Nitrogen and Phosphorus Differentially Affect Annual and Perennial Plants in Tidal Freshwater and Oligohaline Wetlands

Andrew H. Baldwin

Abstract To test species composition and biomass responses to excess nutrients, herbaceous plants of tidal freshwater and oligohaline wetlands in a Chesapeake Bay subestuary were fertilized with nitrogen (N), phosphorus (P), both N and P (N+P), or not fertilized (Control) for 4 years. In marshes, the N treatment increased abundance measures of perennials but decreased those of annuals while the P treatment increased annuals and decreased perennials. In swamps, however, perennials increased in response to P. Total herbaceous aboveground biomass production was not limited by N, P, or N+P in marshes or swamps. These findings suggest that annual species are more susceptible than perennials to P limitation, possibly due to lack of a large perenniating root organ and lower susceptibility to mycorrhizal inoculation. Furthermore, eutrophication effects are likely to vary between swamp and marsh habitats and depend on whether the dominant nutrient supplied is nitrogen or phosphorus.

Keywords Tidal freshwater marsh · Swamp · Wetland · Nitrogen · Phosphorus · Nutrient limitation · Vegetation N/P ratio · Plant communities · Annual · Perennial · Aboveground biomass

A. H. Baldwin (✉)
Department of Environmental Science and Technology,
University of Maryland,
College Park, MD 20742, USA
e-mail: baldwin@umd.edu

Introduction

Human alteration of global nitrogen and phosphorus cycles has altered primary production and other ecosystem functions in many different ecosystems (Vitousek et al. 1997; LeBauer and Treseder 2008; Vitousek et al. 2010). Addition of nitrogen (N) or phosphorus (P) enhances plant production in many terrestrial ecosystems, but potassium limitation may occur or environmental constraints such as light, temperature, water supply, or non-resource stressors may also constrain growth (Chapin and Shaver 1985; Gough et al. 1994; Aerts and Chapin 2000; Peterson-Smith and Baldwin 2006). Changes in vegetation composition and decreases in plant species richness resulting from nutrient addition are due to altered relative competitive ability of individual species depending on their functional traits or due to random loss of species occurring at low initial abundance (DiTomasso and Aarssen 1989; Suding et al. 2005). Different species in a community respond individually to increased nutrients, resulting in compositional change even if community biomass production remains stable over time (Chapin and Shaver 1985; DiTomasso and Aarssen 1989).

In a variety of wetland types, N or P limits plant productivity and excesses in these nutrients alter plant species richness, composition, and production (Bedford et al. 1999; Venterink et al. 2003; Gusewell et al. 2005; Johnson and Rejmankova 2005). Estuarine ecosystems, including Chesapeake Bay on the US mid-Atlantic coast, are hot spots for excess nutrients due to runoff from agricultural and urban runoff and wastewater treatment plants (Howarth et al. 2002; Russell et al. 2008), and coastal development and eutrophication have been demonstrated to change plant species composition and alter fish and invertebrate interactions (Silliman and Bertness 2004;

Deegan et al. 2007). Fertilization experiments have demonstrated that salt and brackish marshes are primarily N limited (Valiela et al. 1978; van Wijnen and Bakker 1999) (in this paper, wetlands dominated by herbaceous plants are referred to as marshes and those dominanted by woody plants as swamps). However, individual species respond differently to N addition within the same wetland type: in North America, the salt marsh grasses *Spartina alterniflora* and *Distichlis spicata* consistently increase and the rush *Juncus roemerianus* (when in mixture with *S. alterniflora*) consistently decreases in response to N (Pennings et al. 2002, 2005).

Fewer studies of nutrient effects have been conducted in tidal freshwater and oligohaline marshes than in salt marshes, but those that have suggest that N is limiting singly or co-limiting with P in these wetlands (Crain 2007; Frost et al. 2009; Graham and Mendelssohn 2010; Ket et al. 2011). In a Louisiana oligohaline marsh, N did not affect species richness but did result in an increase some species while others decreased (Graham and Mendelssohn 2010). Similarly, species diversity was not altered but species composition changed dramatically in response to N addition in oligohaline marshes of two estuaries in Maine on the northeast US Atlantic coast (Crain 2007). Release of *Zizaniopsis miliacea* from N limitation in a tidal freshwater marsh (in Georgia on the US southeastern coast) was accompanied by higher densities of benthic invertebrates (Frost et al. 2009). Nutrient ratios suggest that many forested wetlands (swamps) are P limited (Bedford et al. 1999), and P limitation is suggested by high soil N/P ratios in some tidal freshwater swamps (Baldwin 2007). These findings suggest that patterns of nutrient limitation can differ between wetland types within the same estuary and salinity regime.

Nutrient ratios of plant tissue have been proposed as an indicator of N or P limitation in wetlands (Koerselman and Meuleman 1996) but further investigation indicates that although high N/P ratios (>20) probably indicate P limitation, N limitation and long-term changes in species composition are difficult to predict based on tissue concentrations (Gusewell et al. 2003; Gusewell 2004). In tidal freshwater and oligohaline wetlands, low N/P ratios did not correspond to N or P limitation of biomass production in some marshes (Morse et al. 2004; Crain 2007) but were indicative of N limitation in others (Frost et al. 2009; Graham and Mendelssohn 2010; Ket et al. 2011). Tissue and soil nutrients may nonetheless be useful in identifying the relative scarcity of N and P (Gusewell et al. 2003) and in understanding shifts in relative abundance of species in response to different nutrients.

Furthermore, plants are often subject to multiple resource limitation (MRL; Vitousek et al. 2010), where they are simultaneously limited by N, P, or other resources (e.g., light, CO_2, and water). Under MRL conditions, increases in one limiting resource, such as N, may create greater limitation in others, such as P. The resource ratio hypothesis of succession (Tilman 1985) is consistent with MRL in that it proposes that species in a community have different competitive abilities for various limiting resources and that community composition will change when the relative availability of different resources shifts. Furthermore, different species can experience greater or lesser limitation to different resources depending on their morphology and physiology. For example, plants with larger roots, such as perennials, may be competitively superior in foraging for patchy resources relative to plants with smaller roots (Rajaniemi and Reynolds 2004). Additionally, perennials are often more responsive to mycorrhizal inoculation than annuals and are more likely to benefit from mycorrhizae at low P levels than annuals (Boerner 1992a; Peat and Fitter 1993; Collier et al. 2003) (but see Roumet et al. (2006)). Mycorrhizal responsiveness also correlates with ability to access insoluble forms of P (Boerner 1992b). Thus, perennials may be able to better access insoluble or patchy resources such as P than annuals.

