Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication

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ABSTRACT: We enriched experimental plots in a Sagittaria lancifolia L. dominated oligohaline marsh for 4 yr with one of 4 levels of nitrogen (N) (0, 50, 200, or 1200 kg N ha⁻¹ yr⁻¹) in combination with one of 2 levels of phosphorus (P) (0 or 131 kg P ha⁻¹ yr⁻¹) to investigate nutrient limitation of primary production and plant community- and species-level responses to nutrient enrichment. Overall, significant changes in ecosystem structure and function occurred with N enrichment only; P enrichment had no significant effect alone or in interaction with N. Both 200 and 1200 kg N ha⁻¹ yr⁻¹ stimulated above-ground plant production. Enrichment with 1200 kg N ha⁻¹ yr⁻¹ also increased S. lancifolia tissue N:P ratio, reduced S. lancifolia N and P resorption during senescence, and altered the relative dominance of the 3 dominant species, but had no effect on species richness. We conclude that (1) N limits above-ground primary production in this oligohaline marsh; (2) the plant response to Nenrichment is sequential: moderate N loading stimulated plant production only, while high N loading maintained the elevated production and also altered plant tissue nutrients and species dominance, but not species richness; (3) N enrichment beyond the assimilation capacity of the vegetation drives changes in ecosystem structure caused by altered plant nutrient cycling; and (4) linear changes in species dominance with increasing N enrichment suggest that further nutrient enrichment in time or quantity may result in a shift in species dominance and reduced species richness.

KEY WORDS: Marsh \cdot Nutrient enrichment \cdot Nitrogen limitation \cdot Primary production \cdot Relative dominance \cdot Nutrient resorption \cdot Louisiana

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INTRODUCTION

Human activities and the resulting manipulation of the global environment have greatly altered the flow and cycling of nutrients across the land-sea margin. Global inputs of reactive nitrogen (N) to coastal waters have increased 20-fold since 1860 (Galloway & Cowling 2002), and the flux of phosphorus (P) to the world's oceans has increased by nearly 3-fold in modern times (Howarth et al. 1995, Bennett et al. 2001). As a result, cultural eutrophication, or the biological response to human-induced nutrient over-enrichment, is affecting >400 coastal areas around the world (Diaz & Rosenberg 2008). In the USA alone, 60% of 138 estuaries,

representing >90% of the conterminous US estuarine surface area, are moderately to severely affected by eutrophic conditions (Bricker et al. 1999).

The ecological effects of eutrophication are readily apparent in coastal wetlands. Documented effects of nutrient over-enrichment include increased primary production, community metabolism, and consumer activity, reduced species richness and carbon sequestration, altered nutrient cycling and species composition, and expansions of invasive species, to name a few (Mendelssohn 1979, Whigham & Nusser 1990, Chambers et al. 1999, Morris & Bradley 1999, Pennings et al. 2002, Silliman & Bertness 2004, DeLaune et al. 2005, Bertness et al. 2008, and more). A large body of litera-

ture supports the proposition that in mesohaline (brackish) and polyhaline (salt) marshes, these changes in ecosystem structure and function result from greater N loading because plant growth in these systems is N-limited (Tyler 1967, Sullivan & Daiber 1974, Valiela & Teal 1974, Broome et al. 1975, Patrick & Delaune 1976, Jefferies & Perkins 1977, Boyer et al. 2001, Wigand et al. 2004, and more). In comparison, oligohaline (intermediate) marshes have received much less attention, and general conclusions concerning the effects of coastal eutrophication on them are lacking.

Although a few published oligohaline marsh fertilization studies have documented altered plant community structure and/or function induced by N (DeLaune & Lindau 1990) and N-P-potassium (N-P-K) enrichment (Gough & Grace 1997, Slocum & Mendelssohn 2008), only Crain (2007) applied fully crossed treatments of N and P to determine the growth-limiting nutrient or nutrient combination that affects change, in this case N and P co-limitation. Moreover, no study has emphasized plant community- and species-level response trajectories resulting from the application of multiple nutrient levels in oligohaline marshes. Additional information is needed to provide more comprehensive answers to fundamental questions concerning the dynamics of oligohaline marsh ecology, including 'Are oligohaline marshes in general co-limited limited by N and P?' and 'What drives ecosystem change once nutrient over-enrichment occurs?'

