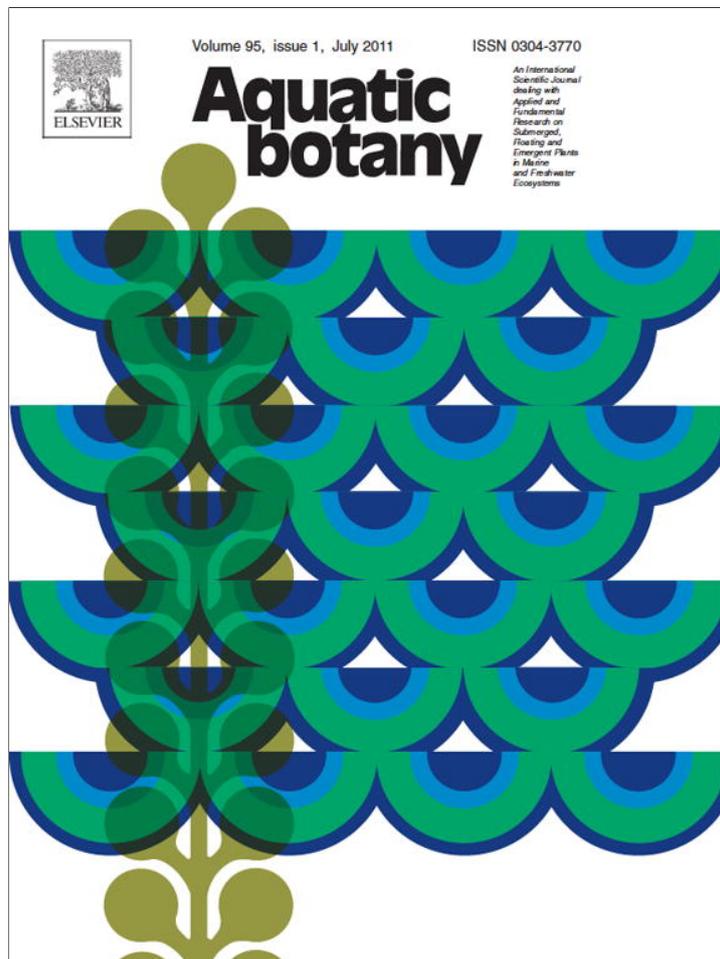


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

# Aquatic Botany

journal homepage: [www.elsevier.com/locate/aquabot](http://www.elsevier.com/locate/aquabot)

## Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh

Wesley A. Ket<sup>a,\*</sup>, Joseph P. Schubauer-Berigan<sup>b</sup>, Christopher B. Craft<sup>a</sup><sup>a</sup> School of Public and Environmental Affairs, Indiana University, 1315 East Tenth Street, Bloomington 47405, IN, USA<sup>b</sup> USEPA, Office of Research and Development National Risk Management Research Laboratory, 26 West Martin Luther King Drive, Cincinnati 45268, OH, USA

### ARTICLE INFO

#### Article history:

Received 28 July 2010

Received in revised form 28 February 2011

Accepted 1 March 2011

Available online 9 March 2011

#### Keywords:

Nutrient limitation

Nitrogen

Phosphorus

Tidal freshwater marsh

Aboveground biomass

Belowground biomass

N:P ratio

Fertilizer

*Zizaniopsis miliacea**Pontederia cordata**Sagittaria lancifolia*

### ABSTRACT

The purpose of this experiment was to determine if nitrogen (N) or phosphorus (P) acts as the limiting nutrient for tidal freshwater marsh vegetation. To answer this question, we added N, P, and N+P to a tidal freshwater marsh dominated by *Zizaniopsis miliacea* (Michx.) (giant cutgrass) in Georgia, USA, for five years to determine their effects on aboveground and belowground biomass and nutrient (N, P) uptake. Nitrogen and P were applied twice per year at an annual rate of 50 g m<sup>-2</sup> year<sup>-1</sup> and 10 g m<sup>-2</sup> year<sup>-1</sup>, respectively. Aboveground biomass and leaf C, N, and P were sampled in August of each year. Belowground biomass and C, N, and P content were measured in August of year five. After two years, plots receiving N and N+P had significantly greater aboveground biomass than the control and P plots. This trend continued through the fifth year of the study and resulted in two to three times more aboveground biomass at the end of the fifth year in the N (1570 g m<sup>-2</sup>) and N+P (1264 g m<sup>-2</sup>) plots relative to P (710 g m<sup>-2</sup>) and control (570 g m<sup>-2</sup>) plots. After five years of nutrient additions, macro-organic matter (MOM), the living plus dead root and rhizome mat (0–10 cm), was significantly lower in the N (1457 g m<sup>-2</sup>) and N+P (994 g m<sup>-2</sup>) plots than the control (2189 g m<sup>-2</sup>) plots. There was less live rhizome biomass in the N+P (23 g m<sup>-2</sup>) plots than the control (1085 g m<sup>-2</sup>) plots. We observed a 31–33% increase in the N content of *Z. miliacea* leaves in years three through five in the N and N+P plots relative to the control plots, but observed no P enrichment of leaves. In the N-treated plots, leaf C:N decreased 20–25% whereas N:P increased 21–64% in years three through five relative to the control and P plots. These findings collectively suggest that N, rather than P, limits productivity of tidal freshwater marsh vegetation. Reduced belowground biomass that accompanies N enrichment is of special concern as it may lead to increased erosion and reduced organic matter inputs to the soil, increasing their susceptibility to disturbances associated with wind, waves, river flooding and rising sea level.

© 2011 Elsevier B.V. All rights reserved.