Perennials also translocate nutrients to rhizomes during leaf senescence while annuals direct resources to reproductive structures and resorb little or none (Aerts and Chapin 2000). Thus, perennials can draw on stored resources for growth, a conservative resource-use strategy, in lieu of foraging, an acquisitive strategy, as annuals must do (Suding et al. 2005; Roumet et al. 2006). A meta-analysis of North American N-addition studies indicated that perennials decreased in response to fertilization and were more likely to be lost from communities than annuals, as might be predicted if the acquisitive strategy allowed annuals to better use pulses of nutrients than perennials (Suding et al. 2005). However, as noted above, annuals may not be as successful as perennials in acquiring patchy resources such as P if their roots are smaller and they are less responsive to mycorrhizal association.

The Nature Conservancy identified excess nutrients as a threat to biologically rich tidal freshwater wetlands in the Nanticoke River subestuary of Chesapeake Bay (TNC 1998), and in response to TNC concerns a broad goal of this research was to quantify eutrophication effects on plant diversity in this system. In this study, I examined the responses of herbaceous plant communities in tidal freshwater marshes and swamps to additions of N and P in a factorial treatment arrangement. The objectives were to (1) determine if N, P, or both were limiting herbaceous plant community biomass production, (2) describe changes in species composition and richness, and (3) relate soil and plant tissue concentrations of N and P to observed fertilization responses.

Methods

Study Sites

The Nanticoke River, a subestuary of Chesapeake Bay, drains a watershed of primarily agricultural land on the Delmarva peninsula, which forms the eastern shore of the Bay. In an analysis of 15 years of water-quality data in ten major Chesapeake Bay tributaries, mean water concentrations of total N were higher (2.2 mg l^{-1}) in the Nanticoke and Chester Rivers than in the eight other tributaries studied (0.4–1.7 mg l^{-1}) (Dauer et al. 2000). Total P concentrations (0.07 mg l^{-1}), in contrast, were within the middle of the range for the other tributaries (0.02–0.13 mg l^{-1}). The fertilization experiment was set up at three replicate marsh and three replicate swamp sites in the tidal freshwater and oligohaline reaches of the Nanticoke River main stem and the Broad Creek and Marshyhope Creek tributaries (Fig. 1; photographs in the Electronic supplementary material). All of the marsh sites are located in the "high marsh" community, which lies above mean high tide and thus is irregularly flooded. The swamp sites have a hummock–hollow microtopography. The hummocks are up to 20 cm higher than the hollows (Peterson and Baldwin 2004) and are flooded rarely (e.g., during spring tides), but the

hollows in many places are below mean high tide and thus are flooded regularly (see Rheinhardt (1992)). Thus, swamp hollows and hummocks exist in an intertidal hydrologic zone roughly comparable to that spanned by low and high marsh communities (Odum et al. 1984). The maximum width of most hollows and hummocks at the study sites was smaller at 2 m, so that most monitoring plots included some of each. Diurnal tidal range is about 0.7 m at Vienna on the Nanticoke River (NOAA tide gauge). Mean daily air temperature ranges from −2°C to 9°C in January up to 20°C to 30°C in July, and precipitation is about 1,100 mm annually (National Climatic Data Center, www.ncdc.noaa.gov).

Salinity in this part of the estuary is generally <1 (fresh to low oligohaline) but during severe droughts may increase to more than 7 at the Marshyhope–Nanticoke confluence (Peterson and Baldwin 2004; Baldwin 2007; Sharpe and Baldwin 2009). During the 4-year period of this study, mean porewater salinity±SE (measured with a portable meter) at marsh experimental sites was 0.5±0.1 (N=142) and 0.7±0.1 (N=144) at swamp sites. Salinity was highest in September 2002 during a drought, when mean salinity increased to 1.1±0.1 in marshes and 1.3±0.2 in swamps (N=36 for each). The highest recorded salinity was 4.5, measured at the swamp site on Marshyhope Creek in
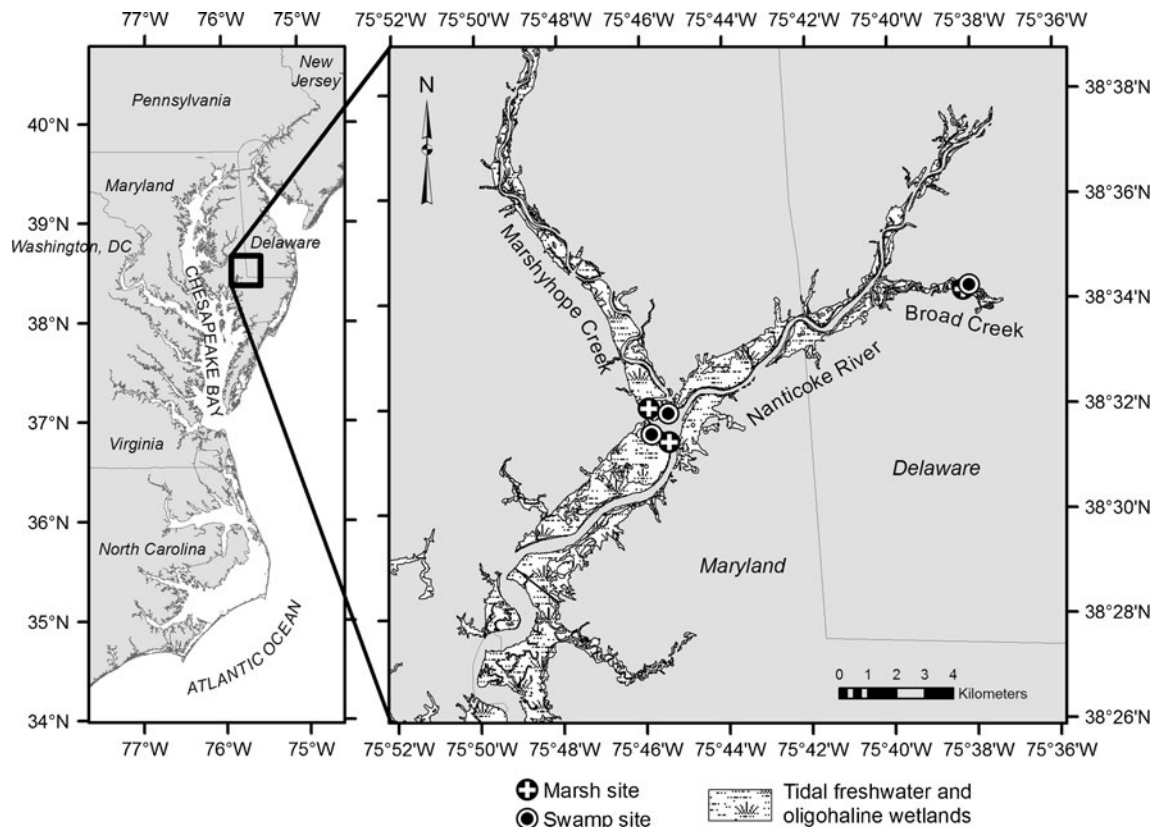

