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## Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay

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1 In press at Estuaries 2 3 Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and 4 macroalgae community structure in Florida Bay 5 6 Anna R. Armitage<sup>1</sup> 7 8 Department of Biological Sciences and Southeast Environmental Research Center, 9 Florida International University, Miami, FL 33199 10 Ph 305-348-7317 Fax 305-348-4096 Email armitage@fiu.edu 11 12 Thomas A. Frankovich 13 Department of Biological Sciences and Southeast Environmental Research Center, 14 Florida International University, Miami, FL 33199 15 16 Kenneth L. Heck, Jr. 17 Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, AL 36528 18 19 James W. Fourgurean 20 Department of Biological Sciences and Southeast Environmental Research Center, 21 Florida International University, Miami, FL 33199 22 <sup>1</sup>Corresponding author 23

Complex nutrient effects on seagrass, algae

Armitage et al.

### **Abstract**

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25 We examined the spatial extent of nitrogen (N) and phosphorus (P) limitation of each of the major benthic primary producer groups in Florida Bay: seagrass, epiphytes, macroalgae, and 26 27 benthic microalgae, and characterized the shifts in primary producer community composition following nutrient enrichment. We established 24 permanent 0.25 m<sup>2</sup> study plots at each of six 28 29 sites across Florida Bay and added N and P to the sediments in a factorial design for 18 months. 30 Tissue nutrient content of the turtlegrass *Thalassia testudinum* revealed a spatial pattern in P 31 limitation, from severe limitation in the eastern bay (N:P > 96:1), moderate limitation in two 32 intermediate sites (~63:1), and balanced with N availability in the western bay (~31:1). P 33 addition increased T. testudinum cover by 50-75% and short-shoot productivity by up to 100%, 34 but only at the severely P-limited sites. At sites with an ambient N:P ratio suggesting moderate P 35 limitation (~63:1), few seagrass responses to nutrients occurred. Where ambient T. testudinum 36 tissue N:P ratios indicated N and P availability was balanced (~31:1), seagrass was not affected 37 by nutrient addition but was strongly influenced by disturbance (currents, erosion). Macroalgal 38 and epiphytic and benthic microalgal biomass were variable between sites and treatments. In 39 general, there was no algal overgrowth of the seagrass in enriched conditions, possibly due to the 40 strength of seasonal influences on algal biomass or regulation by grazers. N addition had little 41 effect on any benthic primary producers throughout the bay. Overall, the Florida Bay benthic 42 primary producer community was P-limited, but P-induced alterations of community structure 43 were not uniform among primary producers or across Florida Bay and did not always agree with 44 expected patterns of nutrient limitation based on stoichiometric predictions from field assays of 45 *T. testudinum* tissue N:P ratios.

### Introduction

Increased nutrient input as a consequence of human land use can cause changes in species
composition and primary productivity in terrestrial and aquatic habitats (Borum and Sand-Jensen
1996; Smith et al. 1999; Kennish 2002). Urban and agricultural development in watersheds
render coastal systems particularly susceptible to nutrient input (Nixon 1995; Smith et al. 1999),
making the prediction of nutrient loading effects on coastal systems a key management goal.
Nitrogen (N) is frequently a limiting nutrient in coastal systems, but increasing evidence for
phosphorus (P) limitation suggests that both N and P enrichment are of concern in nearshore
habitats (Howarth 1988).
Frequently documented responses of tropical and subtropical seagrasses to elevated N or P
supply include increases in biomass and productivity (Powell et al. 1989; Tomasko and Lapointe
1991; Lee and Dunton 2000), though consequences of nutrient enrichment vary widely within
and among species and regions. Seagrass beds in carbonate sediments are generally considered to
be P-limited (Short et al. 1985) and may respond strongly to P enrichment, though N-limitation
in carbonate sediments has been documented as well (Udy et al. 1999; Ferdie and Fourqurean
2004). Different nutrient requirements among seagrass species can cause co-occurring species to
be limited by different nutrients (Udy and Dennison 1997). Furthermore, different levels of N or
P limitation for individual seagrass species can occur along regional nutrient availability
gradients (Lee and Dunton 2000; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004).
Macroalgae are important components of seagrass communities as well, but increases in
nutrient supply can cause algal proliferations that overgrow and displace aquatic vegetation
(Duarte 1995; Valiela et al. 1997; Hauxwell et al. 2001; McGlathery 2001). Evidence for both P-
limitation (Lapointe 1989) and N-limitation (Larned 1998) suggests that tropical macroalgal

69 responses to nutrient enrichment are highly species-specific and vary among regions. Like 70 seagrasses, tropical macroalgae may also exhibit intraspecific variation in responses to nutrient 71 enrichment along gradients corresponding to background nutrient influence (Fong et al. 2003). 72 However, algae do not necessarily exhibit the same limitation patterns as co-occurring seagrasses 73 (Ferdie and Fourgurean 2004). 74 Microalgae, both benthic and epiphytic, comprise another important primary producer in 75 seagrass communities (Moncreiff et al. 1992). Microalgal biomass and productivity can increase 76 when enriched with N or P (Nilsson et al. 1991) and can overgrow other aquatic vegetation 77 (Tomasko and Lapointe 1991). However, microalgal responses to nutrient enrichment may also 78 be strongly limited by biotic factors including herbivory (Williams and Ruckelshaus 1993). 79 Florida Bay is a shallow, semi-enclosed system with extensive seagrass beds dominated by 80 Thalassia testudinum (turtle grass). T. testudinum tissue in much of Florida Bay has a N:P ratio 81 of >>30:1 with a maximum of 115:1 (Fourqurean and Zieman 2002), suggesting severe P-82 limitation (Atkinson and Smith 1983). A complex network of shallow carbonate banks within the 83 bay restricts water flow and creates numerous, effectively isolated basins, such that sites in close 84 proximity may have dramatically different stoichiometric patterns. A spatial gradient in N:P of 85 seagrass tissue (Fourqurean et al. 1992) and the water column (Fourqurean et al. 1993) suggest 86 that P availability is highest along the western marine boundary of the Bay and decreases 87 towards the east and north. From this pattern, it has been hypothesized that the marine waters of 88 the Gulf of Mexico are the major P source for Florida Bay (Fourqurean et al. 1992; Fourqurean 89 et al. 1993); these hypotheses have been supported with budgetary calculations (Rudnick et al. 90 1999). There is evidence, however, that N can limit some components of the pelagic primary 91 producers in the western regions of the Bay (Lavrentyev et al. 1998; Tomas et al. 1999), and

arguments have been made that some of the changes in the Florida Bay ecosystem, including loss of seagrass and increases in phytoplankton abundance, may be a result of increased N loading into western Florida Bay (Brand 2002; Lapointe and Barile 2004). Given the gradient of N:P and the finding of N limitation of benthic primary producers offshore towards the Florida Barrier Reef (Ferdie and Fourqurean 2004), such arguments bear testing experimentally.

