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

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24 **Abstract**

25 We examined the spatial extent of nitrogen (N) and phosphorus (P) limitation of each of the  
26 major benthic primary producer groups in Florida Bay: seagrass, epiphytes, macroalgae, and  
27 benthic microalgae, and characterized the shifts in primary producer community composition  
28 following nutrient enrichment. We established 24 permanent 0.25 m<sup>2</sup> study plots at each of six  
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.

## 46 **Introduction**

47 Increased nutrient input as a consequence of human land use can cause changes in species  
48 composition and primary productivity in terrestrial and aquatic habitats (Borum and Sand-Jensen  
49 1996; Smith et al. 1999; Kennish 2002). Urban and agricultural development in watersheds  
50 render coastal systems particularly susceptible to nutrient input (Nixon 1995; Smith et al. 1999),  
51 making the prediction of nutrient loading effects on coastal systems a key management goal.  
52 Nitrogen (N) is frequently a limiting nutrient in coastal systems, but increasing evidence for  
53 phosphorus (P) limitation suggests that both N and P enrichment are of concern in nearshore  
54 habitats (Howarth 1988).

55 Frequently documented responses of tropical and subtropical seagrasses to elevated N or P  
56 supply include increases in biomass and productivity (Powell et al. 1989; Tomasko and Lapointe  
57 1991; Lee and Dunton 2000), though consequences of nutrient enrichment vary widely within  
58 and among species and regions. Seagrass beds in carbonate sediments are generally considered to  
59 be P-limited (Short et al. 1985) and may respond strongly to P enrichment, though N-limitation  
60 in carbonate sediments has been documented as well (Udy et al. 1999; Ferdie and Fourqurean  
61 2004). Different nutrient requirements among seagrass species can cause co-occurring species to  
62 be limited by different nutrients (Udy and Dennison 1997). Furthermore, different levels of N or  
63 P limitation for individual seagrass species can occur along regional nutrient availability  
64 gradients (Lee and Dunton 2000; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004).

65 Macroalgae are important components of seagrass communities as well, but increases in  
66 nutrient supply can cause algal proliferations that overgrow and displace aquatic vegetation  
67 (Duarte 1995; Valiela et al. 1997; Hauxwell et al. 2001; McGlathery 2001). Evidence for both P-  
68 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 Fourqurean 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

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

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

## 106 **Methods**

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

115 The middle two sites (Region "B") occurred in a region of intermediate P-limitation (Fourqurean  
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*  
122 assemblage. At each site we established 24 0.25 m<sup>2</sup> study plots demarcated with a PVC frame  
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).  
129 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  
130 based on potential sewage loading rates (MCSM 2001) and previous studies in the region (Ferdie  
131 and Fourqurean 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 Fourqurean 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

138 content was not determined due to insufficient algal abundance and the occurrence of different  
139 species at each site. Sediment cores (1 cm diameter, 5 cm deep) were collected from each plot  
140 and dried at 60°C. Two *T. testudinum* short-shoots were collected from each plot and the  
141 epiphytes removed by gently scraping the blades with a razor blade. At site A1, nutrient content  
142 of *S. filiforme* tissue was also determined. Nitrogen content of the sediment, epiphytes, and the  
143 cleaned seagrass tissue were determined separately using a CHN analyzer (Fisons NA1500).  
144 Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a  
145 colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992).

146 Using methods based on extensive monitoring in this region (Fourqurean et al. 2002), we  
147 estimated seagrass (*T. testudinum*, *S. filiforme*, and *Halodule wrightii*) and macroalgal percent  
148 cover with a modified Braun-Blanquet (BB) abundance scale: 0 = absent; 0.1 = one individual,  
149 <5% cover; 0.5 = few individuals, <5% cover; 1 = many individuals, <5% cover; 2 = 5-25%  
150 cover; 3 = 25-50% cover; 4 = 50-75% cover; 5 = 75-100% cover. To measure *T. testudinum*  
151 productivity, two haphazardly selected short-shoots in each plot were marked at the base of the  
152 leaves with an 18-gauge hypodermic needle and labeled with a cable tie for identification. We  
153 collected the shoots after 11-13 days, recorded the increase in leaf lengths, and calculated  
154 productivity (mg short-shoot<sup>-1</sup> d<sup>-1</sup>). We limited our productivity measurements to two shoots to  
155 minimize impact and facilitate future long-term sampling of this experiment