### 1. Introduction

Tidal freshwater marshes occur in estuaries beyond the limits of salt water intrusion yet are still influenced by diurnal astronomical tides (Neubauer et al., 2005). Less is known about these ecosystems than other tidal wetlands such as salt marshes (Odum, 1984; Hopkinson, 1992). These freshwater marshes are known to have high plant productivity and aboveground biomass ranges from 1000 to 3000 g m<sup>-2</sup> year<sup>-1</sup> (Whigham et al., 1978; Keefe, 1972; Sasser and Gosselink, 1984; Hopkinson, 1992). The productivity of these ecosystems is dependent upon the availability of limiting nutrients such as nitrogen (N) and phosphorus (P). In salt marshes, the limiting nutrient has been shown to be nitrogen (Valiela and Teal, 1974; Hopkinson and Schubauer, 1984; Kiehl

et al., 1997; Visser and Sasser, 2006). The source (N versus P) of nutrient limitation in tidal freshwater marshes is less clear as some studies have suggested it is nitrogen (Morse et al., 2004; Frost et al., 2009) while others have suggested it is phosphorus (Paludan and Morris, 1999; Sundareshwar and Morris, 1999). Jordan et al. (2008) suggested that the observed shift from P limitation in freshwaters to N limitation in coastal marine waters is linked to P availability. In freshwaters, P precipitates with Fe-bearing minerals such as vivianite and other ferrous minerals that do not form in saline environments. Tidal freshwater marshes, located in the upper reaches of estuaries where saltwater seldom, but occasionally penetrates, are located where either N or P (or both) could be limiting, in the water column and possibly in the wetlands. The source of nutrient limitation is important as human activities have the ability to change natural nutrient loading in these ecosystems that may lead to changes in plant community structure (species diversity), function (productivity) and nutrient cycling.

\* Corresponding author. Fax: +1 281 255 0055.

E-mail address: [wket@indiana.edu](mailto:wket@indiana.edu) (W.A. Ket).

The ambiguity over nutrient limitation in these marshes is in part due to a lack of long-term nutrient enrichment experiments in these plant communities. Although nutrient enrichment manipulations in tidal freshwater marshes of short duration (i.e., one-year) have been conducted (Chambers and Fourqurean, 1991; Morse et al., 2004), such short-term studies may not allow for conclusive findings. For example, other researchers have shown it takes up to two years for plants to respond to nutrient additions (Craft et al., 1995). A more extended study in these marshes by Frost et al. (2009) examined nutrient limitation over two years. This study showed an increase in aboveground biomass with N fertilization and consistent leaf tissue N:P ratios <30, both of which suggested that N was the more limiting nutrient.

The current study was designed to continue the work of Frost et al. by tracking the effects of annual nutrient (N, P, N + P) additions over five years. We examined the response of marsh plant species to nutrient additions by measuring aboveground and belowground biomass, leaf C, N, P, C:N:P, and diversity. We also compared our results with published studies to determine whether our findings are consistent with other studies of N versus P limitation of tidal freshwater marsh vegetation.

## 2. Methods

### 2.1. Site description and nutrient addition

We established plots in a tidal freshwater marsh on Carrs Island on the northern bank of Hammersmith Creek near the Altamaha River (Georgia, USA) (31.334364°, -81.475900°), in spring 2004. Vegetation at the site is dominated by *Zizaniopsis miliacea* (Michx.) (giant cutgrass) along with small amounts of *Pontederia cordata* (L.) (pickerelweed) and *Sagittaria lancifolia* (L.) (bull tongue arrowhead). Sixteen 2 m × 2 m plots were established to provide four replicates of four treatments (nitrogen-N, phosphorus-P, N + P, and control). A 2 m buffer was established between each plot to minimize nutrient exchange between plots. Nitrogen and P were applied at an annual rate of 50 g m<sup>-2</sup> year<sup>-1</sup> and 10 g m<sup>-2</sup> year<sup>-1</sup>, respectively, by broadcasting it by hand twice a year during the growing season (March and May) in equal amounts. Fertilizer was broadcast at low tide when no water was present on the marsh surface to help ensure nutrient additions stayed within their specific experimental plot. Additionally, the fertilizer pellets we used were heavier than water and thus reduced concerns over it being removed during high tide. Triple Superphosphate (45% P<sub>2</sub>O<sub>5</sub>) was used as the P source. The nitrogen source in year one was ammonium chloride (NH<sub>4</sub>Cl). In order to provide a longer lasting N source we switched to a polymer coated urea CO(NH<sub>2</sub>)<sub>2</sub> (Polyon, Pursell Technologies Inc., Sylacauga, AL, USA) beginning in year two and continuing through the rest of the five year experiment.

### 2.2. Field and lab measurements

Aboveground biomass of emergent vegetation was nondestructively determined in August of each year by measuring the number and height of leaves in a 0.5 m × 0.5 m (0.25 m<sup>2</sup>) subplot within each treatment plot. Height measurements were converted to mass using an allometric equation determined from the height and weight of 509 *Z. miliacea* leaves harvested outside of, but in close proximity, to the treatment plots (Frost et al., 2009). Beginning in 2007, we measured the number and height of *P. cordata* leaves present in the plots. An allometric equation (mass (g) = 0.000001(height (cm)<sup>3</sup>); n = 77, r<sup>2</sup> = 0.85) was used to calculate leaf weight from height for *P. cordata*. The number and height of *S. lancifolia* also was measured in 2007 and 2008. This species was much less abundant in the plots than *P. cordata* (for example,

in 2008, there were a total of seven leaves of *S. lancifolia* in the plots as compared to 105 leaves for *P. cordata*) so we used the same allometric equation for both species which have similar leaf height and shape.

Leaf C, N, and P was measured annually by selecting five random leaves of average height of *Z. miliacea* from each plot. In 2008, one leaf of *P. cordata* and, *S. lancifolia* was collected from each plot, where present, for C, N, and P analysis. Leaves were oven dried at 50 °C and ground using a Thomas Scientific Wiley Mill (Swedesboro, NJ, USA). Leaf C and N were measured using a Perkin-Elmer 2400 CHN analyzer with NIST 1515 Apple Leaves as the standard. We measured P content of leaves following the methods of Sommer and Nelson (1972) using NIST 1575a (pine needles) as the P standard. Recovery of NIST standards was 97% for N (n = 62) and 88% for P (n = 71).