Though global and national inventories of oligohaline marshes do not exist, the latter represent a major coastal marsh type that requires more intensive study. For example, in Louisiana approximately 23 % (276 000 ha) of all coastal wetlands (~1.2 million ha) are oligohaline (Turner 1990). This amount alone represents approximately 2.5% of the total coastal wetland area in the entire conterminous USA (Field et al. 1991). These marshes typically have much greater plant species diversity than their more saline counterparts (Visser et al. 1998, Crain 2007). Multiple studies have documented up to 30 species growing within relatively small sampling areas (Brewer & Grace 1990, Baldwin & Mendelssohn 1998). Nutrient over-enrichment is of particular concern in species-rich oligohaline marshes because nutrient excess may alter competitive hierarchies (Brewer & Grace 1990) and thereby reduce or otherwise modify biodiversity.

The objectives of our research were to determine (1) the nutrient or nutrient combination (N, P, or both) that limits primary productivity in an oligohaline marsh, (2) how experimental nutrient manipulation alters plant nutrient cycling and community composition, and (3) whether these changes occur simultaneously or at different rates depending upon the level of nutrient enrichment applied. By addressing these

objectives, we tested the general hypothesis that N and P co-limit oligohaline marsh primary production. We also identified how enrichment with the limiting nutrient(s) affected individual component species and the vegetative community as a whole, and determined the factors driving changes in community composition following nutrient enrichment. Specifically, we hypothesized that N limits above-ground biomass production, and because of this, N enrichment increases above-ground production, alters plant nutrient dynamics, and affects overall community composition. At high N loading, we hypothesized that P becomes secondarily limiting when N limitation is relieved by fertilization, and enrichment with P in combination with high N induces additional changes in ecosystem structure and function. We also hypothesized that altered ecosystem structure would result from altered plant nutrient cycling once increased production leveled off.

MATERIALS AND METHODS

Study area. The coastal waters of Louisiana receive approximately 1.6 million metric tons (t) N yr⁻¹ and 136 500 t P yr⁻¹ from the Mississippi-Atchafalaya River complex (Goolsby et al. 1999). The nitrate load $(\sim 950\,000 \text{ t yr}^{-1}; \text{Goolsby et al. } 1999) \text{ is now more than}$ twice that discharged in the 1950s (Turner & Rabalais 1991), driving the development of a persistent and reoccurring near-shore hypoxic area that can exceed 20 000 km² (Rabalais 2002, Turner et. al. 2006; www. gulfhypoxia.net, accessed 3 Dec 2009). Prior to human modification of the Lower Mississippi River, much of this water would follow distributaries and crevasses through the vast coastal wetlands of Louisiana's delta before entering the Gulf of Mexico (Welder 1959). However, following the great flood of 1927, floodcontrol levees were constructed almost continuously to the mouth of the river, and today most of Louisiana's wetlands remain hydrologically isolated from the Mississippi River (Kesel 1988, 2003).

One proposed method for reducing the nutrient load to the northern gulf is to reconnect the Mississippi River to its delta through river diversions (Mitsch et al. 2001, CPRA 2007). The rationale is that coastal wetlands will assimilate nutrients in the diverted river water and reduce the nutrient load to the open waters of the gulf (see Day et al. 2007). However, a major concern with this proposal is that the elevated nutrient load could be a driver of wetland eutrophication over the long term (Parsons et al. 2006).

Study site. To address this concern and our objectives, we investigated the effects of nutrient enrichment in a river-fed oligohaline marsh along the

west bank of the Tchefuncte River (30° 23.205′ N, 90° 09.551′ W), approximately 1 km north of Lake Pontchartrain. Soil at the site is classified as a Kenner series Histosol (euic, thermic Fluvaquentic Medisaprist) formed from herbaceous plant material and characterized as 'very poorly drained, rapidly permeable organic soil' (Trahan et al. 1990). The plant community is highly diverse (Slocum & Mendelssohn 2008) and is representative of the Oligohaline Mix vegetation type described by Visser et al. (1998). The species mix is dominated by *Sagittaria lancifolia* L., *Eleocharis fallax* Weatherby, and *Polygonum punctatum* Ell. All 3 dominants are perennial (clonal) herbs that emerge, flower, and senesce at similar times during the growing season at this site.

The marsh floods from water-level fluctuations in the Tchefuncte River and Lake Pontchartrain, which are caused primarily by wind shifts during frontal passages, although a 10 cm microtidal range also affects hydrology (Swenson & Chuang 1983). Average surface water salinity (1999 to 2006) was approximately 1.6 g l $^{-1}$ (LADEQ 2006), indicating oligohaline estuarine conditions (Odum 1988). Nutrient loading to the study marsh is affected by residential development throughout the Tchefuncte River watershed and agriculture in the upper reaches (Table 1). However, Tchefuncte River water has much lower concentrations of inorganic N (NH $_3$ + NO $_3$ + NO $_2$), total N, and total P compared to Mississippi River: 6, 2, and 3 times lower, respectively (Table 1).