Fig. 1 Location of tidal marsh and swamp sites used in the fertilization experiment. The markers for the Broad Creek marsh and swamp sites are partially overlapping

September 2002. Salinity throughout the study was lowest at the most upstream sites, located on Broad Creek (marsh, 0.1 ± 0.1; swamp, 0.1 ± 0.01, $N=46$ for each) and highest at the most downstream sites, located on TNC property on the Nanticoke main stem (marsh, 0.9 ± 0.1; swamp, 1.0 ± 0.1, $N=46$ for each).

Nutrient Addition

At each marsh and swamp site, twelve 2×2-m fertilization plots were marked at random locations (72 plots total). Within each site, three plots were selected randomly to receive one of four nutrient addition treatments: Control (no nutrients added), N (nitrogen only added), P (phosphorus only added), and N+P (both nitrogen and phosphorus added). Pelletized N (40–0–0) and P (0–45–0) fertilizer was broadcast onto study plots at 22.4 gm^{-2} available N and 11.2 gm^{-2} available P three times annually at approximately 2-month intervals during the growing season (April–October) for 2 years, beginning in 2000 and ending before the final harvest. This application rate was selected to be similar to application rates for N and P for crops (Felton and Coale, University of Maryland Extension Service, personal communication) and was used in another tidal freshwater wetland study (Morse et al. 2004). Fertilizer was re-applied at approximately 2-month intervals due to a concern about washing away of nutrients during tidal inundation, resulting in annual net application rates of about 67.2 gm^{-2} $year^{-1}$ N and 33.6 gm^{-2} $year^{-1}$ P (670 N and 340 P kg ha^{-1} $year^{-1}$).

The nitrogen loading rate used in this study is similar to that applied in other tidal freshwater wetlands (50–120 gm^{-2} $year^{-1}$ N) (Graham and Mendelssohn 2010; Ket et al. 2011) but lower than that applied in a study of oligohaline, brackish, and salt marshes (163 gm^{-2} $year^{-1}$ N) (Crain (2007)). The loading rates in the current study are much lower than those applied in some salt marshes (Levine et al. 1998; Pennings et al. 2002), in which application of 60 gm^{-2} of 29–3–4 N–P–K fertilizer biweekly (about 450 gm^{-2} $year^{-1}$ N and 4,500 kg ha^{-1} $year^{-1}$) resulted in no obvious signs of plant burning.

Although differences in hydrology between swamp and marsh sites might have resulted in different rates of nutrient loading resulting from the treatments, their similar position in the intertidal zone suggests that any differences in loading rate were small. In swamps, only the fertilization responses of the herbaceous understory were examined; trees and shrubs were not directly fertilized or measured this study. Although it is possible that the small-scale fertilization of herbaceous understory plots used increased the growth of the few woody plants that were rooted directly in some of the swamp plots, no obvious increases in woody plant growth or shading of herbaceous plants were observed.

Vegetation and Soil Measurements

In each plot, estimates of plant cover were made in a 1×2-m quadrat twice during the first three growing seasons and once during the fourth. Visually estimated plant cover is a robust index of vegetation abundance that has long been used in many community types (Peet et al. 1998; Gurevitch et al. 2006). In August 2003 aboveground biomass was harvested for plant tissue and standing crop analysis in two 0.5×0.5-m quadrats within each 1×2-m cover quadrat. Aboveground biomass samples from marsh plots were separated into the two dominant species at each marsh site (*Polygonum arifolium* L. at all three sites, *Acorus calamus* L. at two sites, and *Typha* spp. at one site). Both *Typha latifolia* L. and *Typha angustifolia* L. and their hybrid *Typha×glauca* were present, and these were combined because it was often difficult to distinguish between them due to lack of flowers and the intermediate characters of the hybrid. *P. arifolium* is an annual species and *A. calamus* and *Typha* spp. are perennials. Other marsh species present were homogenized and analyzed together. At swamp sites no single species consistently dominated and biomass was lower so all species in each plot were homogenized. Samples were dried to a constant mass at 70 C and weighed to the nearest 0.1 g. Samples were ground and analyzed for total N and total P by the University of Pennsylvania Agricultural Analytical Services Laboratory. Surface soil samples were collected annually at the end of each growing season and analyzed for total N and total P by the University of Delaware Soil Testing Program. Each sample consisted of two 15-cm deep by 5-cm diameter bulb-planter cores collected from each plot and mixed together.

Data Analysis

Sites were treated as true replicates for each community type, with plots at each site treated as subsamples nested within sites. The arrangement of treatments was a 2×2 factorial. Data on plant cover and species richness (number of species) per 2-m^2 plot were analyzed using repeated measures analysis of covariance, with the initial cover or richness measured before treatments were applied used as the covariate. For individual species only nonzero values were included so that changes in their abundance where they occurred could be determined. Thus, their mean cover and biomass values do not reflect their average abundance across all plots, only the average of the plots where they occurred. Furthermore, this resulted in different degrees of freedom for each dependent variable. Results are only

presented for species that had significant main effects of N or P or N×P interaction effects. Soil nutrients and ratios were analyzed as univariate repeated measures analysis of variance (ANOVA) and plant tissue concentrations and mass of aboveground plant biomass analyzed as two-way ANOVA. The Tukey's procedure was used to separate treatment means. Simple linear regression was used to relate soil versus vegetation N/P ratios (both determined in 2003). Two-sample t tests were used to compare nutrient concentrations and ratios between unfertilized plots in marshes versus swamps. Analyses were conducted using SAS 9.2 (SAS Institute, Cary, North Carolina) and SigmaPlot 10 (Systat Software, San Jose, California).