Macro-organic matter mass, the living and dead root and rhizome mat (Gallagher and Plumbley, 1979), was sampled in year five by collecting an 11 cm diameter by 70 cm deep core from each plot. Macro-organic matter (MOM) cores were separated into 10 cm sections, washed, and sorted to separate live rhizomes from the remaining MOM following methods outlined in Hopkinson and Dunn (1984) and Schubauer and Hopkinson (1984). Belowground fractions were oven dried at 50 °C to a constant weight and ground using a Wiley Mill. Macro-organic matter and rhizomes were analysed for C, N, and P using the same procedures and standards as for leaves.

### 2.3. Statistical analyses

We used repeated measures analysis of variance (ANOVA) to determine the effect of nutrient additions on stem height, stem density, and aboveground biomass (SAS, 2002). ANOVA was also used to test the effects of treatment and year on leaf C, N, P, and C:N:P ratios (SAS, 2002). MOM and rhizome biomass and nutrient (C, N, P) concentrations were analysed using a 2-way ANOVA based on treatment and sampling depth (SAS, 2002). Means were separated using the Ryan-Einot-Gabriel-Welsch multiple range test (SAS, 2002). All tests of significance were conducted at  $\alpha = 0.05$  level.

## 3. Results

### 3.1. Aboveground and belowground biomass

Nitrogen additions resulted in a significant increase in aboveground biomass of *Z. miliacea*. Aboveground biomass was two times greater in the N and N + P plots than in the control and P plots beginning in year two and continuing through year five (Fig. 1a). Phosphorus additions had no effect on aboveground biomass of *Z. miliacea*. Increased aboveground biomass was attributed to increased leaf height (Fig. 1b) and to some extent the number of leaves. Leaf height in the N plots was greater than the control and P plots in years two to five. Leaf height in the N + P had varied results and was greater than the control and P plots only in years three and five. Over the five year duration of the study, the mean number of leaves per m<sup>2</sup> was significantly greater in the N (241 ± 15) and N + P (238 ± 20) plots than in the control (159 ± 16) plots.

There was no difference in species richness among the treatments. On average, there were 5 species present in the control plots, 4.75 species in the P plots, and 3.75 species in both the N and N + P plots. *Z. miliacea* was the dominant species in all treatments throughout the five-year experiment. In years four and five, it accounted for at least 84% of total aboveground biomass. There was a total of seven other plant species (*Rhynchospora corniculata*, *Polygonum hydropiperoides*, *Ludwigia linearis*, *Smilax tamnoides*, *P. cordata*, *S. lancifolia*, and an unidentifiable *Asteraceae*) present in the

**Table 1**

Mean belowground biomass ( $\text{g m}^{-2}$ ) and standard error of macro organic matter (MOM) and rhizomes as a function of treatment and depth. Means with the same letter are not significantly different ( $p > 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test).

Tissue	Treatment	Depth			
		0–10 cm	10–20 cm	20–30 cm	30–40 cm
MOM	C	2189 ± 663 a	1526 ± 193	1747 ± 286	1494 ± 196
	P	1531 ± 270 a,b	1639 ± 81	1386 ± 102	1571 ± 207
	N	1457 ± 221 b	1381 ± 121	1363 ± 94	1152 ± 62
	N+P	994 ± 55 b	1560 ± 253	1428 ± 136	1260 ± 74
Rhizomes	C	1085 ± 578 a	17 ± 17	1 ± 1	0.0
	P	468 ± 258 a,b	57 ± 54	0.0	1 ± 1
	N	315 ± 197 a,b	17 ± 13	0.0	0.0
	N+P	23 ± 12 b	9 ± 6	25 ± 18	0.0

16 treatment plots, though *P. cordata* and *S. lancifolia* accounted for most of the remaining biomass. In year five (2008) *P. cordata* and *S. lancifolia* accounted for 16% of the aboveground biomass in the control treatments versus 2%, 5%, and 6% in the N, N + P, and P fertilized plots, respectively.

N additions also affected MOM and live rhizomes though in an opposite manner to aboveground biomass. Plots receiving N contained significantly less MOM (0–10 cm) relative to the control plots

(Table 1). Biomass of rhizomes was also significantly less in the N + P treatment compared to the control plots. In the 0–10 cm depth, the N and N + P treatments contained 45–67% of the MOM biomass that we measured in the control plots. Rhizome biomass in the N treated plots was only 2–30% of the live rhizome biomass measured in the control plots. There were no significant differences in MOM and rhizome biomass among the treatments at deeper (>10 cm) depths in the soil.

### 3.2. Plant tissue C, N, P

Leaf N of *Z. miliacea* was significantly greater in the N and N + P plots than the control and P plots, beginning in year three and continuing through year five (Fig. 2a). In year two, we observed a decrease in leaf nitrogen content in the N treated plots as compared to year one that we attribute to dilution caused by the rapidly increasing aboveground biomass (Fig. 1a). Leaf phosphorus concentrations were not consistently affected by N or P nutrient additions (Fig. 2b), though in year five, leaves collected from the N + P plots had a significantly greater phosphorus concentration than the control and N plots.

Leaf C:N:P of *Z. miliacea* also was affected by N additions. Leaf C:N was significantly less in the N and N + P plots than the control and P plots in years three and four, and it was significantly less in the N plots relative to the control plots in year five (Fig. 3a). Similar to C:N, leaf N:P was greater in the N and N + P plots relative to the C and P plots in years three and four and it was significantly greater in the N plots than the control plots in year five (Fig. 3b).