Design and sampling. We applied granulated slowrelease fertilizer by surface broadcast for 4 yr to 1 m² oligohaline marsh plots. Each plot received one of 4 N levels (0, 50, 200, or 1200 kg N ha^{-1} yr⁻¹ applied as Nutralene methylene urea 40-0-0) in combination with one of 2 P levels (0 or 131 kg P ha⁻¹ yr⁻¹ applied as Humaphos 0-5-0) to yield 8 treatment combinations in a completely randomized block design. Treatment combinations were replicated in 5 locations (i.e. blocks) spaced 5 to 10 m apart and parallel to a small drainage canal. Plots were fertilized twice during the growing season in April and July of 2002 through 2005. We delayed sampling until the third and fourth growing seasons after the initiation of nutrient additions (i.e. 2004 or 2005) to increase the chances of detecting treatment effects, as multiple studies have shown that the effects of nutrient enrichment on wetland plant communities become more pronounced with each year of continued enrichment (Valiela et al. 1975, Craft et al. 1995, Kiehl et al. 1997, Crain 2007, Frost et al. 2009). However, we should note that Lindig-Cisneros et al. (2003) found reduced effects of nutrient enrichment on Spartina foliosa total stem length over multiple years of fertilization.

To address Objective 1, we estimated net aboveground primary productivity (NAPP) during the 2005 growing season. Vegetation at this site senesces each Table 1. Average ambient water quality condition of Tchefuncte River at Madisonville, LA (LA040802_00, Site 0106; LADEQ 2006) and Mississippi River at St. Francisville, LA (LA070201 00, Site 0055; LADEQ 2006) from 1999 to 2006. Potential nutrient loading rate to the study marsh was estimated using water level measurements collected on September 22, 2006 at 3 locations within each 1 m² plot at mid-tide. Average water level for each plot was correlated by time with 15 min river gauge height data for Tchefuncte River at Madisonville, approximately 1 km north of the study site (USGS Stn 07375230) to obtain the plot surface elevation relative to the river stage. Relative surface elevation was then averaged across all plots and subtracted from the gauge height data to calculate mean flood depth per plot (0.137 m m⁻²) and flooding frequency (0.59 d⁻¹) for the period of record February 2004 to November 2006. Potential loading rate for each nutrient was then calculated using appropriate unit conversions and the following equation: [nutrient] \times flood depth \times plot area × flooding frequency

Constituent	Sample size (n)	Concentration (mg N or P l ⁻¹)	Potential loading rate (kg ha ⁻¹ yr ⁻¹)
Tchefuncte Riv	er		
NH ₃ -N	64	0.14 ± 0.01	41
$NO_3 + NO_2 - N$	81	0.12 ± 0.01	40
Total N	82	0.79 ± 0.03	231
Total P	90	0.11 ± 0.004	32
Mississippi Riv	er		
NH ₃ -N	42	0.15 ± 0.02^{a}	b
$NO_3 + NO_2 - N$	91	1.39 ± 0.06	
Total N	91	2.26 ± 0.07	
Total P	89	0.21 ± 0.01	

^aAverage NH₃ concentration from 1999 to 2004

winter (Baldwin & Mendelssohn 1998, S. A. Graham pers. obs.), so plant biomass at the beginning of the growing season was zero. Each 1 m² plot was divided into four 0.25 m² sub-plots, and all above-ground biomass within a single randomly chosen sub-plot was clipped approximately every 6 wk, for a total of 4 biomass harvests per plot. Clipped plant biomass was then separated into live and dead categories, dried to a constant weight at 60°C, and weighed. Estimates of NAPP were calculated using the Smalley method, which uses changes in both live and dead biomass over time to determine plant production (Smalley 1959). This method is the most widely used in salt marshes for estimating net production, although it is limited in ability to account for biomass export due to tidal flushing and shoot mortality and decomposition between sampling periods (Linthurst & Reimold 1978). While

^bThe potential nutrient loading rates to this marsh from diverted Mississippi River water were not included due to the hypothetical nature of this calculation. However, to calculate these hypothetical loading rates, recognizing the assumption that these values apply only if similar hydrologic conditions are maintained, plug the Mississippi River nutrient concentration into the potential loading rate equation described above

absolute production may be underestimated (Daoust & Childers 1998), relative differences in production accurately reflect treatment effects.

To address Objectives 2 and 3, we harvested end-ofseason above-ground biomass in October 2004. This end of the growth season biomass harvest had no effect on the following year's NAPP estimation because vegetation at the site senesces each winter. Plant material within a 0.5 m² quadrat placed in the center of each 1 m² plot was clipped to the ground surface and separated into live or dead categories by species, then dried at 60°C and weighed. Species richness was determined as the total number of species per clipped plot. We calculated species' relative dominance as the percent of species-specific biomass per clipped plot. Differential biomass among species is a good measure of dominance, especially when the plants have similar growth forms. However, because many species occurred at low frequency, we statistically analyzed relative dominance of the 3 dominant species only.