156 All epiphytes were removed from one short-shoot from each plot as above. Epiphytes were  
157 freeze dried and pigments extracted with 90% acetone. The chlorophyll *a* concentration was  
158 determined using a Shimadzu RF-Mini 150 fluorometer with low bandwidth filters  
159 (Welschmeyer 1994) following calibration with a chlorophyll *a* standard using a Shimadzu UV

160 Mini 1240 spectrophotometer and the spectrophotometric equations of Jeffrey and Humphrey  
 161 (1975).

162 To determine benthic microalgal biomass, we collected a 2.5 cm diameter, 1 cm deep core  
 163 haphazardly located within each plot. Sediments were freeze dried, pigments extracted with 90%  
 164 acetone, and chlorophyll *a* concentration determined as for the epiphytes.

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

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

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

## 181 Results



182 Sediment, seagrass, and epiphyte nutrient content reflected nutrient treatments, though the  
183 magnitude of the responses varied among regions and sites. Significant region\*P and site\*P  
184 interactions for sediment %P (Table 1) suggested that although sediment P content increased at  
185 all sites when P was added, the magnitude of increase varied among sites within regions. All  
186 sites had low P content (<0.05%) prior to P addition, but sites A2 and B1 appeared to have the  
187 largest increases when P was added (Fig. 2a). Sediment N content was significantly affected by  
188 region, site, and N addition. Overall, sediment %N increased when N was added, but the increase  
189 was generally less than 50% over control levels (Fig. 2b). Sediment N content was lower in  
190 region C than in regions A and B and highest overall in sites A2 and B1.

191 Significant region\*P and site\*P\*N interactions suggested that responses of *T. testudinum*  
192 tissue %P content to nutrient treatments varied among sites within regions but that there was no  
193 clear regional pattern (Table 1). Overall, *T. testudinum* P content increased by up to 300% when  
194 P was added at all sites except A1, where control and enriched levels of P were similar to each  
195 other and to enriched P levels at other sites (Fig. 3a). However, at sites B2 and C2, P addition did  
196 not increase tissue P content as much when N was also added. Tissue %N content significantly  
197 increased in response to N addition, though the change was small (10-20% increase, Fig. 3b),  
198 relative to the P responses. Significant site\*P and region\*P interactions suggested that tissue %N  
199 content response to P enrichment varied among sites within regions. Tissue %N content  
200 increased when P was also added, but only at sites B2 and C1. In addition, at site A1, N content  
201 decreased when P was added. Significant region\*P and site\*P\*N interactions for *T. testudinum*  
202 molar N:P ratios closely followed the site-specific but not region-specific tissue %P responses. In  
203 control plots, the *T. testudinum* N:P ratio was ~30:1 at site A1, ~60:1 at sites A2 and B1, and  
204 ~100:1 at sites B2, C1, and C2 (Fig. 3c). At all sites except A1, P addition lowered *T. testudinum*

205 N:P ratios to values approaching those at A1, though the largest relative decreases occurred at  
206 sites B2 and C2. At those two sites, N:P ratios appeared to be lower when P was added alone  
207 than when P and N were added together. *S. filiforme* tissue nutrient content did not vary with  
208 nutrient treatment, but did have slightly higher P (mean  $0.24 \pm 0.01$  SE %P) and N ( $2.45 \pm 0.09$   
209 %N) content and a lower N:P ratio ( $22.58 \pm 1.27$  N:P) than *T. testudinum* at site A1.