Our objectives were to evaluate the spatial extent of nutrient (N or P) limitation of each of the major benthic primary producer groups in Florida Bay: seagrass, epiphytes, macroalgae, and benthic microalgae, and characterize the shifts in primary producer community composition following fertilization of the seagrass community. We hypothesized that N and P enrichment would stimulate acute primary producer responses corresponding to the degree of nutrient limitation as predicted by ambient *T. testudinum* tissue N:P ratios along the gradient of P availability in the Bay. We also predicted that nutrient enrichment would shift primary producer community composition towards micro- and macroalgal species with high turnover rates (sensu Duarte 1995).

### Methods

To evaluate the effects of N and P enrichment across a P-availability gradient within Everglades National Park in Florida Bay, we used a three-way split-plot ANOVA design, where the factors were P addition, N addition, and region of the bay. In October 2002 we established six study sites (all depths <2 m), with two sites nested in each of three major regions of the bay: Northeast, Interior/Central, and Gulf, as defined by Zieman et al. (1989) based on macrophyte and sediment characteristics. The two eastern sites (Region "C," Fig. 1) were characterized by a sparse, short *Thalassia testudinum* canopy with some calcareous green macroalgae, primarily *Penicillus* spp., and occurred in an area of severe P-limitation (Fourqurean and Zieman 2002).

The middle two sites (Region "B") occurred in a region of intermediate P-limitation (Fourgurean 115 116 and Zieman 2002) but varied in their vegetation characteristics. The easternmost of these sites 117 (B2) had vegetation characteristics similar to those in region C, while the westernmost site (B1) 118 featured a dense, tall *T. testudinum* canopy and little macroalgae. The westernmost two sites 119 (Region "A") were located in a region that may experience both N- and P-limitation. The eastern 120 site in region A (A2) had vegetation characteristics similar to site B1. The western site (A1) was 121 characterized by a dense macroalgal-Syringodium filiforme (manatee grass)-T. testudinum assemblage. At each site we established 24 0.25 m<sup>2</sup> study plots demarcated with a PVC frame 122 123 secured to the benthos at one meter intervals. 124 We randomly assigned treatments [control (C), nitrogen only (N), phosphorus only (P), both 125 nitrogen and phosphorus (NP)] to six plots per site (at site A1, n = 3 due to the loss of 12 plots 126 from erosion and boat disturbance over the course of the study). Nitrogen was added in the form 127 of slow release nitrogen fertilizer (Polyon™, Pursell Technologies Inc., 38-0-0, 94% N as urea) 128 and phosphorus as granular phosphate rock (Multifos™, IMC Global, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 18% P). Loading rates of 1.43 g N m<sup>-2</sup> day<sup>-1</sup> and 0.18 g P m<sup>-2</sup> day<sup>-1</sup> (molar N:P ratio 17.6:1) were selected 129 130 based on potential sewage loading rates (MCSM 2001) and previous studies in the region (Ferdie 131 and Fourgurean 2004). We began bimonthly applications of fertilizer in October 2002 by 132 sprinkling granular fertilizer evenly on the sediment surface and gently working it into the 133 sediment by hand. Sediment in the control plots was similarly disturbed but no fertilizer was 134 added. Benthic fertilizer applications ensured accessibility of nutrients to both above-ground and 135 benthic primary producers (Ferdie and Fourgurean 2004; Mutchler et al. 2004). 136 In February 2004 we determined sediment, seagrass tissue, and seagrass epiphyte N and P 137 content to assess the effectiveness of the enrichment treatments. Macroalgal tissue nutrient

content was not determined due to insufficient algal abundance and the occurrence of different species at each site. Sediment cores (1 cm diameter, 5 cm deep) were collected from each plot and dried at 60°C. Two T. testudinum short-shoots were collected from each plot and the epiphytes removed by gently scraping the blades with a razor blade. At site A1, nutrient content of S. filiforme tissue was also determined. Nitrogen content of the sediment, epiphytes, and the cleaned seagrass tissue were determined separately using a CHN analyzer (Fisons NA1500). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourgurean et al. 1992). Using methods based on extensive monitoring in this region (Fourgurean et al. 2002), we estimated seagrass (T. testudinum, S. filiforme, and Halodule wrightii) and macroalgal percent cover with a modified Braun-Blanquet (BB) abundance scale: 0 = absent; 0.1 = one individual, <5% cover; 0.5 = few individuals, <5% cover; 1 = many individuals, <5% cover; 2 = 5-25% cover; 3 = 25-50% cover; 4 = 50-75% cover; 5 = 75-100% cover. To measure *T. testudinum* productivity, two haphazardly selected short-shoots in each plot were marked at the base of the leaves with an 18-gauge hypodermic needle and labeled with a cable tie for identification. We collected the shoots after 11-13 days, recorded the increase in leaf lengths, and calculated productivity (mg short-shoot<sup>-1</sup> d<sup>-1</sup>). We limited our productivity measurements to two shoots to minimize impact and facilitate future long-term sampling of this experiment All epiphytes were removed from one short-shoot from each plot as above. Epiphytes were freeze dried and pigments extracted with 90% acetone. The chlorophyll a concentration was determined using a Shimadzu RF-Mini 150 fluorometer with low bandwidth filters (Welschmeyer 1994) following calibration with a chlorophyll a standard using a Shimadzu UV

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Mini 1240 spectrophotometer and the spectrophotometric equations of Jeffrey and Humphrey
 (1975).
 To determine benthic microalgal biomass, we collected a 2.5 cm diameter, 1 cm deep core
 haphazardly located within each plot. Sediments were freeze dried, pigments extracted with 90%

acetone, and chlorophyll a concentration determined as for the epiphytes.