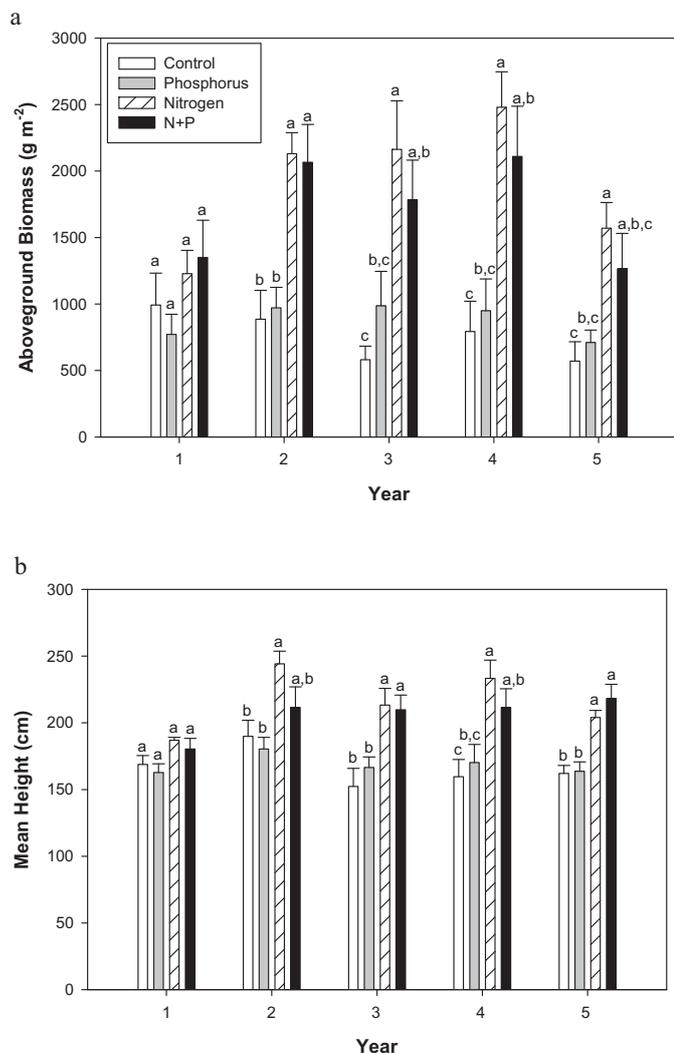
In contrast to leaf N and P, we did not find any difference in the nutrient content of belowground tissues (rhizomes and MOM) among treatments. However, we did note that across all treatments, N and organic C content of MOM and rhizomes increased with depth. For example, MOM N increased from  $0.98\% \pm 0.03\%$  in the 0–10 cm depth to  $1.60\% \pm 0.07\%$  in the 60–70 cm depth. Rhizome P also increased from 1.02% in the 0–10 cm depth to 1.65% in the 30–40 cm depth, lower extent of rhizomes.

Greater biomass production and leaf N in N treated plots resulted in N and P pools ( $\text{g m}^{-2}$ ) that were significantly greater in the N and N + P plots relative to the control plots in year five of the study (Table 2). Aboveground N pools were two to three times higher in the N plots and N + P plots than the P plots and control plots. Owing to greater aboveground biomass, P pools were two to two and a half times higher in the N plots and N + P plots than the P plots and the control plots. We observed no significant difference in N or P pools of MOM among the experimental plots.

## 4. Discussion

### 4.1. Above- and belowground response to nutrient additions

Findings from our five-year study corroborate and expand on the results of Frost et al. (2009) who documented the response of



**Fig. 1.** (a) Mean aboveground biomass ( $\text{g m}^{-2}$ ) and (b) leaf height (cm) in response to nutrient additions. Error bars show standard error. Means with the same letter are not significantly different ( $p > 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test).

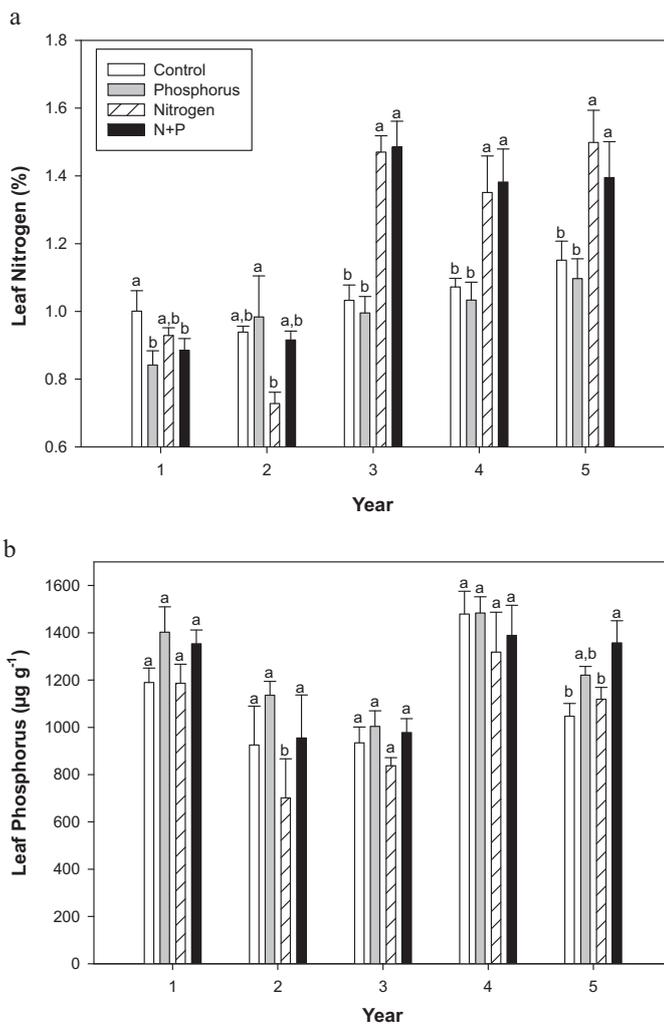
**Table 2**  
Mean year five aboveground and belowground (Macro-organic matter – MOM) plant tissue nutrient (N and P) content ( $\text{g m}^{-2}$ ) and standard errors organized by treatment. Means with the same letter are not significantly different ( $p > 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test). No significant difference was observed in MOM N and P among treatments.