Results

Cover, Richness, and Biomass

In marshes, cover of perennials was significantly higher and annuals significantly lower in the N treatment compared with the Control (Fig. 2). Cover of both annuals and perennials in the N+P treatment did not differ significantly from the Control, resulting in a significant N×P interaction for annuals (Table 1). In swamps, cover of annuals was
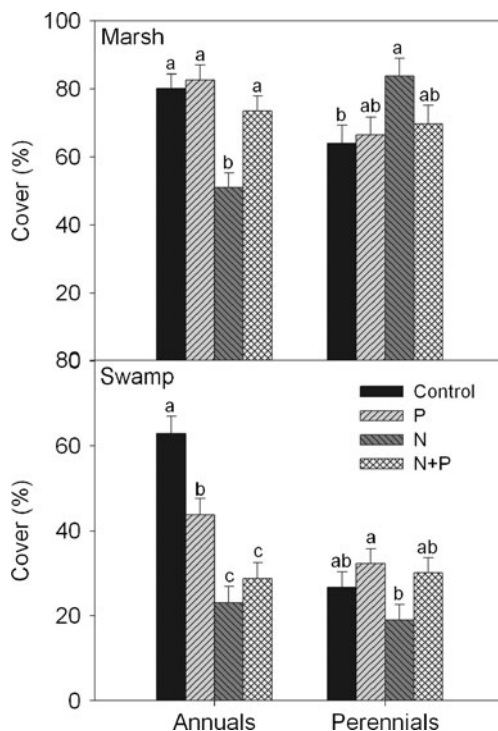

Fig. 2 Variation in cover of annual and perennial herbaceous species in response to N and P addition treatments. Plotted values are mean+ SE of cover in 2-m² plots over a 4-year period. Within each life history duration means with different letters are significantly different (Tukey's, $p<0.05$)

lowest in the N and N+P treatments while cover in the P treatment was intermediate to these and the Control, resulting in a significant N×P interaction. The N effect on swamp annuals also varied over time ($p<0.05$, N×time interaction) but was negative throughout the study (results not plotted). Compared with other treatments, the N treatment in swamps had the lowest cover of perennials while the P treatment had the highest.

Only four of the more than 75 species observed showed significant cover effects of N, P, or N×P (Table 1). In marshes, added N increased cover of the dominant perennials *A. calamus* and *Typha* spp. over the 4-year study ($p<0.05$, main effect of N; Fig. 3; Table 1). Although the main effect of P on *Typha* spp. cover was not significant, its cover was highest in the N+P treatment, suggesting co-limitation by N and P. In contrast to these two perennial species, cover of the annual species *P. arifolium* in marshes decreased due to N addition ($p<0.05$, main effect of N; Table 1), and was significantly lower in the N treatment than in the P treatment (Fig. 3). Added N also reduced *P. arifolium* cover in swamps but the magnitude of reduction was greater in the N than in the N+P treatment ($p<0.0001$, N×P interaction). The N effect on *P. arifolium* cover in swamps varied over time ($p<0.05$, N×time interaction) but was always negative (results not plotted). Cover of the annual *Zizania aquatica* in swamps increased in the N and P treatments but not in the N+P treatment, resulting in a significant N×P interaction ($p=0.001$, N×P interaction) but no significant main effects (Fig. 3; Table 1).

Species richness of marsh plants was unaffected by nutrient addition treatments but N reduced richness of swamp herbaceous plants, although to a lesser extent when P was also added (Fig. 4; Table 1; $p<0.001$, N×P interaction). Species richness was similar in marsh and swamp plots (about seven species per 2-m² plot).

Total aboveground biomass of herbaceous plant communities of marsh and swamp habitats determined after 4 years of nutrient addition did not significantly increase in response to addition of N, P, or N+P (Fig. 5; Table 2). However, in marshes biomass of the two dominant perennials, *A. calamus* and *Typha* spp., was significantly reduced by P addition ($p<0.05$, main effect of P) and *Typha* spp. biomass increased significantly due to N ($p<0.05$, main effect of N). Addition of N, P, or N+P did not significantly affect swamp total biomass, and there were no significant biomass responses of *P. arifolium* or "other species" due to nutrient addition treatments. Total mean biomass was higher in marshes (380–590 g m⁻²) than in swamps (20–100 g m⁻²).

Nutrient Concentrations and Ratios

Addition of N and P generally increased concentrations of these elements in plant tissues (Fig. 6; Table 3). Adding N

Table 1 *F* values from repeated measures analysis of covariance of effects of N, P, time, and their interactions, and the initial value (covariate) on plant cover and species richness in tidal freshwater marshes and swamps over a 4-year period (except *Typha* spp., analyzed only for years 1–3 due to missing cells in year 4)

| Variable	Habitat	df	N	P	N×P	Time	N×Time	P×Time	N×P×Time	Initial
Cover of annuals and perennials										
Annuals	Marsh	1, 179	18.68****	8.37**	4.79*					0.46
		5, 179				25.73****	0.93	0.37	0.82	
Perennials	Marsh	1, 179	4.69*	1.16	2.57					1.16
		5, 179				16.23****	1.50	0.31	0.19	
Annuals	Swamp	1, 119	50.22****	2.97	10.07**					9.47**
		5, 119				19.15****	2.81*	1.36	1.60	
Perennials	Swamp	1, 119	1.96	5.63*	0.58					88.39****
		5, 119				9.09****	0.58	0.99	0.33	
Cover of individual species										
Acorus calamus	Marsh	1, 117	3.99*	3.32	0.08					0.86
		5, 117				37.67****	0.59	0.37	0.12	
Polygonum arifolium	Marsh	1, 172	4.20*	3.86	0.05					1.80
		5, 172				23.80****	1.30	0.30	0.38	
Typha spp.	Marsh	1, 69	9.46**	0.07	0.52					1.79
		4, 69							0.15	
		5, 69				4.81***	1.17	1.07		
P. arifolium	Swamp	1, 134	56.00****	0.92	15.93****					9.79**
		5, 134				3.91**	2.66*	1.67	0.95	
Zizania aquatica	Swamp	1, 48	0.24	1.11	12.32**					26.51****
		5, 48				5.71***	0.66	0.21	1.76	
Species richness										
	Marsh	1, 179	0.81	1.07	2.50					213.88****
		5, 179				13.21****	0.17	0.68	1.38	
	Swamp	1, 191	19.06****	0.12	13.66***					333.13****
		5, 191				13.69****	0.32	0.15	0.82	