The variances of all data were tested for homoscedasticity using the  $F_{max}$  test and log transformed if necessary to conform to the assumptions of ANOVA. All data were analyzed with three-way split-plot ANOVA, where the factors were  $\pm$  P,  $\pm$  N, and region (A, B, C) with sites (A1, A2, B1, B2, C1, C2) nested within regions. Region, P, and N factors were considered fixed and sites were random for this ANOVA model. The linear model for this design was:

(response variable) $_{ijklm} = \mu + (\text{region})_i + (P \text{ addition})_k + (N \text{ addition})_l + (\text{interaction between region and P})_{ik} + (\text{interaction between region and N})_{il} + (\text{interaction between P and N})_{kl} + (\text{interaction between region, P, and N})_{ikl} + (\text{site within region})_{j(i)} + (\text{interaction between site within region and P})_{j(i)k} + (\text{interaction between site within region and N})_{j(i)kl} + (\text{interaction between site within region and N})_{j(i)kl} + (\text{interaction between site within region, P, and N})_{j(i)kl} + \epsilon_{ijklm}$ 

where  $\mu$  is the overall mean,  $\epsilon$  is the unexplained error, i, j, k, and l represent the levels within each factor, and m is the number of observations per site per nutrient treatment (Quinn and Keough 2002). Site A1 was excluded from analyses of  $\underline{T}$ .  $\underline{testudinum}$  productivity and epiphyte nutrient content due to insufficient replication, as several plots at that site contained only one  $\underline{T}$ .  $\underline{testudinum}$  short-shoot, and we prioritized the epiphyte chlorophyll a and seagrass tissue nutrient analyses.

#### Results

Sediment, seagrass, and epiphyte nutrient content reflected nutrient treatments, though the magnitude of the responses varied among regions and sites. Significant region\*P and site\*P interactions for sediment %P (Table 1) suggested that although sediment P content increased at all sites when P was added, the magnitude of increase varied among sites within regions. All sites had low P content (<0.05%) prior to P addition, but sites A2 and B1 appeared to have the largest increases when P was added (Fig. 2a). Sediment N content was significantly affected by region, site, and N addition. Overall, sediment %N increased when N was added, but the increase was generally less than 50% over control levels (Fig. 2b). Sediment N content was lower in region C than in regions A and B and highest overall in sites A2 and B1. Significant region\*P and site\*P\*N interactions suggested that responses of *T. testudinum* tissue %P content to nutrient treatments varied among sites within regions but that there was no clear regional pattern (Table 1). Overall, T. testudinum P content increased by up to 300% when

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P was added at all sites except A1, where control and enriched levels of P were similar to each other and to enriched P levels at other sites (Fig. 3a). However, at sites B2 and C2, P addition did not increase tissue P content as much when N was also added. Tissue %N content significantly increased in response to N addition, though the change was small (10-20% increase, Fig. 3b), relative to the P responses. Significant site\*P and region\*P interactions suggested that tissue %N content response to P enrichment varied among sites within regions. Tissue %N content increased when P was also added, but only at sites B2 and C1. In addition, at site A1, N content decreased when P was added. Significant region\*P and site\*P\*N interactions for T. testudinum molar N:P ratios closely followed the site-specific but not region-specific tissue %P responses. In control plots, the *T. testudinum* N:P ratio was ~30:1 at site A1, ~60:1 at sites A2 and B1, and ~100:1 at sites B2, C1, and C2 (Fig. 3c). At all sites except A1, P addition lowered T. testudinum

N:P ratios to values approaching those at A1, though the largest relative decreases occurred at sites B2 and C2. At those two sites, N:P ratios appeared to be lower when P was added alone than when P and N were added together. S. filiforme tissue nutrient content did not vary with nutrient treatment, but did have slightly higher P (mean  $0.24 \pm 0.01$  SE %P) and N ( $2.45 \pm 0.09$ %N) content and a lower N:P ratio (22.58  $\pm$  1.27 N:P) than T. testudinum at site A1. The nutrient content of <u>T. testudinum</u> epiphytes also showed complex responses to nutrient addition. Significant region\*P and site\*P interactions for epiphyte P content stemmed from larger increases in %P following P addition at sites B2 and C2 than at the other sites with no distinct regional pattern (Table 1, Fig. 4a). No epiphyte elemental analyses were performed for site A1 due to insufficient T. testudinum tissue available for collection. Epiphyte N content was lower in region C than in the other regions and variable among sites, with the highest content at sites A2 and B1, but was not affected by nutrient addition treatment (Fig. 4b). Significant region\*P and site\*P interactions for epiphyte molar N:P ratios were driven by large P-induced decreases in N:P ratios at all sites except A2 (Fig. 4c). In addition, epiphyte N:P ratios were significantly higher when N was added in all regions, though the magnitude of the N effect was smaller than the P effect. T. testudinum cover and productivity generally responded positively to P addition but were unaffected by N addition. A significant interaction between site and P for T. testudinum cover stemmed from large P-induced increases in cover at sites B2, C1, and C2 (Table 2, Fig 5a). The strong site-specific responses of *T. testudinum* cover to P addition obscured any regional patterns. At sites B2, C1, and C2, control plots had a Braun-Blanquet (BB) score of ~2, which corresponds to about 25% cover. P addition plots had BB scores of 3 or 4, corresponding to 50-

75% cover. T. testudinum productivity varied significantly with P and N addition but strong site-