Treatment	Aboveground ( $\text{g m}^{-2}$ )		MOM ( $\text{g m}^{-2}$ )			
	N	P	0–10 cm		10–40 cm	
			N	P	N	P
C	7.22 ± 1.48 b	0.67 ± 0.17 b	19.94 ± 4.62	9.69 ± 4.77	51.40 ± 8.04	12.66 ± 2.63
P	8.28 ± 0.92 b	0.89 ± 0.09 a,b	15.69 ± 3.15	4.77 ± 1.50	51.12 ± 3.52	10.15 ± 0.91
N	24.25 ± 3.35 a	1.80 ± 0.25 a	13.85 ± 2.91	3.88 ± 1.51	43.42 ± 4.66	7.31 ± 1.02
N + P	18.98 ± 4.37 a	1.80 ± 0.42 a	9.78 ± 0.12	3.42 ± 0.62	42.11 ± 1.37	7.63 ± 0.79

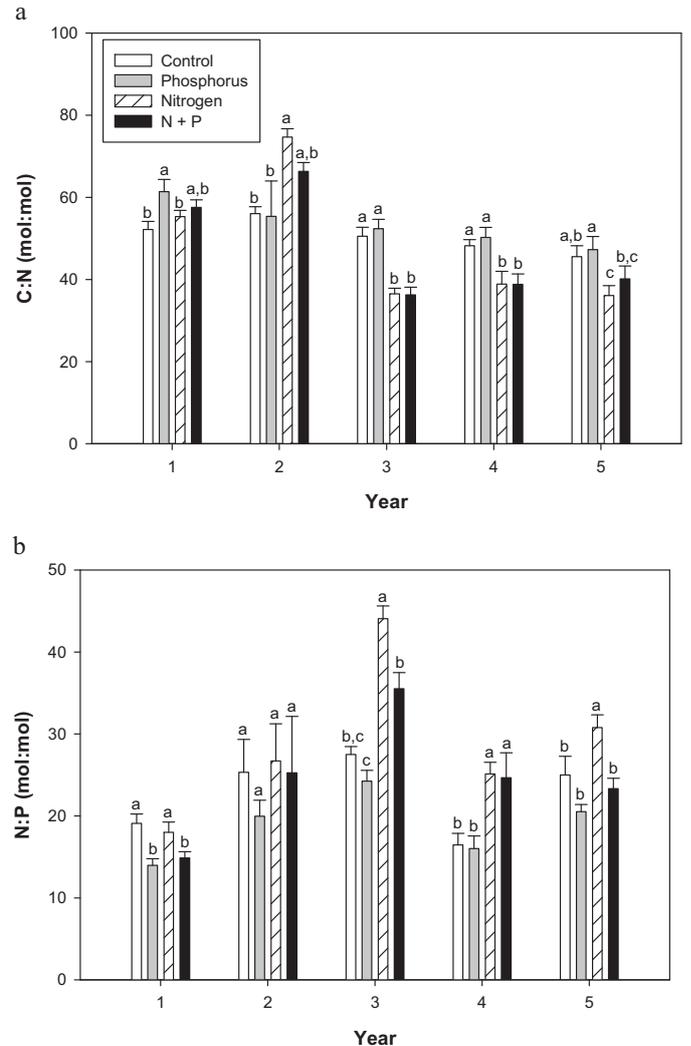
aboveground vegetation during the first two years of the experiment. Our five-year study provides compelling evidence that N rather than P is the primary limiting nutrient for vegetation in tidal freshwater marshes dominated by *Z. millicea*. Our results are similar to Frost et al. (2009) in that N additions continued to promote significantly greater growth response than P additions or control conditions. Frost et al. (2009), however based their nutrient (N) limitation conclusions solely on this increased growth response as they did not observe significant increases in leaf N content or leaf N:P ratios as we did in our five year study. We also observed reduced belowground biomass in N-treated plots, not measured by

Frost et al. that further supports the idea that N rather than P is the primary limiting nutrient for tidal freshwater marsh vegetation.

Belowground biomass declined in response to five years of N additions but only in the 0–10 cm depth. Macro-organic matter dry weight (0–10 cm) was less in the N and N+P plots relative to the control plots. Biomass of rhizomes was less in the N+P treatment relative to the control. When nutrients are abundant, as is the case in fertilization experiments, plants do not forage for nutrients so they produce fewer roots (Morris and Bradley, 1999; Darby and Turner, 2008a). Furthermore, in these conditions, plants do not store as much N and therefore produce less rhizome biomass



**Fig. 2.** (a) Mean nitrogen and (b) phosphorus concentration ( $\mu\text{g P/g}$  leaf) of *Zizaniopsis millicea* leaves. Error bars show standard error. Means with the same letter are not significantly different ( $p > 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test).



**Fig. 3.** (a) Mean C:N ratio and (b) N:P ratio of *Zizaniopsis millicea* leaves. Error bars show standard error. Means with the same letter are not significantly different ( $p > 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test).