More than 75 species were observed during the study; results are presented only for those having significant effects of N, P, or N×P

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

increased N concentrations in *A. calamus* and *Typha* spp. (*p*<0.05, main effect of N). Adding P significantly increased P concentration in all marsh vegetation, both grouped and for individual species. Swamp vegetation N concentration was higher in the N treatment than in the Control and N+P treatments, resulting in a significant N×P

Fig. 3 Effects of nutrient addition treatments on cover of plant species that demonstrated any significant effect of nitrogen (N), phosphorus (P), or the N×P interaction in marshes or swamps. Values are mean+SE of cover of plant species in 2-m² plots over a 4-year period (except *Typha* spp., years 1–3 only). Within each species means with different letters are significantly different (Tukey's, *p*<0.05)
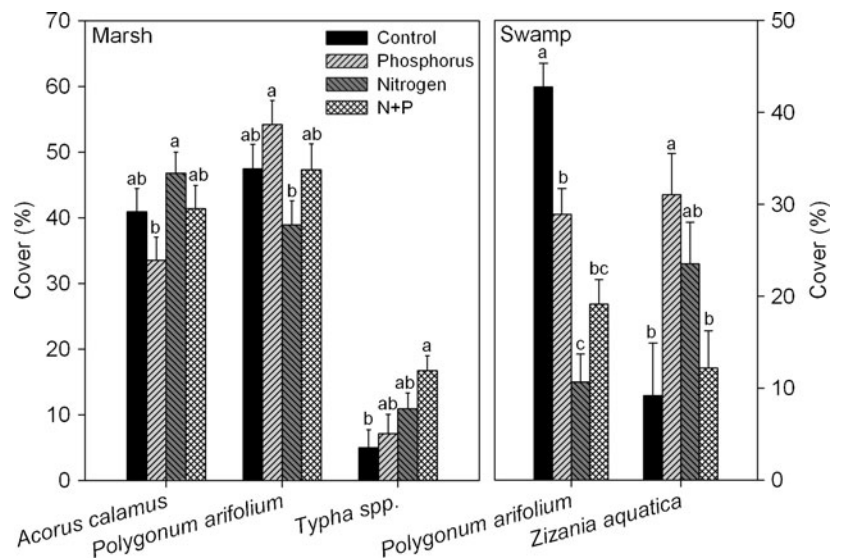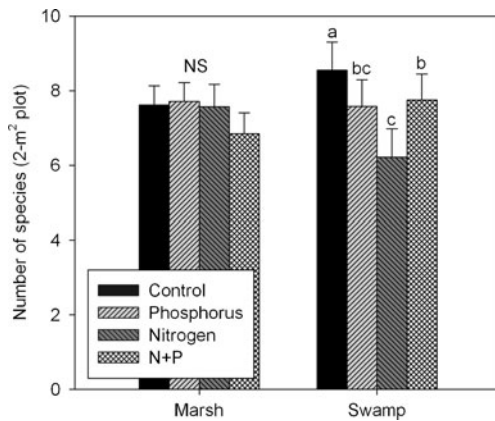
Fig. 4 Variation in species richness of herbaceous plants in marsh and swamp habitats. Values are mean+SE of number of species in 2-m² plots sampled over a 4-year period. Within each habitat means with different letters are significantly different (Tukey's, $p<0.05$). *NS* no significant difference between means

interaction. Neither N nor P addition resulted in changes in P concentrations in swamp plant tissue.

Addition of P singly or in combination with N reduced N/P ratios of soil and vegetation while the N treatment resulted in soil and vegetation N/P ratios similar to or higher than those of Control plots (Fig. 7). Vegetation N/P was similar to soil N/P at low soil N/P (about 5) but proportionally decreased to less than half of soil N/P at higher soil N/P (15–20). Concentrations of N in Control plots were similar in soil and vegetation but P concentration was generally higher in vegetation than soil (Fig. 8). An exception was *Typha* spp., which had lower concentrations of both N and P than other species. Soil N/P ratios in Control plots ranged from about 10 to 24 in marshes and 15 to 24 in swamps but did not differ significantly (equal-variance two-sample *t* test: $t_{15}=1.16$, $p=0.2637$, $p>0.05$). However, soil P concentration in Control plots was lower in

swamps than marshes (swamps, 0.079±0.002%; marshes, 0.107±0.009%; unequal-variance two-sample *t* test, $t_{8.5}=3.22$, $p=0.0114$). Vegetation N/P in control plots ranged from about 4 to 12 and nutrient concentrations in marsh and swamp plants were similar.

Discussion

Changes in Plant Community Composition

Nutrient treatments resulted in significant shifts in plant community composition. In marshes, which had higher soil P concentrations than swamps, the N treatment increased the cover of perennial species as a group and the dominant perennials *A. calamus* and *Typha* spp. (and the biomass of *Typha* spp.), suggesting that these species were limited by N. There was a corresponding decrease in the cover of annual plants (including *P. arifolium*) in response to the N treatment in marshes, suggesting a shift in relative competitive ability toward perennials. This is the opposite pattern observed in a meta-analysis of N-addition studies, which found that perennials were more likely to be lost than annuals across four herbaceous plant-dominated North American non-wetland sites (Suding et al. 2005). No species were lost from the one wetland ecosystem included in the study, Atlantic salt marsh.