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228 specific responses masked regional trends. Productivity increased with both N and P addition 229 relative to controls, though productivity tended to be similar between P and NP plots at sites B2, 230 C1, and C2 (Fig. 5b). In addition, productivity tended to be higher at sites A2 and B1 than at the 231 other sites. Site A1 was excluded from the productivity analysis due to insufficient T. testudinum 232 tissue available for collection. Two additional species of seagrass occurred at some study sites. Syringodium filiforme was 233 234 found in all plots at site A1 throughout the study period, but percent cover was similar across 235 nutrient treatments (average BB score  $3.1 \pm 0.3$ ). <u>Halodule wrightii</u> colonized one NP plot at 236 both sites A2 and B1 and comprised 25-50% cover in those plots. 237 Macroalgal cover showed group-specific responses to nutrient treatment that varied widely 238 among regions and between sites within regions. Filamentous and other uncalcified, branching 239 red algae (Div. Rhodophyta) and calcified green algae (Div. Chlorophyta) were the most 240 common groups. A significant region\*P interaction suggested that cover of red macroalgae 241 (especially the epiphytic species *Polysiphonia binneyi*, *Ceramium brevizonatum* var. *caribicum*, 242 and *Chondria* sp.) increased in response to P addition, but primarily at sites in region C (Table 2; 243 Fig. 5c). Percent cover tended to be highest overall at site A1, and red algae were not detected at 244 site B1 or in the control and N only plots at site B2. A site\*N interaction suggested that calcified 245 green macroalgal cover (especially the benthic genus *Penicillus* spp.) response to nutrient 246 addition differed among sites, but high variability among sites obscured regional patterns (Fig. 247 5d). A slight N-induced increase in cover occurred at site C2 and a small N-induced decrease in 248 cover occurred at site A1. Calcareous green algae were not detected at sites A2 or B1. 249 Epiphyte loads were highly variable among regions and sites and exhibited complex

responses to nutrient treatments. A significant site\*P\*N interactions suggested that epiphyte

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chlorophyll *a* concentration exhibited strong site-specific responses to nutrients that masked regional patterns (Table 2). The highest chlorophyll *a* concentrations occurred at sites A1 and B2 (Fig. 5e). There was a strong decrease in chlorophyll *a* in the P and NP treatments at site B2, an increase in the P and NP treatments at site C1, and no nutrient effects at sites A1, A2, B1, or C2.

Benthic microalgal biomass, as estimated by benthic chlorophyll *a* concentration, exhibited region-specific but generally weak responses to P addition, as suggested by a significant region\*P interaction (Table 2). P addition slightly increased benthic chlorophyll *a* in region C (Fig. 5f). Similar trends occurred in region A, though variability was high at site A1. Benthic microalgal biomass did not respond to P addition treatment at sites in region B, and no N effects were detected throughout the bay. A significant site effect stemmed from higher benthic chlorophyll *a* concentrations at sites A1 and B2 than the other sites.

### **Discussion**

Stoichiometric evidence from seagrass tissue N:P ratios suggests widespread phosphorus limitation in tropical seagrass communities on carbonate sediments (Atkinson and Smith 1983; Fourqurean et al. 1992). This prediction has been corroborated by experimental evidence that demonstrated positive seagrass responses to P addition (Short et al. 1985) and low porewater P concentration in carbonate sediments (McGlathery et al. 2001). P-limitation in these cases may be attributed to the adsorption of phosphate to carbonate sediments (Koch et al. 2001) and the augmentation of N supply through nitrogen fixation in systems like Florida Bay with long water residence times (Howarth 1988). Accordingly, in our study there was little evidence for N limitation for any of the benthic primary producers throughout the bay. This agrees with predictions from studies of seagrass tissue nutrient content, which suggest that %N content above 1.8% indicates that N is not limiting (Duarte 1990), and seagrass tissue %N at all of our study

sites was ~2%. However, we underestimated the complexity of the benthic primary producer responses to P enrichment. Recent work finds that there is no general pattern of P-limitation on carbonate sediments (Ferdie and Fourqurean 2004), possibly due to an active dissolution of carbonate sediments in the rhizosphere of seagrass beds (Burdige and Zimmerman 2002), making P sorbed to sediments available for root uptake (Jensen et al. 1998). Thus, relative supply of N and P are probably the most important determinants of the limiting nutrient, even in carbonate sediments (Erftemeijer et al. 1994).

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T. testudinum responses to P enrichment exhibited strong site-specific variation that corresponded with the ambient seagrass N:P ratio at each site but did not follow the regional patterns we expected. The eastern bay was severely P-limited, as indicated by the high (~100:1) seagrass N:P ratios in unenriched plots, and P addition caused marked increases in seagrass cover and growth. However, the impacts of P addition were not restricted to the two sites in region C; site B2 had a similarly high ambient N:P ratio and increase in cover and productivity following P enrichment. In contrast, there was little cover or growth response to P addition in the three western sites, despite tissue P content that fell well below the 0.2% DW threshold that suggests P limitation (Duarte 1990) at all sites except A1. Variation in growth responses was introduced by sampling only two shoots per plot in order to preserve the integrity of the canopy and facilitate future observations in this long term study, but relative differences among sites suggest that productivity responses to nutrients were much less pronounced in the western than in the eastern sites. At sites A2 and B1, T. testudinum had similar N:P ratios in control plots (~60:1) and exhibited similar responses to nutrient addition, despite the presence of a large, shallow bank between the two sites that limited oceanic and Gulf of Mexico water input to the Interior Bay region (region B). It is not unusual to encounter site-specific patterns of nutrient

limitations in tropical seagrass communities (Agawin et al. 1996; Udy and Dennison 1997), but the spatial complexity we encountered did not follow topographic contours as we expected.