**Table 3**  
Comparison of findings from other tidal freshwater marsh nutrient studies that used fertilizer.

Reference	Location and vegetation	Duration of study	Treatment	Plant growth	Findings	Limiting nutrient
Ket et al. (current Study)	Altamaha River, Georgia, USA <i>Zizaniopsis miliacea</i>	5 Years	50 g N m <sup>-2</sup> year <sup>-1</sup> 10 g P m <sup>-2</sup> year <sup>-1</sup>	1250–2500 g m <sup>-2</sup> in fertilized plots	Fertilization increased aboveground biomass and leaf N content, decreased belowground biomass, and N:P <30 (mol: mol).	Nitrogen limited
Frost et al. (2009)	Altamaha River, Georgia, USA <i>Zizaniopsis miliacea</i>	2 Years	50 g N m <sup>-2</sup> year <sup>-1</sup> 10 g P m <sup>-2</sup> year <sup>-1</sup>	1250–2100 g m <sup>-2</sup> in fertilized plots	Fertilization increased aboveground biomass and N:P < 30 (mol: mol).	Nitrogen limited
Morse et al. (2004)	Mattaponi River, Virginia, USA	1 Year	22.5 g N m <sup>-2</sup> 11.2 g P m <sup>-2</sup>	950–1350 g m <sup>-2</sup> in fertilized plots	Fertilization resulted in no biomass response, no leaf N and P change, N:P <30 (mol: mol).	Inconclusive
Chambers and Fourqurean (1991)	Chickahominy River, Virginia, USA <i>Peltandra virginica</i>	2 Years total, 1 Year fertilization	1400 g m <sup>-2</sup> of N:P 14:14 Fertilizer	750–1550 g m <sup>-2</sup> in all plots	Fertilization resulted in no biomass response, no leaf N and P change, N:P <30 (mol: mol) but no change over time. After fertilization N:P ratios increased to >35 (mol: mol).	Inconclusive
Booth (1989)	Pamunkey River, Virginia, USA <i>Spartina cynosuroides</i> and <i>Peltandra virginica</i>	1 Year	Unknown	1634 g m <sup>-2</sup>		P limitation

(Hopkinson and Schubauer, 1984). Accordingly, our belowground biomass data also supports the idea that N not P limits primary productivity. This is in contrast to a short-term (5 month) fertilization experiment in a *Spartina alterniflora* salt marsh in Louisiana where nutrients other than N (P, Fe and various combinations of N, P and Fe) led to fewer roots and rhizomes relative to the control and N-treated plots (Darby and Turner, 2008a). In a separate study, Darby and Turner (2008b) reported that additions of P and N+P to salt marsh vegetation in eastern North America (Nova Scotia, MA, VA, LA) resulted in reduced belowground biomass relative to unfertilized plots. However, no plots received N alone so it is difficult to infer the relative effects of N versus P on belowground biomass from this study. Darby and Turner (2008a,b) suggested that reduced belowground biomass has important consequences for the future of tidal salt marshes; (1) the diminished root mat has less ability to withstand erosion from wind, waves and storms and (2) diminished belowground biomass means less organic matter available to be incorporated into the soil. The organic matter derived from roots and rhizomes is necessary for tidal marshes, especially tidal freshwater and brackish marshes, to maintain their elevation in the face of rising sea level (Craft, 2007; Darby and Turner, 2008a,b).

Leaf N also responded to nutrient additions in a way that suggests N limitation. Leaf N increased by 21–43% in the N and N+P plots relative to the control plots. Leaf P, on the other hand did not show any clear trends across the treatments. Gerloff and Krombholz (1966) suggested that nutrient limitation could be inferred by the amount of N or P incorporated into leaf tissue. According to their research, the addition of a limiting nutrient will lead to an increased uptake and incorporation of the nutrient into plant tissue as well as an increase in biomass. We have shown both a significant increase in biomass and tissue nutrient content (N), which suggests N limitation according to criteria established by Gerloff and Krombholz (1966).

Nitrogen to phosphorus ratios have also been used to infer N or P limitation in a variety of ecosystems following Redfield's early work with phytoplankton (Redfield, 1958). Koerselman and Meuleman (1996) reviewed the literature and suggested that higher plants are N limited when N:P ratios are <30 (mol: mol) and are P limited when N:P > 35. During the five year duration of our study, the leaf

N:P ratios of the control and P plots remained below 30 (mol: mol) suggesting N limitation.

#### 4.2. Vegetation response to nutrients through time

There have been relatively few long term studies investigating nutrient limitation of estuarine wetland vegetation (Vince and Valiela, 1981; Valiela et al., 1982, 1985; Morris and Bradley, 1999; Feller, 1995; Feller et al., 1999), including three studies that fertilized for more than 10 years (Valiela et al., 1985; Sarda et al., 1996; Morris and Bradley, 1999) but none that we are aware of for tidal freshwater wetlands. In our experiment, after a lag time of one year, additions of N consistently increased aboveground biomass. Our measured trends in aboveground biomass are consistent with other long-term nutrient enrichment studies in wetlands. For example, Valiela et al. (1982, 1985) found that *S. alterniflora* salt marshes (MA) responded in the second year of N additions, applied as urea, during a 12 year fertilization experiment. They added N+P, beginning in year 5, that increased biomass even more, suggesting co-limitation with N and P. However, Valiela et al. did not add P alone so we cannot know whether P is limiting singly. Feller (1995) added N, P and K for 5 years to dwarf mangroves (Belize) and, like the Everglades, found P to be the primary limiting nutrient with the biomass response occurring in year 2. Morris and Bradley (1999) fertilized *S. alterniflora* (SC) with N+P for 12 years and measured three times as much aboveground biomass in the fertilized plots than in the control plots. After 12 years, MOM (0–5 cm) declined from 1201 g m<sup>-2</sup> in the control plots to 726 g m<sup>-2</sup> in the fertilized plots. In all studies, the limiting nutrient sustained high levels of high aboveground biomass for the duration of the experiment.