210 The nutrient content of *T. testudinum* epiphytes also showed complex responses to nutrient  
211 addition. Significant region\*P and site\*P interactions for epiphyte P content stemmed from  
212 larger increases in %P following P addition at sites B2 and C2 than at the other sites with no  
213 distinct regional pattern (Table 1, Fig. 4a). No epiphyte elemental analyses were performed for  
214 site A1 due to insufficient *T. testudinum* tissue available for collection. Epiphyte N content was  
215 lower in region C than in the other regions and variable among sites, with the highest content at  
216 sites A2 and B1, but was not affected by nutrient addition treatment (Fig. 4b). Significant  
217 region\*P and site\*P interactions for epiphyte molar N:P ratios were driven by large P-induced  
218 decreases in N:P ratios at all sites except A2 (Fig. 4c). In addition, epiphyte N:P ratios were  
219 significantly higher when N was added in all regions, though the magnitude of the N effect was  
220 smaller than the P effect.

221 *T. testudinum* cover and productivity generally responded positively to P addition but were  
222 unaffected by N addition. A significant interaction between site and P for *T. testudinum* cover  
223 stemmed from large P-induced increases in cover at sites B2, C1, and C2 (Table 2, Fig 5a). The  
224 strong site-specific responses of *T. testudinum* cover to P addition obscured any regional  
225 patterns. At sites B2, C1, and C2, control plots had a Braun-Blanquet (BB) score of ~2, which  
226 corresponds to about 25% cover. P addition plots had BB scores of 3 or 4, corresponding to 50-  
227 75% cover. *T. testudinum* productivity varied significantly with P and N addition but strong site-

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.

233 Two additional species of seagrass occurred at some study sites. *Syringodium filiforme* was  
234 found in all plots at site A1 throughout the study period, but percent cover was similar across  
235 nutrient treatments (average BB score  $3.1 \pm 0.3$ ). *Halodule wrightii* 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  
250 responses to nutrient treatments. A significant site\*P\*N interactions suggested that epiphyte

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

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

## 262 **Discussion**

263 Stoichiometric evidence from seagrass tissue N:P ratios suggests widespread phosphorus  
264 limitation in tropical seagrass communities on carbonate sediments (Atkinson and Smith 1983;  
265 Fourqurean et al. 1992). This prediction has been corroborated by experimental evidence that  
266 demonstrated positive seagrass responses to P addition (Short et al. 1985) and low porewater P  
267 concentration in carbonate sediments (McGlathery et al. 2001). P-limitation in these cases may  
268 be attributed to the adsorption of phosphate to carbonate sediments (Koch et al. 2001) and the  
269 augmentation of N supply through nitrogen fixation in systems like Florida Bay with long water  
270 residence times (Howarth 1988). Accordingly, in our study there was little evidence for N  
271 limitation for any of the benthic primary producers throughout the bay. This agrees with  
272 predictions from studies of seagrass tissue nutrient content, which suggest that %N content above  
273 1.8% indicates that N is not limiting (Duarte 1990), and seagrass tissue %N at all of our study

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

281 *T. testudinum* responses to P enrichment exhibited strong site-specific variation that  
282 corresponded with the ambient seagrass N:P ratio at each site but did not follow the regional  
283 patterns we expected. The eastern bay was severely P-limited, as indicated by the high (~100:1)  
284 seagrass N:P ratios in unenriched plots, and P addition caused marked increases in seagrass  
285 cover and growth. However, the impacts of P addition were not restricted to the two sites in  
286 region C; site B2 had a similarly high ambient N:P ratio and increase in cover and productivity  
287 following P enrichment. In contrast, there was little cover or growth response to P addition in the  
288 three western sites, despite tissue P content that fell well below the 0.2% DW threshold that  
289 suggests P limitation (Duarte 1990) at all sites except A1. Variation in growth responses was  
290 introduced by sampling only two shoots per plot in order to preserve the integrity of the canopy  
291 and facilitate future observations in this long term study, but relative differences among sites  
292 suggest that productivity responses to nutrients were much less pronounced in the western than  
293 in the eastern sites. At sites A2 and B1, *T. testudinum* had similar N:P ratios in control plots  
294 (~60:1) and exhibited similar responses to nutrient addition, despite the presence of a large,  
295 shallow bank between the two sites that limited oceanic and Gulf of Mexico water input to the  
296 Interior Bay region (region B). It is not unusual to encounter site-specific patterns of nutrient