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Further complexity in responses to P enrichment was revealed by the generally weak microand macroalgal responses to nutrient addition throughout the bay. Epiphytic and macroalgal overgrowth of seagrass frequently occurs in nutrient enriched conditions in both temperate (Valiela et al. 1997; Hauxwell et al. 2001; Cardoso et al. 2004) and tropical (Tomasko and Lapointe 1991; McGlathery 2001) habitats, yet this expected algal proliferation did not occur in our study. In fact, a decrease in epiphyte load occurred in P-addition treatments at site B2. Increased T. testudinum leaf productivity may have reduced leaf life span and decreased the amount of time for epiphyte accumulation, a pattern similar to that seen in N-limited seagrass beds offshore from the Florida Keys (Ferdie and Fourgurean 2004). However, differences in T. testudinum specific leaf productivity in Florida Bay account for less variation in epiphyte loading than grazer abundance and nutrients (Frankovich and Zieman 2005), suggesting that increased leaf productivity in enriched plots at site B2 only partially explains lower epiphyte loads. Benthic microalgal biomass can also increase in enriched conditions (Nilsson et al. 1991), but we detected little microphytobenthic response. Although we disturbed the sediments every two months during the addition of fertilizer, our activities were unlikely to obscure microalgal responses to nutrient addition because benthic microalgae are often dominated by biraphid pennate diatoms that have well-developed motility, allowing rapid migration back to the sediment surface following small-scale bioturbation events (Admiraal 1984). It is possible that the lack of response of the epiphytic and macroalgal primary producers may have been a result of the mode of nutrient delivery via fertilization of the sediments. It has been suggested that seagrass epiphytes and some macroalgae are unable to utilize sediment nutrient pools

(Erftemeijer et al. 1994). In our study, substantial portions of the added nutrients, particularly phosphorus, were retained in the sediment, but we did elicit changes in the nutrient content and N:P ratios of the seagrass and epiphyte assemblages in a pattern consistent with increased N and P availability in fertilized plots. Furthermore, Ferdie and Fourqurean (2004) used an identical fertilization protocol in higher energy sites near the Florida Barrier Reef and detected nitrogen responses in both seagrass and epiphytes, demonstrating that this fertilization technique was effective in enriching both benthic and aboveground producers. Thus, the unexpected lack of responses to P addition by both seagrass and algae in seemingly P-limited habitats was probably not due to inaccessibility of the added nutrients but was the likely result of multiple alternative biotic and abiotic factors.

Phosphorus storage in seagrass and algal tissue without subsequent growth or other morphological responses at some sites suggests that another nutrient may have been regulating plant growth. Iron availability may limit seagrasses or algal assemblages, particularly those in carbonate sediments (Duarte et al. 1995). However, experimental Fe additions have yielded few micro- or macroalgal (Kuffner and Paul 2001) or seagrass (Chambers et al. 2001) responses in tropical habitats. Silica availability can control diatom growth when N and P are in excess (Carrick and Lowe 1988). This mechanism may have been important in the benthic microalgal community in our study, which is diatom-dominated (Lewis et al. 2000), but Florida Bay seagrass epiphyte loads are typically dominated by calcium carbonate (coralline algae and adhered sediment) rather than diatoms (Frankovich and Zieman 1994), so silica availability probably did not limit epiphyte responses to N and P addition.

Competition for other limiting resources, such as light (Ibarra-Obando et al. 2004) and space (Marbà and Duarte 2003) may have prevented primary producers from responding to P addition

in the western portions of the bay. Sites A2 and B1 were generally less turbid than sites in the eastern bay, but <u>T. testudinum</u> shoot densities at those sites (~500-800 short-shoots m<sup>-2</sup>) approached the maxima recorded in other studies in this region (Zieman et al. 1999), suggesting that the canopy may have been saturated and that self-shading was occurring. In addition, shading from a P-enriched <u>T. testudinum</u> canopy may have inhibited the response of benthic microalgae to enriched conditions across all sites.

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Grazing can structure temperate and tropical seagrass communities through direct consumption of seagrass (McGlathery 1995; Valentine and Heck 2001), controlling epiphyte growth (Williams and Ruckelshaus 1993; Heck et al. 2000), and regulating benthic microalgal production (Nilsson et al. 1991). Therefore, grazing pressure may have limited *T. testudinum* and algal responses to excess nutrients at the middle bay sites and algal responses throughout the bay. Grazers known to regulate epiphyte assemblages, including snails (van Montfrans et al. 1982) and grass shrimp (Zupo and Nelson 1999) are frequently found in Florida Bay (McClanahan 1992; Matheson et al. 1999; Frankovich and Zieman 2005). Preferential grazing on nutrientenriched plant tissue, a pattern observed in algal (Boyer et al. 2004) and seagrass assemblages (McGlathery 1995), may compensate for nutrient-induced increases in plant biomass. Though this may have contributed to the control of nutrient-induced micro- or macroalgal growth, the most common seagrass grazer in Florida Bay, the pink urchin Lytechinus variegatus, does not exhibit a preference for nutrient-enriched seagrass (McGlathery 1995; Valentine and Heck 2001) and was seldom observed in the study areas (Armitage et al., unpub. data) and was therefore unlikely to mitigate *T. testudinum* response to nutrients.

Species-specific seasonal fluctuations in primary producer biomass and productivity may result in temporally variable responses to excess nutrients. Wintertime peaks in epiphyte

(Frankovich and Zieman 1994) and rhodophyte (Collado-Vides et al. 1994) biomass in tropical regions suggest that algal responses to nutrient enrichment might have been most pronounced at the time of year of our sampling effort. Our continued monitoring of this project supports this assertion, as the proliferations of red algae in P addition treatments in the eastern bay had largely senesced by May 2004 (Armitage et al., unpub. data). In addition, we monitored this experiment on a bimonthly basis since its inception and no qualitative algal proliferations were observed at any time in this study until our sampling in February 2004, further suggesting that the macroalgal responses were ephemeral.

T. testudinum may have responded to nutrient addition in a manner our measurements did not detect because belowground characteristics were not sampled in order to minimize impact and facilitate future long-term sampling of this experiment. Thus, it is possible that nutrient addition was disproportionately allocated to the root/rhizome complex, particularly at sites A2 and B1, where no aboveground response was detected. However, fertilizer-induced changes in shoot:root allocation generally result from an increase in shoot biomass (Lee and Dunton 2000)

allocation generally result from an increase in shoot biomass (Lee and Dunton 2000). Alternatively, excess P may have been translocated along the rhizome system to new growth outside the treatment plot (Marbà et al. 2002) and was thus undetected in our sampling protocol, though our qualitative observations suggest that the fertilization effects were strongly localized around the treatment plots.