In our study, leaf N and C:N:P did not respond until the third year of N additions as, at this time, leaf N and N:P increased and C:N decreased. This runs counter to some studies where the limiting nutrient is taken up and concentrated in the leaves in the first or second year of nutrient additions (Feller, 1995). However, other studies report an initial decline in leaf N in response to N additions as increased aboveground biomass dilutes the N content of the leaves (Valiela and Teal, 1974; Frost et al., 2009). This can lead to a decline in leaf C:N, as we observed in year 2. It is not clear why it took

three years for leaf N to increase in the N-treated plots. Vince and Valiela (1981) measured increased leaf N in N-treated plots after four years of fertilization but they did not measure it during the first three years of their experiment. Compared to the studies above, our tidal freshwater marsh has a complex and dynamic hydrology with tides and river flow. It is likely that significant N (and P) is lost to the water column even though we used a slow release source of N (polymer coated urea) and P (rock phosphorus). It is also important to note that many of the long term studies described above added N and P at much higher rates, 136–420 g N m<sup>-2</sup> year<sup>-1</sup> (Vince and Valiela, 1981; Valiela et al., 1982, 1985; Morris and Bradley, 1999) than we did (50 g N m<sup>-2</sup> year<sup>-1</sup>, 10 g P m<sup>-2</sup> year<sup>-1</sup>) that may help explain why the response to N additions in our study took longer compared to some other studies.

Finally, during our five year experiment, we observed marked variation in aboveground biomass in the control plots among years. This inter-annual variability may be due to natural variation from year to year, reported for other tidal freshwater marsh vegetation (Whigham and Simpson, 1992) or to environmental factors such as mean sea level that affects salinity (Morris et al., 1990). During the five year period, aboveground biomass in the control plots was inversely related to annual salinity (0.07–0.45 psu,  $r^2 = 0.70$ ) measured continuously (15 min intervals) in the nearby river. Regressions of aboveground biomass versus salinity for the fertilized plots were not as strong ( $r^2 = 0.19$ – $0.33$ ). We cannot conclusively say that salinity drives inter-annual variations in tidal freshwater marsh aboveground biomass though it is a strong candidate since it is known to stress tidal freshwater marsh vegetation (Spalding and Hester, 2007).

#### 4.3. Comparison with other tidal freshwater marsh nutrient addition experiments

Few studies have specifically examined nutrient limitation in tidal freshwater marshes using fertilizer applications (Table 3). The intent of these studies is to provide a controlled source of the limiting nutrient, which in turn is expected to produce measurable differences in primary productivity. However, a host of difficulties associated with these experiments has caused other researchers to use alternative methods such as plant tissue nutrient content and N:P ratios as proxies for nutrient limitation (Koerselman and Meuleman, 1996; Chambers and Fourqurean, 1991). The current study used a combination of diagnostic tools (aboveground and belowground biomass, leaf N and P, C:N:P) to determine nutrient limitation. The results from these tools indicate that N is the limiting nutrient. Other studies, however, have used similar approaches but found no results or conflicting results. Chambers and Fourqurean (1991) for example, did not observe a significant biomass response nor did they find any differences in tissue nutrient content in plots of *Peltandra virginica* that were fertilized. Morse et al. (2004), on the other hand found the sediment and soil N:P ratios suggested N limitation, yet aboveground biomass did not increase when N fertilizer was applied. Finally, Booth (1989) observed N:P ratios that suggested P limitation, but did not observe a significant biomass response.

In conclusion, our study is among the first to conclusively demonstrate N limitation in tidal freshwater marshes based on changes in (1) aboveground biomass, (2) belowground biomass, (3) leaf N content, and (4) leaf N:P ratios. Our work suggests tidal freshwater marshes are limited by N and therefore may be susceptible to eutrophication in the face of increasing anthropogenic N inputs. Another concern is that the reduction in belowground biomass in response to N enrichment may make these marshes more susceptible to disturbance from wind and wave action associated with storm surge, river flooding and sea level rise. In the future, N induced eutrophication and climate change induced sea

level rise may put these N limited marshes at risk of degradation and loss.

#### Acknowledgements

We thank Nate Knowles, John Marton, and Iza Redlinski for their help in the lab and field and two anonymous reviewers for their guidance on the manuscript. This research was supported by grants RD 83222001-0 from the U.S. Environmental Protection Agency's Science to Achieve Results (STAR) program and OCE-9982133 from the National Science Foundation to the Georgia Coastal Ecosystem LTER program to CC and support from the U.S. EPA ORD to JSB. This is contribution 1003 of the University of Georgia Marine Institute. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the USEPA.