Dried green and senesced leaf and stem tissue from the plant community dominant species, *Sagittaria lancifolia*, was ground using a Wiley mill and analyzed for total N using a Costech 4010 CHNS/O Elemental Combustion System and total P using Inductively Coupled Plasma (ICP) Spectrometry (Spectro Ciros) following nitric acid digestion. Tissue N and P concentrations were then used to calculate N:P ratios (mol:mol) and dry weight-based nutrient resorption efficiencies (i.e. the relative percent difference between live and dead tissue nutrient concentrations; van Heerwaarden et al. 2003).

Statistical analysis. All statistical analyses were conducted using SAS (version 9.1.3, SAS Institute). We used multivariate analysis of variance (MANOVA) (PROC GLM) to determine overall effects of N, P, and their interaction $(N \times P)$ on the following dependent variables as a group: net above-ground primary production, species richness, relative dominance of the 3 most dominant species (Sagittaria lancifolia, Eleocharis fallax, and Polygonum punctatum), and S. lancifolia tissue N and P concentrations, ratios, and resorption efficiencies. Overall treatment effects were determined using the Wilks' lambda test statistic. Where a significant overall effect was identified, individual mixed-model ANOVAs (PROC MIXED) were used to identify the specific dependent variables that contributed to the significant overall effect. Treatment means in PROC MIXED were tested using the least-squares (LS) means procedure with a Tukey-Kramer adjustment to maintain an experiment-wise error rate of 5 %. We also used linear and curvilinear regression analyses (PROG REG) to identify predictive relationships with increasing N enrichment, and Pearson correlation analysis (PROC CORR) to identify bivariate relationships among calculated nutrient resorption efficiencies. When necessary,

these data were logarithmically or square root-transformed prior to analysis to improve homogeneity of variance and goodness of fit to a normal distribution. All measures of significance were identified at p < 0.05.

RESULTS

MANOVA results indicated a significant overall N effect on the various measures of oligohaline marsh ecosystem structure (e.g. species richness and percent dominance) and function (e.g. NAPP and *Sagittaria lancifolia* tissue nutrient concentrations, ratios, and resorption efficiencies) (Table 2). P had no significant effect alone or in interaction with N. Thus, only N effects are discussed. ANOVA results showing the individual dependent variables that contributed to the significant overall N effect are displayed in Table 2.

Net above-ground primary productivity

Primary production increased from 1243 ± 75 to 1912 ± 152 g m⁻² yr⁻¹ with increasing N enrichment,

Table 2. Multivariate analysis of variance (MANOVA) results showing the overall effects of N, P, and their interaction on all (n = 10) dependent variables as a group. Significant (p < 0.05, p-values in bold) treatments effects were determined using Wilks' lambda test statistic. Individual ANOVA results are displayed for the N effect only to identify the specific dependent variables that contributed to the significant overall N effect

	Numerator df, denominator df	p		
MANOVA				
N	30, 56.4	0.0001		
P	10, 19	0.21		
$N \times P$	30, 56.4	0.78		
ANOVA (N effect)				
(1) Net above-ground	3, 28	0.0006		
primary production				
(2) Species richness	3, 28	0.87		
(3) Relative dominance				
(a) Sagittaria lancifolia	3, 28	0.08		
(b) Eleocharis fallax	3, 28	0.0004		
(c) Polygonum punctatum	3, 28	0.001		
(4) S. lancifolia tissue nutrients				
(a) Green-tissue N	3, 28	0.03		
(b) Green-tissue P	3, 32ª	0.36		
(c) Green-tissue molar N:P rati	io 3, 28	0.0003		
(d) N resorption efficiency	3, 28	0.01		
(e) P resorption efficiency	3, 28	0.004		
and the state of t				

^aDenominator df = 32 because the block covariance parameter estimate σ_{ρ}^{2} = 0. Covariance parameters with zero variance do not contribute to df computed by the Satterthwaite method