The western site with an ambient <u>T. testudinum</u> tissue N:P ratio of 30:1 was not affected by N or P addition, suggesting a balance between N and P supply (Atkinson and Smith 1983).

Accordingly, <u>T. testudinum</u> tissue phosphorus content at that site was high (Duarte 1990), and the diverse macroalgal assemblage and the abundance of <u>Syringodium filiforme</u>, a seagrass species associated with enriched conditions (Ferdie and Fourqurean 2004), reflected the elevated

nutrient supply. However, this site was heavily impacted by factors other than nutrient supply, particularly frequent disturbances, both through current and erosion processes as well as boat traffic, illustrating the range of factors that may regulate benthic primary producer assemblages.

The replacement of slower growing species with more opportunistic ones in nutrient enriched conditions is a commonly observed occurrence in terrestrial (Bargali 1997), freshwater (Craft and Richardson 1997), and marine habitats (Duarte 1995; Valiela et al. 1997; McGlathery 2001). However, in our study, there was no suggestion of macroalgal replacement of seagrass, possibly due to the seasonal nature of macroalgae in Florida Bay. Shoalgrass *Halodule wrightii* began to colonize some of the NP plots, and though its occurrence was patchy at the time of this study, continued fertilization may eventually lead to *H. wrightii* replacement of *T. testudinum*, a phenomenon observed following two years of increased nutrient supply in a separate study in Florida Bay (Fourqurean et al. 1995).

Our results bear directly on the hypothesis that increased freshwater, and therefore N loading from the Everglades ecosystem as a result of restoration efforts could change the nature of seagrass communities of Florida Bay (Brand 2002; Lapointe and Barile 2004). Direct addition of P to eastern Florida Bay seagrass beds caused marked changes in community structure in the short time course of our experiments, but N addition had almost no impact on primary producers in any region of the Bay. These results suggest that an increase in N loading to Florida Bay would have very little direct impact on seagrass communities. However, some bioassay experiments have shown N limitation within the western Bay phytoplankton communities (Lavrentyev et al. 1998; Tomas et al. 1999), so it is still possible that N loading could negatively impact seagrass communities by promoting phytoplankton growth, leading to light limitation of benthic plants. The spatial scale of our experiments was not appropriate for testing this idea, as

any phytoplankton bloom stimulated in our small study plots would have quickly been washed away. Further, as the seagrass communities of the adjacent Florida Keys National Marine Sanctuary are N-limited (Ferdie and Fourqurean 2004), increases in N loading to Florida Bay could have an effect on the adjacent, downstream communities without directly altering seagrass communities within the Bay.

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Stoichiometric ratios are widely used to predict nutrient limitation, where a N:P ratio of 30:1 represents the modified Redfield ratio for seagrasses (e.g., Atkinson and Smith 1983; Fourqurean and Zieman 2002). However, our study adds to the growing body of evidence that although very high or very low stoichiometric ratios represent P or N (respectively) limitation, a broad middle range of N:P ratios (~20-60:1) does not yield consistent predictions of patterns of nutrient limitation in seagrasses. Although the breadth of that middle range might be a function of the relatively short time period of this study, several experimental studies provide further support for this conclusion. For example, four species of seagrass in Australia had similar (~20:1) molar N:P ratios, but each species exhibited a unique response to N and P addition: strong N limitation, weak N limitation, N and P balance, and no nutrient response were all observed (Udy and Dennison 1997; Udy et al. 1999). In another study on the ocean side of the Florida Keys, seagrass tissue N:P ratios (~40:1) suggested P-limitation in nearshore seagrass beds (Fourqurean and Zieman 2002). However, experimental nutrient enrichment demonstrated N-limitation in the macro- and microalgae but few effects of N or P enrichment on the seagrass (Ferdie and Fourquean 2004), illustrating that predictions of nutrient limitation from stoichiometric ratios may not apply uniformly to all primary producers within a community. In addition, other biotic or abiotic factors, such as grazing pressure, space, or the level of disturbance, may exert strong influences on primary producer responses to nutrient enrichment. Supplementing stoichiometric

field assays with experimental manipulations will enable more accurate predictions of the impacts of nutrient enrichment on coastal habitats.

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Table 1: Results from three-way split-plot ANOVA of P, N, and bay region, with site nested within region, on N and P content of the sediment, *Thalassia testudinum* tissue, and epiphytes.

	df	MS	F	p	df	MS	F	p
	Sedir	nent %P			Sedir	nent %N		
Region	2	373.91	365.20	< 0.0001	2	3.83	106.36	< 0.0001
P	1	1640.11	1601.92	< 0.0001	1	0.01	0.20	0.6580
N	1	1.60	1.57	0.2134	1	0.82	22.71	<0.0001
P*N	1	0.35	0.35	0.5577	1	< 0.01	0.01	0.9403
Region*P	2	300.37	293.38	<0.0001	2	0.07	1.87	0.1597
Region*N	2	1.75	1.71	0.1851	2	0.01	0.22	0.8053
Region*P*N	2	0.36	0.35	0.7025	2	0.01	0.35	0.7078
Site(Region)	3	60.95	59.53	< 0.0001	3	1.52	42.17	< 0.0001
Site(Region)*P	3	19.58	19.12	< 0.0001	3	0.04	1.03	0.3821
Site(Region)*N	3	0.81	0.79	0.5002	3	0.08	2.24	0.0874
Site(Region)*P*N	3	0.25	0.24	0.8663	3	0.03	0.85	0.4723
Residual	108	1.02			108	0.04		

