is degraded. The plateau told us how much of the pool was relatively recalcitrant. Plateaus for all sites were high relative to initial levels, meaning that only a small portion of the total DOC pool was degraded. Therefore, it is important that both the Span and Plateau be reported along with K , or else DOC decomposition may be overestimated.

The bioavailability of the ambient DOC (Control) was not significantly different across sampling sites (Fig. 7). Ambient BDOC ranged from 2.0–12.9% with a median of 6.5% (Table 2). Bioavailability in the control incubations across Florida Bay did not appear have a pattern that is regional in scale. The highest bioavailability and one of the very lowest control bioavailability we observed across the study period occurred at northeastern central study site of Joe Bay (1.73 and 14.06%). Joe Bay is in the ecotone between the Everglades mainland and Florida Bay and is cloistered behind miles of winding mangrove waterway. It is interesting that BDOC was highest in the western Florida Bay sites and in those sites closest to the mangrove estuary and was lowest in the central Florida Bay further from the freshwater ecotone. This suggests that the amount of BDOC from the marsh and mangrove that is transported to Florida Bay is low. This concept was born out in the

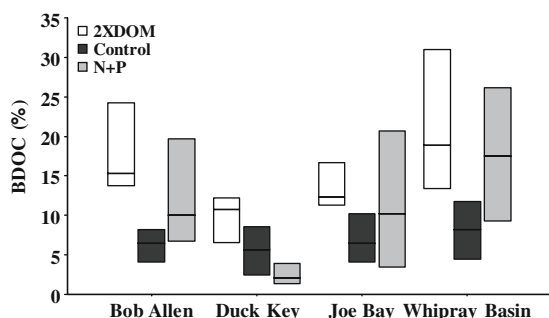


Figure 7. Box and whisker plots of BDOC (%) by site and treatment showing effects of increased nutrient and DOM on bioavailability.

fluorescence characterizations, where we saw the 2×DOM amendment was very similar in molecular behavior and fluorescence properties to the ambient DOM in the controls. This provides some validation that indeed our 2×DOM amendments were similar in structure to the ambient DOM.

There was a significant response in BDOC, relative to Controls, for both the N + P and 2×DOM treatments (Table 1). BDOC at all sites was stimulated by the 2×DOM addition (Fig. 7). Except for Duck Key, BDOC at all sites was also stimulated by the addition of N + P. Overall, the 2×DOM addition provoked greater response than did N + P addition, but it was not always significant. There was no significant difference between the N + P and 2×DOM treatments at Joe Bay and Whipray Basin sites, although the mean BDOC was higher for the 2×DOM treatment. Some of the differences between treatment effects may be

due to differences in actual amounts of N and P added. In addition to DOC, the 2×DOM addition added 20 μM DON and 0.2 μM DOP to the incubation bottles. Therefore, the potential for N stimulation in the 2×DOM treatment was twice that of the N + P amendment whereas, the potential P added as organic matter was only 20% that of the N + P treatment.

We tested bioavailability across time and found that there was a significant difference between the dry and wet seasons. BDOC was lower in the dry season than in the wet season (5.56% vs. 16.86%). This may be explained by the distinct chemical characteristics of the DOM produced at different times of year (Maie et al., 2006). The highest bioavailability that we observed was from the Whipray Basin site with 2×DOM treatment (35.5%). The bioavailability seen at this site in the first wet season experiment was twice what we observed at any other site under any treatment over time and suggests that this site responds more quickly to ‘fresh DOC’ and more intensely to organic additions.

In our incubation assays, the initial rate of degradation (Table 2) was much slower than from our previous results from freshly leached DOC from mangrove, sawgrass, and seagrass leaves (Sculley et al., 2004). This is consistent with the general idea that increased environmental exposure of DOM results in decreased biological reactivity. The age distribution of the DOM pool may be a significant factor in explaining the variability of measured BDOC estimates. The preponderance

Table 2. Means of single phase exponential decay constants for all BDOC incubations

Treatment	Site	K (d^{-1})	Span (μM)	Plateau (μM)	BDOC (%)
Control	Duck Key	0.001	50.6	554.4	6.9
	Joe Bay	1.480	36.1	504.6	7.0
	Whipray Basin	0.003	77.9	724.5	9.8
	Bob Allen	0.005	78.7	910.0	6.7
N + P	Duck Key	0.001	121.5	492.9	17.0
	Joe Bay	1.420	10.9	499.0	2.2
	Whipray Basin	0.003	169.8	662.9	20.4
	Bob Allen	0.826	259.6	741.9	26.0
DOM	Duck Key	0.088	286.3	1404.4	16.3
	Joe Bay	0.295	183.5	1401.5	18.7
	Whipray Basin	0.796	213.6	1635.0	11.7
	Bob Allen	0.545	772.3	1264.6	36.2

Table 3. Range of bioavailability results of our study compared with those of researchers in estuaries reported across the globe with + standard error when reported or available

Location of study	BDOC (%)	Focus of study	Citation
Cape Fear River, USA	9.0 ± 4.5	Total microbial community	Avery et al. (2003)
Rio de Janeiro, Brazil	6.6 ± 17.4	Bacteria only	Fajalla et al. (2002)
Baltic Sea	2–72	Heterotrophic community	Stephanaukas (2000)
Sacramento-San Joaquin River Delta	11–15	Total microbial community	Sobzjak et al. (2002)
Duck Key, Florida Bay USA	1.3–12.7	Total microbial community Dark incubations	This study
Joe Bay, Florida Bay, USA	2.7–25.3	Total microbial community Dark incubations	This study
Whipray Basin, Florida Bay, USA	3.1–35.1	Total microbial community Dark incubations	This study
Bob Allen Keys, Florida Bay USA	2.5–27.8	Total microbial community Dark incubations	This study

of older DOM during the dry season may also help explain the seasonal differences in bioavailability (Maie et al., 2006).

BDOC estimates from this study were low but comparable to other studies with low anthropogenic impacts (Table 3). del Giorgio & Davis (2003) provided an exhaustive review of BDOC results from various ecosystems. They found large differences in initial bioavailability between lakes and marine systems vs. rivers, marshes and estuaries with lakes and marine being 3- to 5-fold higher. On an average, 9–10% of DOC pool was biodegraded after 3 days. Most of these differences were attributed to proportion of algal DOC to the system. In general, autochthonous DOC was more rapidly and more completely utilized than allochthonous DOC.

Our BDOC for the DOM amendment is as high as any reported value in the literature (Table 3). This suggests a feedback loop where higher concentrations of DOC introduced to Florida Bay from the mainland Everglades, either through pulse events such as hurricanes, massive rains, or human altered water events, can lead to higher bioavailability and eventually higher DOC concentrations. Nutrients provided in the form of organic matter stimulated increases in BDOC that were equivalent to inorganic nutrients alone. An increase in DOM loading from the Everglades, especially during the wet season, may therefore produce a response equal to or greater than the response from inorganic nutrient enrichment because it is transporting fresher material to the estuary. This suggests that both inorganic and organic forms of nutrients can be limiting factors for bacteria and phytoplankton populations in the area. Further, it supports the argument that the

microbial population of Florida Bay responds to allochthonous carbon inputs to the water column, such as those conditions that may exist in pulsed energy events such floods and hurricanes.

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