Surprisingly, the P treatment increased cover of the annual *P. arifolium* and reduced biomass of the perennials *A. calamus* and *Typha* spp. This suggests that *P. arifolium* was limited by P and was able to outcompete the dominant perennials when its P limitation was relieved. *P. arifolium* and other annual species that lack large root organs may be less effective than perennials in foraging for P, which can be relatively immobile compared with N in wetlands (the

Fig. 5 Aboveground biomass responses to N and P addition treatments. Plotted values are mean+SE of herbaceous plant biomass in August 2003 in marsh and swamp habitats. Only nonzero values were included in analyses so changes in biomass of individual species where they occurred could be assessed. Within a community component, means with different letters are significantly different (Tukey's, $p<0.05$). *NS* no significant difference between means within a community component
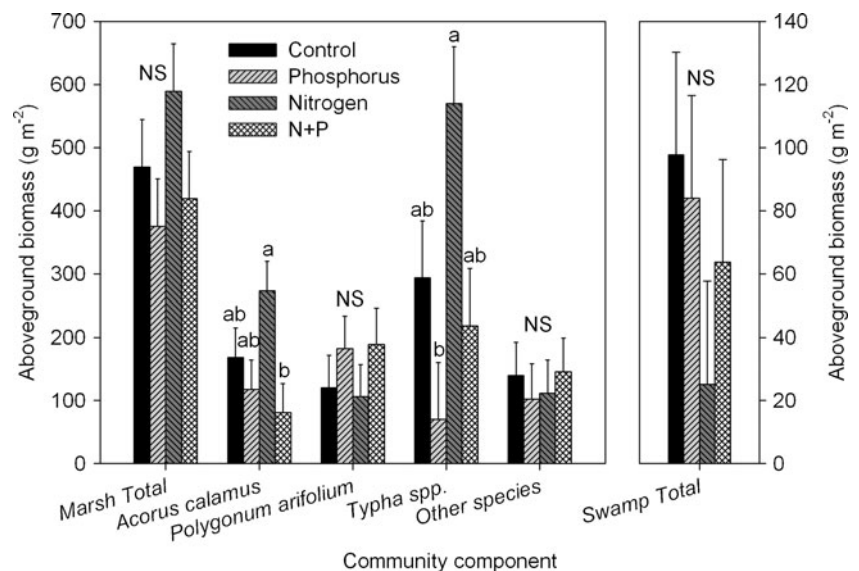
Table 2 Results of two-way ANOVA for effects of N and P addition treatments and their interaction (N×P) on final aboveground biomass

Type	Community component	df	Nitrogen (N) F	Phosphorus (P) F	N×P F
Marsh	Total	1, 32	1.19	3.11	0.26
	Acorus calamus	1, 19	0.52	6.80*	2.32
	Polygonum arifolium	1, 28	0.01	1.88	0.04
	Typha spp.	1, 7	5.52*	10.15*	0.51
	Other species	1, 30	0.02	0.001	0.44
Swamp	Total	1, 28	2.04	0.15	0.65

*$p < 0.05$

greater dissipation of N and accumulation of P is evident from the soil N/P ratios in Fig. 7). The smaller roots of annuals also store less P and annuals are often less responsive to mycorrhizal association than perennials (Boerner 1992a; Peat and Fitter 1993), reducing their capacity for acquiring insoluble P (Boerner 1992b). Addition of N+P in marshes resulted in no change in cover of *A. calamus* and *P. arifolium*, suggesting that

alleviating both N and P limitation resulted in a competitive balance between the two species.

Although relative abundance of marsh species changed due to nutrient treatments, species richness was not altered, similar to results in other tidal fresh and oligohaline marshes (Crain 2007; Graham and Mendelssohn 2010).

The P treatment in swamps, which had significantly lower soil P concentrations than marshes, promoted

Fig. 6 Nitrogen (N) and phosphorus (P) concentration in plant tissues in plots receiving different N and P addition treatments. Values are mean+SE. Within each community component means with different letters are significantly different (Tukey's, $p < 0.05$). Where main effects of N or P were significant but N×P means were not separated by the Tukey's procedure, the factors and their level of significance are noted above the group of bars for that community component (*$p < 0.05$). *NS* no significant difference between means within a community component
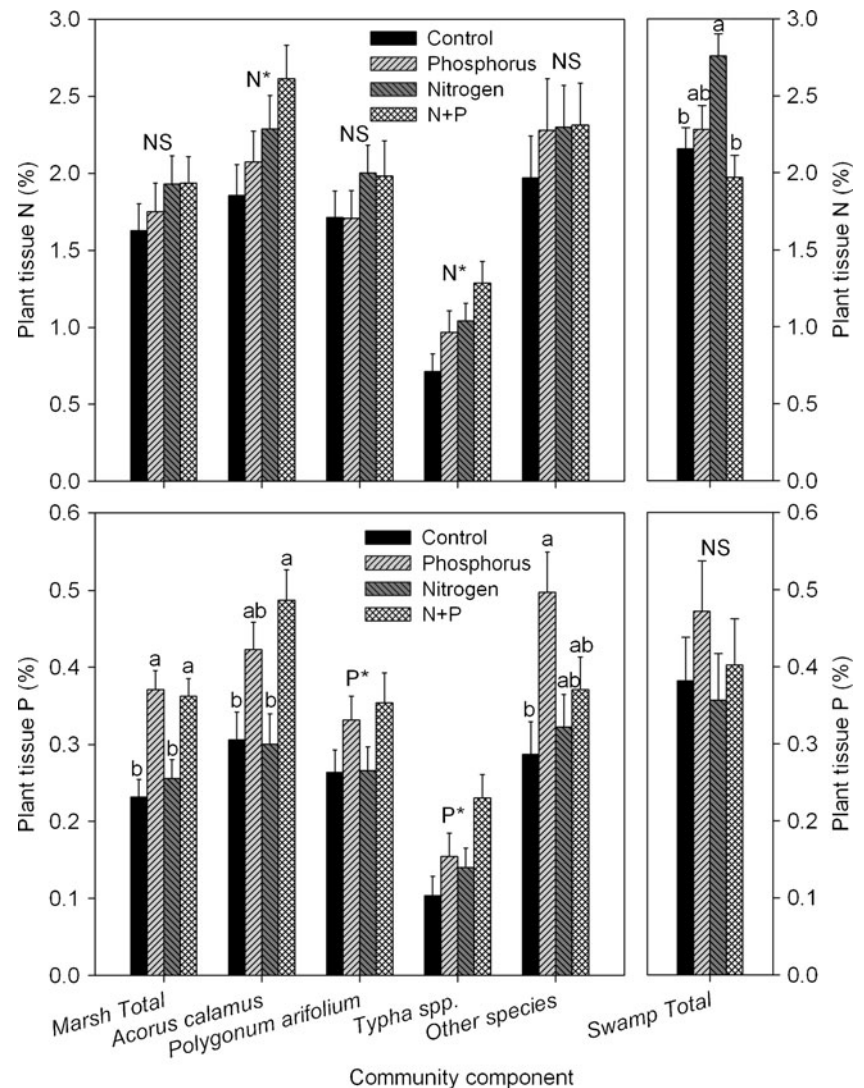
**Table 3** Two-way ANOVA results for effects of N and P treatments on plant tissue nutrient concentrations