### Table 1 (cont.):

	df	MS	F	p	df	MS	$\boldsymbol{\mathit{F}}$	P	df	MS	F	P	
	<u>T. tes</u>	<u>tudinum</u>	%P		T. tes	<u>studinum</u>	%N	%N <u>T. tes</u>			studinum N:P		
Region	2	0.71	70.98	< 0.0001	2	0.03	25.73	< 0.0001	2	0.55	60.89	<0.0001	
P	1	1.75	175.44	< 0.0001	1	< 0.01	2.46	0.1196	1	1.61	177.76	< 0.0001	
N	1	0.11	10.69	0.0014	1	0.01	8.47	0.0044	1	0.18	20.24	< 0.0001	
P*N	1	0.05	4.67	0.0329	1	<0.01	<0.01	0.9807	1	0.05	5.20	0.0245	
Region*P	2	0.25	25.53	<0.0001	2	0.01	5.93	0.0036	2	0.18	19.56	<0.0001	
Region*N	2	0.01	1.13	0.3277	2	< 0.01	0.15	0.8603	2	0.01	1.56	0.2152	
Region*P*N	2	0.02	2.37	0.0985	2	<0.01	0.58	0.5616	2	0.02	2.29	0.1059	
Site(Region)	3	0.20	20.13	<0.0001	3	0.05	45.63	<0.0001	3	0.41	45.67	< 0.0001	
Site(Region)*P	3	0.08	8.45	< 0.0001	3	<0.01	2.72	0.0481	3	0.07	7.63	< 0.0001	
Site(Region)*N	3	0.01	0.96	0.4164	3	<0.01	1.17	0.3231	3	0.01	1.29	0.2832	
Site(Region)*P*N	3	0.03	3.33	0.0225	3	<0.01	0.66	0.5811	3	0.03	3.30	0.0233	
Residual	107	0.01			107	< 0.01			107	0.01			

### Table 1 (cont.):

	df	MS	F	p	df	MS	F	p	df	MS	F	p
	Epip	phyte %	P		Epij	ohyte %l	N	Epiphyte N:P				
Region	2	0.55	19.24	< 0.0001	2	0.21	16.57	< 0.0001	2	0.12	6.23	0.0030
P	1	1.32	45.93	< 0.0001	1	0.02	1.72	0.1932	1	1.13	56.86	< 0.0001
N	1	0.04	1.38	0.2427	1	0.03	2.02	0.1584	1	0.11	5.45	0.0219
P*N	1	< 0.01	0.07	0.7988	1	0.04	2.84	0.0951	1	0.01	0.68	0.4127
Region*P	2	0.13	4.62	0.0124	2	0.01	0.59	0.5578	2	0.16	8.01	0.0006
Region*N	2	< 0.01	0.05	0.9466	2	0.01	1.04	0.3577	2	<0.01	0.20	0.8217
Region*P*N	2	< 0.01	0.04	0.9638	2	0.01	0.96	0.3858	2	0.01	0.45	0.6368
Site(Region)	2	0.11	3.64	0.0303	2	0.07	5.60	0.0050	2	0.18	9.10	0.0003
Site(Region)*P	2	0.21	7.31	0.0012	2	0.03	1.95	0.1482	2	0.11	5.73	0.0046
Site(Region)*N	2	0.07	2.41	0.0958	2	< 0.01	0.23	0.7978	2	0.02	1.20	0.3069
Site(Region)*P*N	2	0.01	0.17	0.8445	2	0.01	0.37	0.6905	2	0.01	0.65	0.5228
Residual	87	0.03			97	0.01			87	0.02		

Table 2: Results from three-way split-plot ANOVA of P, N, and bay region, with site nested within region, on *Thalassia testudinum*, macroalgae, epiphytic microalgal, and benthic microalgal characteristics.

	df	MS	F	p	df	MS	F	p	df	MS	F	p	
	<u>T. tes</u>	<u>studinum</u>	Cover		<u>T. tes</u>	<u>studinum</u>	Producti	vity	Rhod	Rhodophyta Cover			
Region	2	0.77	41.76	< 0.0001	2	0.42	9.19	0.0002	2	45.34	83.05	<0.0001	
P	1	0.48	26.00	< 0.0001	1	1.18	25.85	< 0.0001	1	2.04	3.73	0.0560	
N	1	<0.01	0.15	0.7027	1	0.31	6.72	0.0110	1	0.43	0.79	0.3767	
P*N	1	<0.01	0.21	0.6519	1	<0.01	0.03	0.8515	1	0.54	0.98	0.3234	
Region*P	2	0.03	1.67	0.1933	2	0.05	1.06	0.3503	2	3.15	5.77	0.0042	
Region*N	2	0.04	2.03	0.1359	2	0.01	0.19	0.8279	2	1.12	2.04	0.1345	
Region*P*N	2	0.01	0.60	0.5491	2	0.08	1.81	0.1683	2	0.30	0.56	0.5753	
Site(Region)	3	1.78	95.72	< 0.0001	2	0.56	12.32	<0.0001	3	15.33	28.09	<0.0001	
Site(Region)*P	3	0.22	11.63	< 0.0001	2	0.05	1.08	0.3436	3	0.95	1.75	0.1613	
Site(Region)*N	3	<0.01	0.17	0.9159	2	<0.01	<0.01	0.9987	3	0.70	1.29	0.2826	
Site(Region)*P*N	3	0.02	1.23	0.3023	2	0.01	0.14	0.8724	3	0.35	0.64	0.5894	
Residual	108	0.02			98	0.05			108	0.55			

Table 2 (cont.):

	df	MS	F	p	df	MS	F	p	df	MS	F	p
	Chlo	rophyta (	Cover		Epipl	yte chlo	orophyll a		Benth			
Region	2	10.33	72.73	< 0.0001	2	0.92	34.28	< 0.0001	2	0.17	7.97	0.0006
P	1	0.01	0.07	0.7906	1	0.10	3.62	0.0598	1	0.33	15.23	0.0002
N	1	0.15	1.09	0.2988	1	0.02	0.69	0.4073	1	0.01	0.28	0.6000
P*N	1	0.01	0.09	0.7594	1	0.06	2.09	0.1513	1	0.01	0.22	0.6433
Region*P	2	0.43	3.01	0.0532	2	0.25	9.39	0.0002	2	0.09	4.31	0.0159
Region*N	2	0.47	3.27	0.0417	2	0.03	1.08	0.3447	2	0.02	0.91	0.4054
Region*P*N	2	0.11	0.75	0.4772	2	0.03	0.94	0.3921	2	0.02	0.99	0.3760
Site(Region)	3	16.66	117.29	< 0.0001	3	4.10	152.69	< 0.0001	3	0.72	32.90	< 0.0001
Site(Region)*P	3	0.27	1.92	0.1312	3	0.31	11.56	< 0.0001	3	0.02	0.79	0.5033
Site(Region)*N	3	0.45	3.20	0.0262	3	0.03	1.03	0.3835	3	0.04	1.78	0.1549
Site(Region)*P*N	3	0.13	0.93	0.4304	3	0.09	3.38	0.0211	3	0.01	0.50	0.6856
Residual	108	0.14			108	0.03			108	0.02		