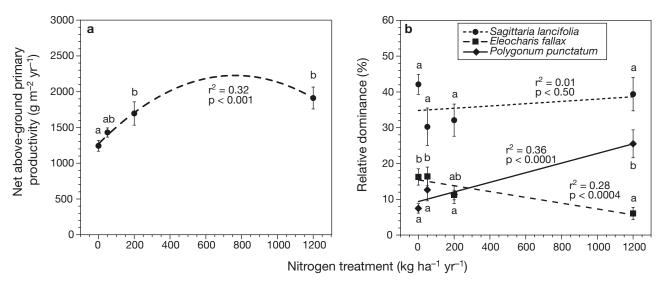


Fig. 1. (a) Macrophyte net above-ground primary productivity (NAPP) and (b) relative dominance of the 3 dominant macrophytes. Means (\pm SE, n = 10) are averaged over P treatment levels (no significant P-effect). Means separated by different letters are significantly different (p \leq 0.05, Tukey-Kramer multiple comparison test). Different letters in (b) indicate significant differences in relative dominance within (not across) species. Coefficients of determination (r^2) and p-values represent the best fit to (a) $y = -0.00165x^2 + 2.5091x + 1270.3$ (NAPP), and (b) y = 0.00319x + 34.810 (Sagittaria lancifolia), y = -0.00812x + 15.410 (Eleocharis fallax), and y = 0.01344x + 9.353 (Polygonum punctatum)

and enrichment with 200 kg N ha⁻¹ yr⁻¹ resulted in significantly greater NAPP compared to control plots (Fig. 1a). Further enrichment with 1200 kg N ha⁻¹ yr⁻¹ also increased NAPP compared to the control, but had no additional effect compared to the 200 kg N ha⁻¹ yr⁻¹

treatment. Rather, NAPP increased asymptotically with N enrichment. The quadratic regression suggests that maximum community primary production occurred within our range of N enrichment treatments (Fig. 1a).

Community composition

Within the plant community we distinquished 20 distinct taxonomic categories representing 17 identified species in 11 families (Table 3). N had no significant effect on species richness. The total number of species per 0.5 m² clip plot averaged 7.9 ± 0.2 regardless of treatment. Although most species were rare and represented only a small fraction of each plot's total biomass, the 3 dominant plant species (Sagittaria lancifolia, Eleocharis fallax, and Polygo-num punctatum) were present in almost every plot, and when combined, accounted for >60% of the total aboveground biomass on average. S. lancifolia was clearly the dominant plant, representing $36.0 \pm 2.2\%$ of the total biomass, regardless of treatment (Fig. 1b). Regression analysis did not identify any significant trends or any variability in *S. lancifolia* relative dominance due to N enrichment, which indicates that *S. lancifolia* biomass increased at the

Table 3. Plant species identified within study plots and their relative dominance presented as the average (range) for all plots

Species	Family	Relative dominance		
1	1	(%) (range)		
Sagittaria lancifolia	Alismataceae	36 (12–60)		
Polygonum punctatum	Polygonaceae	14 (0-48) ^a		
Eleocharis fallax	Cyperaceae	12 (1–31)		
	Ameranthaceae	` ,		
Alternanthera philoxeroides		- ()		
Symphyotrichum subulatum	Asteraceae Fabaceae	4 (0–18)		
Vigna luteola		2 (0-6)		
Lythrum lineare	Lythraceae	2 (0-34)		
Echinochloa crus-galli	Poaceae	1 (0-4)		
Panicum dichotomiflorum	Poaceae	<1 (0-6)		
Ipomoea sagittata	Convolvulaceae	(' ' ')		
Galium tinctorium	Rubiaceae	<1 (0 to <1)		
Phyla nodiflora	Verbenaceae	<1 (0 to <1)		
Cyperus odoratus	Cyperaceae	<1 (0 to <1)		
Dioda virginiana	Rubiaceae	<1 (0 to <1)		
Schoenoplectus tabernaemontani	Cyperaceae	<1 (0 to <1)		
Spartina patens	Poaceae	<1 (0 to <1)		
Ambrosia artemisiifolia	Asteraceae	<1 (0 to <1)		
Unknown grass (1) ^b	Poaceae	<1 (0 to <1)		
Unknown grass (2) ^b	Poaceae	<1 (0 to <1)		
Unknown spp.b	Unknown	<1 (0 to <1)		
^a Polygonum punctatum was present in all but 1 plot ^b Unidentifiable plant fragments				

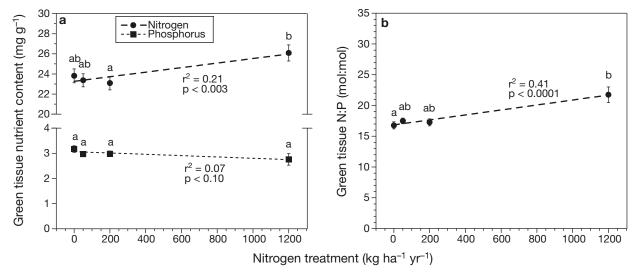


Fig. 2. Sagittaria lancifolia green-tissue (a) N and P content and (b) N:P ratio (mol:mol). Means (\pm SE, n = 10) are averaged over P treatment levels (no significant P-effect). Means separated by different letters are significantly different (p \leq 0.05, Tukey-Kramer multiple comparison test). Coefficients of determination (r²) and p-values are displayed for (a) y = 0.00226x + 23.271 (N content) and y = -0.00026x + 3.064 (P content), and (b) y = 0.00404x + 16.853 (N:P ratio)