Tissue nutrient	Habitat	Community component	df	Nitrogen (N) F	Phosphorus (P) F	N×P F
Nitrogen	Marsh	Total	1, 30	1.84	0.13	0.11
		Acorus calamus	1, 18	5.39*	1.71	0.07
		Polygonum arifolium	1, 26	2.13	0.004	0.002
		Typha spp.	1, 6	6.43*	3.82	0.001
		Other species	1, 29	0.39	0.32	0.26
	Swamp	Total	1, 28	1.03	5.30*	10.10**
Phosphorus	Marsh	Total	1, 30	0.11	26.88****	0.48
		A. calamus	1, 18	0.61	16.34***	0.87
		P. arifolium	1, 26	0.15	5.72*	0.09
		Typha spp.	1, 6	4.09	6.37*	0.51
		Other species	1, 29	1.02	8.32**	3.27
	Swamp	Total	1, 28	0.62	1.26	0.14

$*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$

herbaceous perennial growth compared with the Control, presumably due to release from P limitation. The cover of swamp annuals as a group and *P. arifolium* was lower in the P treatment than in the Control possibly because of increased competitive pressure from the positively responding perennials. Unexpectedly, swamp species richness and cover of annuals and *P. arifolium* was negatively affected by N addition. This suggests that N fertilization resulted in osmotic dehydration, ammonium toxicity, or other negative influence on some swamp herbaceous species. On one occasion wilting was observed in some plants in a single plot immediately following fertilization, suggesting an osmotic effect rather than toxicity, but this was not observed elsewhere at any other time. Not all species were negatively affected; cover of another important understory annual species, *Z. aquatica*, did not decline due to N addition. In a mesic arctic tundra ecosystem, a negative influence of fertilization was observed at the highest rate applied (250 kg/ha NPK) for evergreen shrubs even while herbaceous and one woody species responded positively (Henry et al. 1986). Ammonia toxicity occurred at levels above 100 mg/L in some freshwater wetland plants (Clarke and Baldwin 2002), but toxicity effects have not been noted in other wetland fertilization studies at similar and higher application rates than were used in the current study (Pennings et al. 2002; Crain 2007; Ket et al. 2011). However, added to the non-resource constraints of frequent inundation and low light on understory herbaceous communities in tidal freshwater swamps (Peterson-Smith and Baldwin 2006), N addition may have resulted in death or growth reduction of some swamp herbs.

Lower surface soil P concentration in swamps than in marshes likely resulted from preferential settling of mineral sediments (and their adsorbed P) during flood events in the marsh-covered natural levee typically found between the main river channel and the swamps. Because the marsh and

Fig. 7 Relationship between soil and vegetation N/P ratios (both determined in 2003) in tidal freshwater marshes and swamps expressed on a mass basis (g/g)
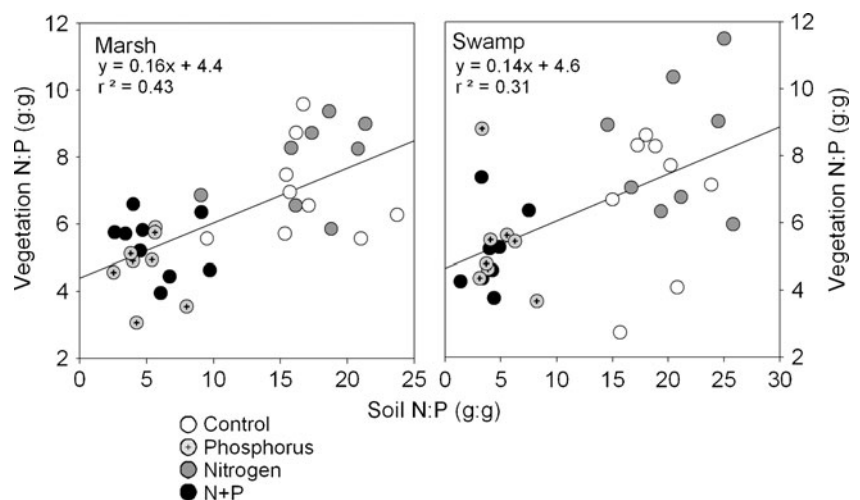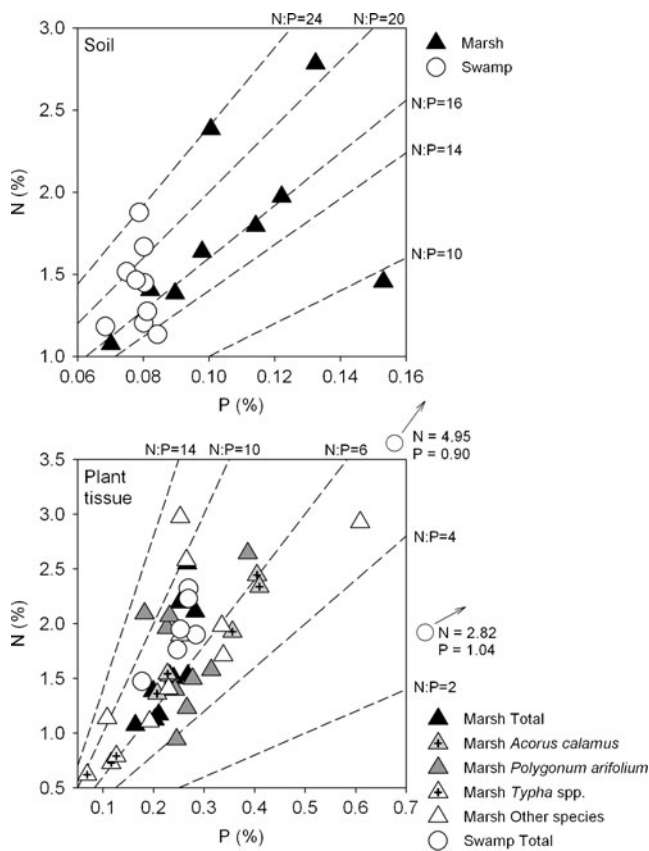
Fig. 8 Nutrient concentrations and ratios (g/g) in soil and plant tissue samples collected from unfertilized (control) plots in tidal freshwater marsh and swamp habitats

swamp soils are both organically derived and developed due to organic and mineral deposition, it is unlikely that there were differences in soil minerarology due to different parent material or soil forming processes between marshes and swamps. Trees and shrubs may also contribute to P removal in swamps, although the relative rates of N and P uptake in swamp trees compared with herbaceous plants are not known.