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

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

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

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

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

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

349 Grazing can structure temperate and tropical seagrass communities through direct  
350 consumption of seagrass (McGlathery 1995; Valentine and Heck 2001), controlling epiphyte  
351 growth (Williams and Ruckelshaus 1993; Heck et al. 2000), and regulating benthic microalgal  
352 production (Nilsson et al. 1991). Therefore, grazing pressure may have limited *T. testudinum* and  
353 algal responses to excess nutrients at the middle bay sites and algal responses throughout the bay.  
354 Grazers known to regulate epiphyte assemblages, including snails (van Montfrans et al. 1982)  
355 and grass shrimp (Zupo and Nelson 1999) are frequently found in Florida Bay (McClanahan  
356 1992; Matheson et al. 1999; Frankovich and Zieman 2005). Preferential grazing on nutrient-  
357 enriched plant tissue, a pattern observed in algal (Boyer et al. 2004) and seagrass assemblages  
358 (McGlathery 1995), may compensate for nutrient-induced increases in plant biomass. Though  
359 this may have contributed to the control of nutrient-induced micro- or macroalgal growth, the  
360 most common seagrass grazer in Florida Bay, the pink urchin *Lytechinus variegatus*, does not  
361 exhibit a preference for nutrient-enriched seagrass (McGlathery 1995; Valentine and Heck 2001)  
362 and was seldom observed in the study areas (Armitage et al., unpub. data) and was therefore  
363 unlikely to mitigate *T. testudinum* response to nutrients.

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



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

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

380 Alternatively, excess P may have been translocated along the rhizome system to new growth  
381 outside the treatment plot (Marbà et al. 2002) and was thus undetected in our sampling protocol,  
382 though our qualitative observations suggest that the fertilization effects were strongly localized  
383 around the treatment plots.

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

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

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

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

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

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

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

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

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448

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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
	Sediment %P				Sediment %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	<i>F</i>	p	df	MS	<i>F</i>	P	df	MS	<i>F</i>	P
	<u><i>T. testudinum</i> %P</u>				<u><i>T. testudinum</i> %N</u>				<u><i>T. testudinum</i> N:P</u>			
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	<i>F</i>	p	df	MS	<i>F</i>	p	df	MS	<i>F</i>	p
	Epiphyte %P				Epiphyte %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><i>T. testudinum</i> Cover</u>				<u><i>T. testudinum</i> Productivity</u>				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	<i>F</i>	p	df	MS	<i>F</i>	p	df	MS	<i>F</i>	p
	Chlorophyta Cover				Epiphyte chlorophyll <i>a</i>				Benthic chlorophyll <i>a</i>			
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 *Thalassia testudinum* 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 *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) *Thalassia testudinum* percent cover, estimated by Braun-Blanquet (BB) score, b) *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.  $\S$  indicates no macroalgae present.