same rate as the plant community. In plots receiving $1200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, the relative dominance of *E. fallax* fell by 10% while the dominance of *P. punctatum* increased by 18% compared to control plots (Fig. 1b). N-induced changes in the relative dominance of both species displayed highly significant linear trends that ultimately resulted in a shift in dominance between the 2 species at the greatest N enrichment level of $1200 \text{ kg ha}^{-1} \text{ yr}^{-1}$.

Sagittaria lancifolia tissue nutrients

Although the main effect of N enrichment on greentissue N was significant (Table 2), no N treatment level was significantly different from the control (Fig. 2a). Tissue N was significantly higher only in plots enriched with 1200 kg N ha⁻¹ yr⁻¹ compared to plots enriched with 200 kg N ha⁻¹ yr⁻¹, but both were similar to the control. Green-tissue P content did not change significantly following enrichment with N. However, on average, green-tissue N and P concentrations were approximately 10% higher and 13% lower, respectively, in plots enriched with 1200 kg N ha⁻¹ yr⁻¹ compared to control plots (Fig. 2a). Overall, tissue N had a significant positive linear relationship with N enrichment, but the regression analysis accounted for only 21% of the variability. The linear relationship between tissue P and N enrichment was not significant.

Sagittaria lancifolia N:P ratios increased significantly with 1200 kg N ha^{-1} yr⁻¹ compared to the control due to the combined non-significant changes in tissue N and P (Fig. 2b). Plots enriched with intermediate levels of N

(50 and 200 kg ha^{-1} yr⁻¹) contained *S. lancifolia* plants with intermediate tissue N:P ratios that were not statistically different from either the control or the high-N plots. A highly significant positive linear relationship with increasing N enrichment accounted for 41% of the variability in tissue N:P.

In control plots, resorption of N (NRE) to perennating structures by *Sagittaria lancifolia* during senescence was little more than half as efficient as P resorption

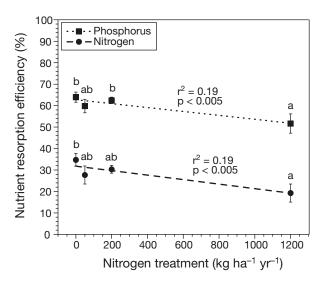


Fig. 3. Sagittaria lancifolia P and N resorption efficiencies (PRE and NRE respectively) averaged over P treatment levels. Means separated by different letters are significantly different (p \leq 0.05, Tukey-Kramer multiple comparison test). Coefficients of determination (r²) and p-values represent the best fit to y = -0.00915x + 62.778 (PRE) and y = -0.01048x + 31.796 (NRE)

(PRE): NRE = 35%, PRE = 64%. Both NRE and PRE decreased following enrichment with 1200 kg N ha⁻¹ yr⁻¹ to where *S. lancifolia* resorbed significantly less N and P compared to the control plots (Fig. 3). Linear regressions for both NRE and PRE were highly significant, but each explained only 19% of the variability in nutrient resorption with increasing N enrichment. There was also a strong correlation between *S. lancifolia* NRE and PRE (r = 0.68, p < 0.0001; data not shown). Higher NRE corresponded to higher PRE, although not at a 1:1 relationship. On average, each 10% change in NRE corresponded to a 6% change in PRE.

DISCUSSION

Nutrient limitation status

The results from the present study confirm our hypothesis that N limits primary production in this oligohaline marsh, but refute the hypothesis of secondary P limitation. This conclusion is based primarily on the significant increase (36 to 54%) in NAPP after 4 yr of N enrichment; no changes were detected following P enrichment, and no additional changes were detected following N and P enrichment. Our findings are consistent with a number of experiments in mesohaline (brackish) and polyhaline (salt) marsh systems that have documented increased plant primary production or standing crop following N enrichment (Sullivan & Daiber 1974, Valiela & Teal 1974, Cargill & Jefferies 1984, Boyer et al. 2001, Wigand et al. 2004). Our results are also consistent with studies that have applied N to oligohaline marshes in Louisiana. Fertilizing with N only, DeLaune & Lindau (1990) doubled the biomass of Sagittaria lancifolia-dominated marsh. Outside of Louisiana, N enrichment increased Zizaniopsis miliacea (giant cutgrass) biomass by 2-fold in a Georgia tidal freshwater marsh after 2 yr of N and/or P enrichment (Frost et al. 2009).