#### References

- Booth Jr. P.M., 1989. Nitrogen and Phosphorus Cycling Strategies in *Peltandra virginica* and *Spartina cynosuroides*. Ph.D. Dissertation, College of William and Mary, Williamsburg, VA.
- Chambers, R.M., Fourqurean, J.W., 1991. Alternative criteria for assessing nutrient limitation of a wetland macrophyte (*Peltandra virginica* (L.) Kunth). *Aquat. Bot.* 40, 305–320.
- Craft, C.B., 2007. Freshwater input structures soil properties, vertical accretion and nutrient accumulation of Georgia and United States (U.S.) tidal marshes. *Limnol. Oceanogr.* 52, 1220–1230.
- Craft, C.B., Vymazal, J., Richardson, C.J., 1995. Response of Everglades plant communities to nitrogen and phosphorus additions. *Wetlands* 15, 258–271.
- Darby, F.A., Turner, R.E., 2008a. Below- and aboveground biomass of *Spartina alterniflora*: response to nutrient addition in a Louisiana salt marsh. *Estuar. Coast.* 31 (2), 326–334.
- Darby, F.A., Turner, R.E., 2008b. Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Mar. Ecol. Prog. Ser.* 363, 63–70.
- Feller, I.C., 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). *Ecol. Monogr.* 65, 477–505.
- Feller, I.C., Whigham, D.F., O'Neill, J.P., McKee, K.L., 1999. Effects of nutrient enrichment on within-stand cycling in a mangrove forest. *Ecology* 80, 2193–2203.
- Frost, J.W., Schleicher, T., Craft, C., 2009. Effects of nitrogen and phosphorus additions on primary production and invertebrate densities in a Georgia (USA) tidal freshwater marsh. *Wetlands* 20, 196–203.
- Gallagher, J.L., Plumbly, F.G., 1979. Underground biomass profiles and productivity in Atlantic coastal marshes. *Am. J. Bot.* 66, 156–161.
- Gerloff, G.C., Krombholz, P.H., 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnol. Oceanogr.* 11, 529–537.
- Hopkinson, C.S., Dunn, E.L., 1984. Rapid sampling of organic matter in flooded soils and sediments. *Estuaries* 7, 181–184.
- Hopkinson, C.S., Schubauer, J.P., 1984. Static and dynamic aspects of nitrogen cycling in the salt marsh graminoid, *Spartina alterniflora*. *Ecology* 65, 961–969.
- Hopkinson, C.S., 1992. A comparison of ecosystem dynamics in freshwater wetlands. *Estuaries* 15, 549–562.
- Jordan, T.E., Cornwell, J.C., Boynton, W.R., Anderson, J.T., 2008. Changes in phosphorus biogeochemistry along an estuarine salinity gradient: the iron conveyor belt. *Limnol. Oceanogr.* 53, 172–184.
- Keefe, C., 1972. Marsh production: a summary of the literature. *Contrib. Mar. Sci.* 16, 163–181.
- Kiehl, K., Esselink, P., Bakker, J.P., 1997. Nutrient limitation and plant species composition in temperate salt marshes. *Oecologia* 111, 325–330.
- Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* 33, 1441–1450.
- Morris, J.T., Bradley, P.M., 1999. Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnol. Oceanogr.* 44, 699–702.
- Morris, J.T., Kjerfve, B., Dean, J.M., 1990. Dependence of estuarine productivity on anomalies in mean sea level. *Limnol. Oceanogr.* 35, 926–930.
- Morse, J.L., Megonigal, J.P., Walbridge, M.R., 2004. Sediment nutrient accumulation and nutrient availability in two tidal freshwater marshes along the Mattaponi River, Virginia, USA. *Biogeochemistry* 69, 175–206.
- Odum, W.E., 1984. Comparative ecology of tidal freshwater and salt marshes. *Annu. Rev. Ecol. Syst.* 19, 147–176.
- Paludan, C., Morris, J.T., 1999. Distribution and speciation of phosphorus along a salinity gradient in inter tidal marsh sediments. *Biogeochemistry* 45, 197–221.
- Neubauer, S.C., Anderson, I.C., Neikirk, B.B., 2005. Nitrogen cycling and ecosystem exchanges in a Virginia tidal freshwater marsh. *Estuaries* 28, 909–922.
- Redfield, A.C., 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46, 25–222.
- SAS (Statistical Analysis Systems), 2002. The SAS 9.1 system for Windows. SAS Institute Inc., Cary, NC.
- Sarda, R., Valiela, I., Foreman, K., 1996. Decadal shift in a salt marsh macro in faunal community in response to sustained long-term experimental nutrient enrichment. *J. Exp. Mar. Biol. Ecol.* 205, 63–81.

- Sasser, C.E., Gosselink, J.G., 1984. Vegetation and primary production in a floating freshwater marsh in Louisiana. *Aquat. Bot.* 20, 245–255.
- Schubauer, J.P., Hopkinson, C.S., 1984. Above- and belowground emergent macrophyte production and turnover in a coastal marsh ecosystem, Georgia. *Limnol. Oceanogr.* 29, 1052–1065.
- Sommer, L.E., Nelson, D.W., 1972. Determination of total phosphorus in soils: rapid perchloric acid digestion procedure. *Soil Sci. Soc. Am. J.* 36, 902–904.
- Spalding, E.A., Hester, M.W., 2007. Interactive effects of hydrology and salinity on oligohaline plant species productivity: implications of relative sea level rise. *Estuar. Coast* 30, 214–225.
- Sundareshwar, P.V., Morris, J.T., 1999. Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient. *Limnol. Oceanogr.* 44, 1693–1701.
- Valiela, I., Teal, J.M., 1974. Nutrient Limitation in salt marsh vegetation. In: Reimold, R.J.N.W., Queen (Eds.), *Ecology of Halophytes*. Academic Press, New York, pp. 563–574.
- Valiela, I., Howes, B., Howarth, R., Giblin, A., Foreman, K., Teal, J.M., Hobbie, J.F., 1982. Regulation of primary production and decomposition in a salt marsh ecosystem. In: Gopal, B., Turner, R.E., Wetzel, R.G., Whigham, D.F. (Eds.), *Wetlands: Ecology and Management*. International Scientific Publications, Jaipur, India, pp. 151–168.
- Valiela, I., Teal, J.M., Cogswell, C., Hartman, J., Allen, S., Van Etten, R., Goehring, D., 1985. Some long term consequences of sewage contamination in salt marsh ecosystems. In: Godfrey, P.J., Kaynor, E.R., Pelczarski, S., Benforado, J. (Eds.), *Ecological Considerations in Wetlands Treatment of Municipal Wastewaters*. Van Nostrand Reinhold, New York, pp. 301–316.
- Vince, S.W., Valiela, I., 1981. An experimental study of the structure of herbivorous insect communities in a salt marsh. *Ecology* 62, 1662–1678.
- Visser, Y.M., Sasser, C.A., 2006. The effect of multiple stressors on salt marsh end-of-season biomass. *Estuar. Coast* 29, 331–342.
- Whigham, D.F., McCormick, J., Good, R.E., Simpson, R.L., 1978. Biomass and primary productivity in freshwater wetlands of the middle Atlantic coast. In: Good, R.E., Whigham, D.F., Simpson, R.L. (Eds.), *Freshwater Wetlands*. Academic Press, New York, NY, USA, pp. 3–20.
- Whigham, D.F., Simpson, R.L., 1992. Annual variation in biomass and production of a tidal freshwater wetland and comparison with other wetland systems. *Virginia J. Sci.* 43, 5–14.