Figure 1

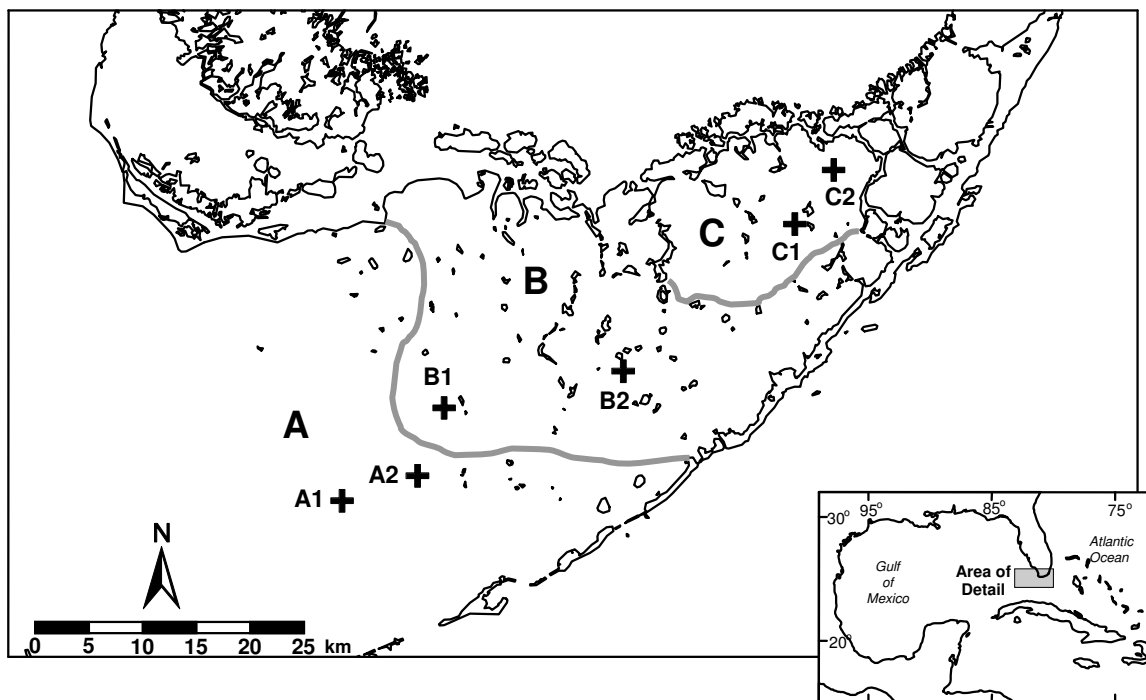


Figure 2