Although our results are consistent with more-saline ecosystems in general, and other oligohaline marshes in Louisiana, they are contrary to results from the only other oligohaline marsh fertilization study designed to determine oligohaline marsh nutrient limitation. Crain (2007) concluded that oligohaline marshes along 2 estuaries in southern Maine were co-limited by N and P after 3 yr of combined nutrient enrichment had increased above-ground biomass by 300%. This inconsistency indicates that the relative importance of P to oligohaline marsh primary production differs between locations. Potential factors contributing to differential nutrient loading include tidal flushing, nutrient source, eutrophic condition, and nutrient inputs (Bricker et al. 1999).

Compared to results from other oligohaline marsh fertilization studies (DeLaune & Lindau 1990, Crain 2007), we observed a relatively small, but significant, increase in NAPP following enrichment, which can most likely be attributed to differences in ambient marsh productivity. Ambient potential nutrient-loading rates calculated for this marsh in Table 1 coupled with high NAPP (~1250 g m⁻² yr⁻¹) in control plots indicate that fertile conditions existed at our site prior to N and/or P enrichment. Furthermore, control plots at our site had approximately 16% and 35 to 80% more biomass than the control plots harvested by DeLaune & Lindau (1990) and Crain (2007), respectively, during comparable biomass harvests in mid-summer. Güsewell & Bollens (2003) determined that the absolute supply of the limiting nutrient was most important when ambient marsh fertility was low. Therefore, nutrient enrichment should have less of an impact on more productive marshes than those that are naturally less productive.

Under the current assemblage of plant species, NAPP appears to have reached a maximum at enrichment levels ≥200 kg N ha⁻¹ yr⁻¹. NAPP in plots enriched with 200 kg N ha⁻¹ yr⁻¹ was not significantly different from NAPP in plots enriched with 1200 kg N ha⁻¹ yr⁻¹, but both were significantly greater than the control. However, we cannot eliminate the possibility that plant production could have been greater at some level of enrichment between 200 and 1200 kg N ha⁻¹ yr⁻¹ as suggested by the regression analysis (Fig. 1a). According to the quadratic fit, NAPP would have peaked at approximately 2220 g m⁻² yr⁻¹ with 760 g N ha⁻¹ yr⁻¹ enrichment. Regardless, the observed reduction in the rate that NAPP increased when enrichment exceeded 200 kg N ha⁻¹ yr⁻¹ is an indication that N limitation was alleviated and the vegetation's nutrientassimilation capacity was surpassed.

Plant nutrient cycling and community composition

We measured *Sagittaria lancifolia* green-tissue N:P ratios of approximately 17 (mol:mol) in control plots, corroborating that N was the primary limiting nutrient under ambient conditions (N:P < 31 mol:mol; Koerselman & Meuleman 1996). Adding additional N increased tissue N:P ratios to approximately 22 (mol: mol), but the ratio remained well below that necessary to indicate P limitation (N:P > 36 mol:mol; Koerselman & Meuleman 1996), even though NAPP measurements indicate that N limitation was alleviated. Similarly, Frost et al. (2009) were unable to increase *Zizaniopsis miliacea* N:P ratios in an N-limited tidal freshwater marsh to a level beyond the P-limitation threshold. Others have found that vegetation N:P ratios do not correspond with nutrient limitation determined by fer-

tilization, suggesting that N:P ratios have limited application in understanding the nutrient dynamics of these and perhaps other systems (Morse et al. 2004, Crain 2007). During the present study, a corresponding decrease in N and P resorption occurred as tissue N:P ratios increased with increasing N enrichment. Thus, under conditions of high N loading, *S. lancifolia* conserves less N and P and returns more to the soil during senescence, which may explain why tissue N:P ratios did not exceed the P-limitation threshold.

Reduced nutrient resorption efficiency is also an indication that Sagittaria lancifolia's optimal N supply rate has been surpassed. A meta-analysis of fertilization experiments by Aerts (1996) revealed that only about one-third of the species tested responded to increased nutrient availability by lowering nutrient resorption. Concurrent changes in relative dominance of the 2 subdominant species at the 1200 kg N ha⁻¹ yr⁻¹ enrichment level suggest that these species have different optimal N supply rates than S. lancifolia and the plant community as a whole (Bedford et al. 1999), and may explain why the meta-analysis by Aerts (1996) revealed that only a small portion of plant species reduce nutrient resorption under conditions of higher nutrient availability. The linear increase in Polygonum punctatum dominance with increasing N enrichment indicates that this species prefers a higher nutrient environment, and therefore, would not down-regulate nutrient resorption under such conditions (Fig. 1b). If this linear trend persists with further N enrichment in time or quantity, a shift in species dominance is likely to occur. P. punctatum could eventually achieve competitive advantage and possibly reduce species richness by displacing species that utilize nutrients less efficiently or that prefer a lower nutrient environment (e.g. Eleocharis fallax). Studies have shown that nutrient-aggressive plants such as Typha spp. and Phragmites australis are capable of reducing species richness by displacement under high nutrient-loading rates (Chambers et al. 1999). In fact, increased Polygonum dominance relative to other component species characterized nutrient enrichment in areas of the Florida Everglades (Vaithiyanathan & Richardson 1999).