Figure Captions:

- Fig. 1: Map of Florida Bay and study sites. Bay regions are defined based on delineations from Zieman et al. (1989). Region A: Gulf/Atlantic, Region B: Interior/East Central, Region C: Northeast.
- Fig. 2: Responses of sediment nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight. At site A1, n = 3 per nutrient treatment; at all other sites, n = 6. In all figures, significant p-values are indicated and all bars represent standard error.
- Fig. 3: Responses of <u>Thalassia testudinum</u> nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight, c) N:P molar ratio. At site A1, n = 3 per nutrient treatment; at all other sites, n = 6.
- Fig. 4: Responses of  $\underline{Thalassia\ testudinum}$  epiphyte nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight, c) N:P molar ratio. n = 6 per site per nutrient treatment.  $\Psi$  indicates insufficient replication for analysis. Fig. 5: Responses of primary producers to region and P and N addition: a)  $\underline{Thalassia\ testudinum}$  percent cover, estimated by Braun-Blanquet (BB) score, b)  $\underline{T.\ testudinum}$  short-shoot (ss) productivity, c) uncalcified red algae (Rhodophyta) cover, d) calcified green algae (Chlorophyta) cover, e) epiphyte chlorophyll a concentration, f) benthic chlorophyll a concentration. At site A1, n = 3 per site per nutrient treatment; at all other sites, n = 6.  $\Psi$  indicates insufficient replication for analysis. § indicates no macroalgae present.

Figure 1

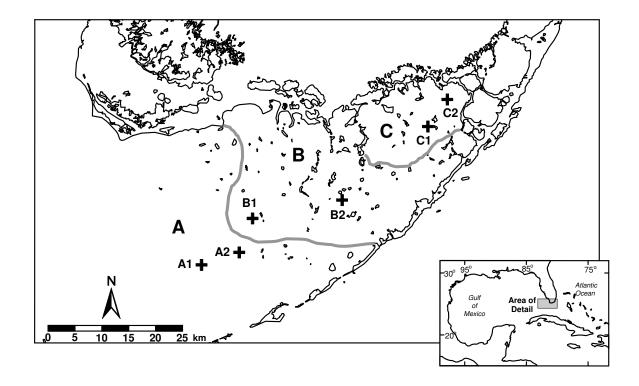


Figure 2

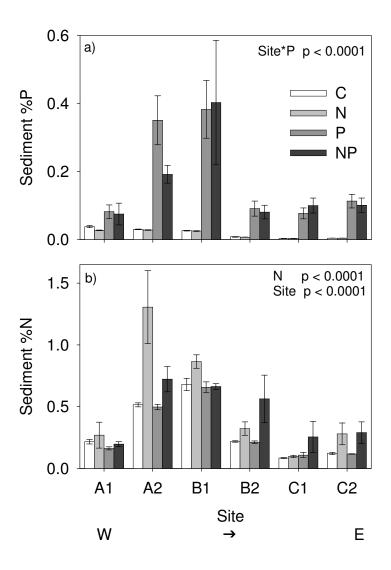


Figure 3

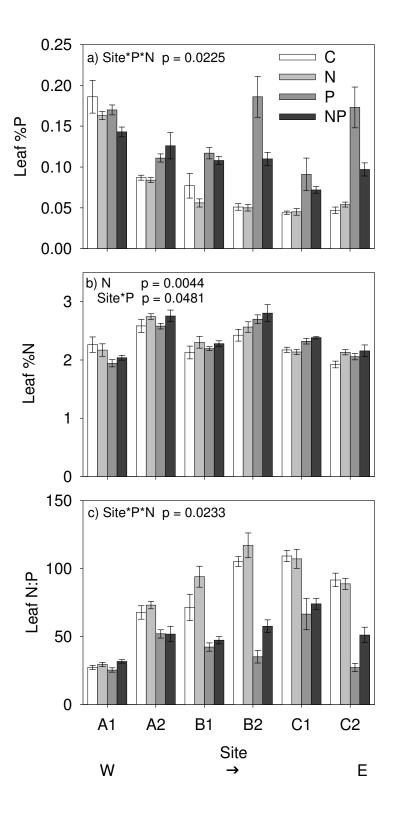


Figure 4

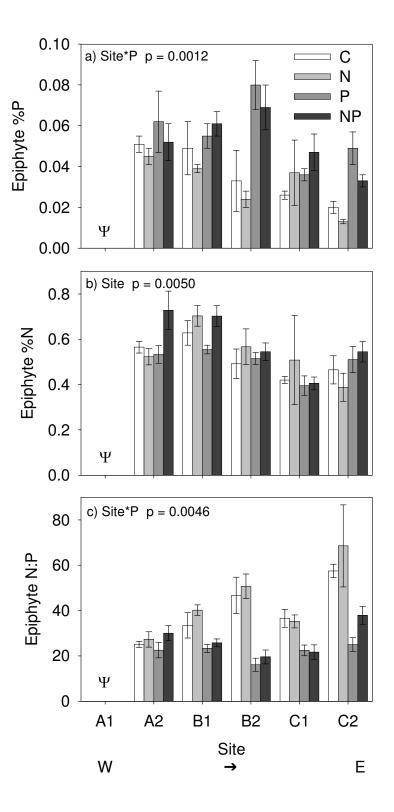


Figure 5