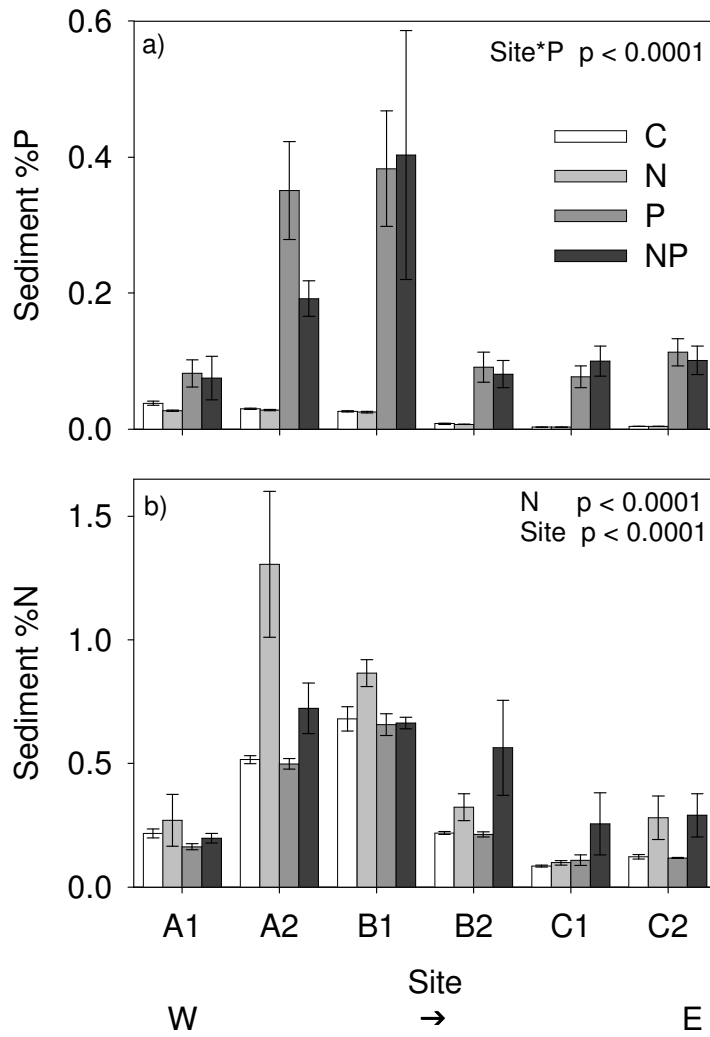


Figure 3

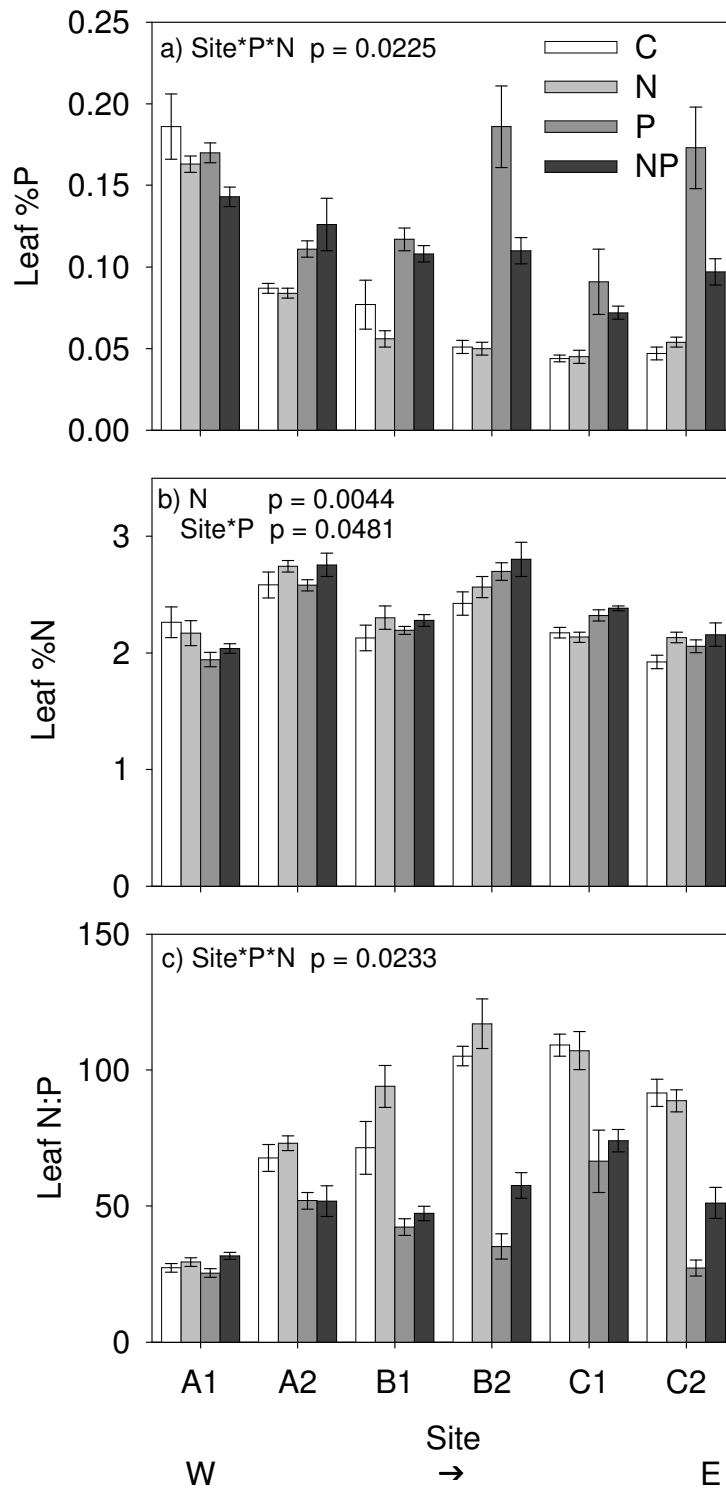