Eutrophication potential

When viewed collectively, these data show that N enrichment affects different aspects of the plant community at different rates depending on the loading rate (Table 4). Adding 200 kg N ha $^{-1}$ yr $^{-1}$ to this marsh stimulated NAPP, but no other significant changes were detected, showing that at this level of enrichment the additional N was utilized primarily to increase plant production. Adding 1200 kg N ha $^{-1}$ yr $^{-1}$, on the other

Table 4. Eutrophication potential of various functional and structural vegetative characteristics. Initial response level indicates the N enrichment level at which a significant (p < 0.05, Tukey-Kramer multiple comparison test) change first occurred. nr: no response

Vegetation parameter	Initial response level (kg N ha ⁻¹ yr ⁻¹)
Net above-ground primary productiv	vity 200
Tissue N:P ratio	1200
N and P resorption efficiency	1200
Relative dominance	1200
Species richness	nr

hand, not only stimulated NAPP to a similar degree as with 200 kg N ha⁻¹ yr⁻¹, but also increased *Sagittaria lancifolia* tissue N:P ratios, decreased *S. lancifolia* nutrient resorption efficiencies, and altered the relative dominance of the dominant species. Therefore, N enrichment beyond that which contributes to plant growth is stored in plant tissues, which in turn, alters plant nutrient-utilization strategies, and ultimately results in changes in plant community structure. Although we detected no significant changes in species richness, Slocum & Mendelssohn (2008) observed reduced species richness at a nearby site receiving the same N loading rate (1200 kg N ha⁻¹ yr⁻¹) applied as N-P-K over a comparable time frame.

Similar to the results from the present study, Aerts et al. (1992) explained that the eutrophication process in Nlimited European Sphagnum bogs can be viewed as a chronosequence. The results from their study showed that N enrichment initially increased Sphagnum production and tissue N:P ratios. With further N enrichment in time or quantity, the N:P ratio became so high that P became limiting, ultimately reducing Sphagnum growth and leading to its disappearance. Contrary to the sequence described by Aerts et al. (1992), we observed an increase in production prior to detecting any changes in nutrient utilization and storage. Furthermore, Sagittaria lancifolia's N:P ratio never indicated P limitation, despite the fact that NAPP measurements indicated that N limitation had been alleviated. Unlike vascular plants, Sphagnum mosses have no root system, and therefore, are unable to translocate nutrients during senescence via nutrient resorption, at least in the traditional sense (see Rydin & Clymo 1989). The observed reduction in nutrient resorption in the present study may be a mechanism by which the effects of elevated nutrient loading are counteracted, slowing this sequence of eutrophication and helping Sagittaria lancifolia maintain relative dominance in the short term. Over the long term, however, reduced nutrient resorption could potentially create a positive feedback loop and accelerate eutrophication by returning more N and P to the soil during senescence.

CONCLUSIONS

We conclude that this marsh and possibly others dominated by Sagittaria lancifolia in Louisiana are N-limited. Therefore, our results refute generalized N and P co-limitation of oligohaline marsh primary production and suggest that local factors, such as ambient marsh fertility, may dictate the relative importance of N and P in these systems. The various measured plant characteristics indicate that a sequence of eutrophication occurs as enrichment with the limiting nutrient increases. This marsh is capable of assimilating at least 200 kg ha⁻¹ yr⁻¹ more N than the current loading rate without significant changes to ecosystem structure. Increased NAPP following N addition, while significant, was rather small in magnitude, and there were signs that N limitation was alleviated. N enrichment above this amount (i.e. 1200 kg N ha⁻¹ yr⁻¹) surpassed the nutrient-assimilation capacity of the vegetation, altering plant nutrient cycling, which caused changes in ecosystem structure. This suggests that oligohaline marshes such as this one may have limited potential for removing excess nutrients, even if some of the species relax their nutrient resorption efficiencies. Although it is unlikely that natural sources of eutrophication (e.g. the Mississippi River) will surpass our experimental N-loading rates in magnitude, the cumulative load over the long term (decades) could be much higher, and result in equivalent or more pronounced changes in ecosystem structure and function.

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