Figure 4

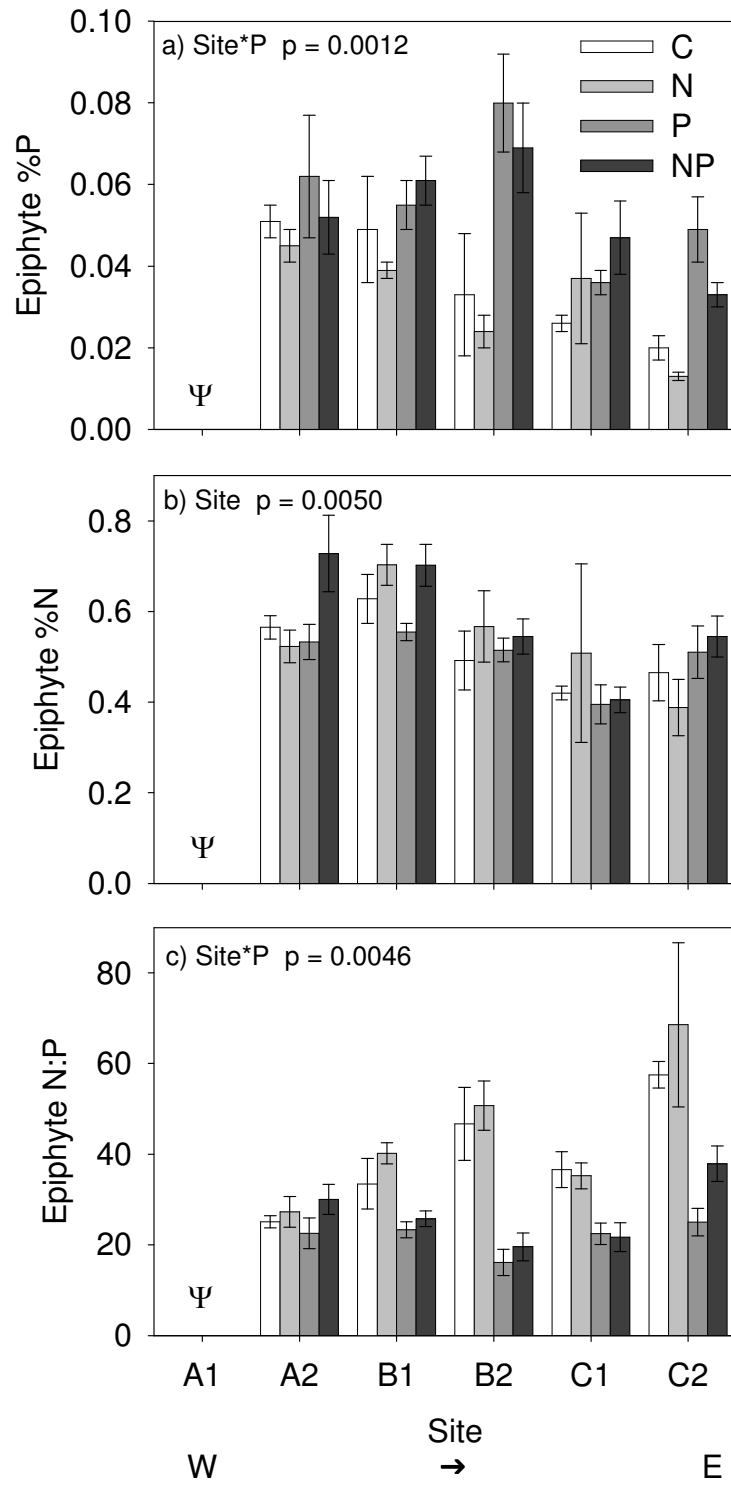


Figure 5