These findings indicate that the phenomenon of MRL (Vitousek et al. 2010) is important in controlling the responses of annuals and perennials in tidal freshwater wetlands. In marshes, an increase in N increases soil N/P ratio, promoting growth of perennials that can access sufficient P and thereby competitively inhibit growth of annuals, which are less able to access P. Conversely, an increase in P promotes marsh annuals at the expense of perennials. Adding both N+P leads to a balance of competitive ability between perennials and annuals but no increase in total biomass due to other constraints (e.g., light or other limiting nutrient). These responses are also consistent with Tilman's resource ratio hypothesis of plant succession (Tilman 1985), which suggests that species in a community each respond differently to limiting resources,

resulting in plant community composition changes due to shifts in the relative availability of different resources.

Nutrient Limitation of Plant Community Biomass

Addition of N, P, or N+P did not increase total aboveground biomass production of tidal freshwater and oligohaline marsh or swamp communities associated with the Nanticoke River. Nutrient additions in other Chesapeake Bay tidal freshwater marshes in Virginia also did not significantly increase biomass production (Morse et al. 2004), albeit at a lower application rate than used in the current study. The lack of a detectable fertilization effect on community biomass at the Nanticoke sites may be due in part to an excess of nutrients already in the system, as noted in the site description, which may have alleviated nutrient limitation. However, total aboveground biomass of unfertilized marsh plots (260–750 gm^{-2} and mean 470 gm^{-2}) was similar to that of other tidal freshwater marshes (Whigham 2009), suggesting the Nanticoke marshes are not eutrophic by comparision. Furthermore, the aboveground biomass observed in unfertilized plots in the current study was similar or lower than that reported in other fertilization studies that did detect a positive fertilization response (Crain 2007; Graham and Mendelssohn 2010; Ket et al. 2011). In swamps, light, inundation, and propagule availability can limit biomass production (Peterson-Smith and Baldwin 2006), and these likely were non-nutrient constraints on swamp herbaceous biomass production.

An important limitation of the current study is that belowground responses to fertilization were not examined. Nutrient addition has been found to reduce belowground biomass in salt marshes (Valiela et al. 1976; Darby and Turner 2008). In a tidal freshwater marsh dominated by *Z. miliacea*, the belowground macro-organic matter (living and dead roots and rhizomes) mat at 0–10 cm depth was significantly lower in plots receiving N or N+P additions for 5 years than in unfertilized control plots, even though aboveground biomass was higher in the N and N+P fertilized plots (Ket et al. 2011). Thus, it is likely that in the current study fertilization responses of roots and rhizomes did not parallel those of leaves and shoots.

Soil and Vegetation N/P Ratios

The use of vegetation N/P ratios as an indicator of the dominant nutrients limiting or co-limiting community biomass production is not supported by the results of this study. Plant tissue N/P ratios in unfertilized plots were all lower than the N/P threshold of 14 below which N limitation occurs in some other wetlands (Koerselman and Meuleman 1996) but an increase in community biomass due to N addition was not observed in marshes (although

some perennials responded positively), and swamp perennials appeared to be P limited, despite similarly low vegetation N/P ratios. Soil N/P ratios varied more between marsh and swamp habitats than did vegetation N/P ratios, and the absolute concentration of soil P in control plots corresponded to some fertilization responses (e.g., P promoted perennial growth in swamps but not in marshes). Furthermore, vegetation N/P ratio was only similar to soil N/P ratio at low values, above which plants appear to take up proportionally more P than N, accumulating higher concentrations of P than in soil and resulting in greater divergence of soil and vegetation N/P ratios as soil N/P increases. Thus, in tidal freshwater wetlands soil P concentration is likely to be a better indicator of which nutrient is limiting than soil N concentration and soil or vegetation N/P ratios.

Conclusions

Aboveground biomass of herbaceous plant communities of tidal freshwater wetlands of the Nanticoke subestuary of Chesapeake Bay was not limited or co-limited by N or P. However, addition of N or P singly caused shifts in the relative abundance of annual and perennial species. These findings are consistent with hypotheses of multiple resource limitation and resource ratios relating to the influence of different nutrients on plant community composition. In general, marsh perennials responded positively in the N treatment while marsh annuals declined, suggesting a shift in relative competitive ability toward perennials in response to N added singly. Abundance of at least one dominant marsh annual increased in the P treatment while two dominant perennials declined, suggesting that P added singly shifts competitive ability toward annuals. Adding N+P generally resulted in no change in the abundance of annual and perennial plants compared with the Control, suggesting a competitive balance was maintained. In swamps, where soil P concentration was lower, perennials responded positively to P, suggesting P limitation. Annuals may have lower ability than perennials to forage for and store immobile P due to their smaller size and are often less capable of developing P-acquiring mycorrhizal associations. Absolute concentration of soil P was a better indicator of which nutrient limited some plants than soil N and soil or vegetation N/P ratios. These findings demonstrate that eutrophication can alter plant species composition of both tidal freshwater marshes and swamps without causing detectable increases in aboveground biomass. Furthermore, annuals and perennials coexisting in the same wetland may be limited by different nutrients, and the dominant nutrient limiting perennials may differ between swamps and marshes due to differences in the availability of P in these habitats.

References

Aerts, R., and F.S. Chapin. 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research* 30: 1–67.

Baldwin, A.H. 2007. Vegetation and seed bank studies of salt-pulsed swamps of the Nanticoke River, Chesapeake Bay. In *Ecology of tidal freshwater forested wetlands of the southeastern United States*, ed. W.H. Conner, T.W. Doyle, and K.W. Krauss, 139–160. Berlin: Springer.

Bedford, B.L., M.R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology* 80: 2151–2169.

Boerner, R.E.J. 1992a. Plant life span and response to inoculation with vesicular–arbuscular mycorrhizal fungi. I. Annual vs. perennial grasses. *Mycorrhiza* 1: 153–161.

Boerner, R.E.J. 1992b. Plant life span and response to inoculation with vesicular–arbuscular mycorrhizal fungi. III. Responsiveness and residual soil P levels. *Mycorrhiza* 1: 169–174.

Chapin, F.S., and G.R. Shaver. 1985. Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* 66: 564–576.

Clarke, E., and A.H. Baldwin. 2002. Responses of wetland plants to ammonia and water level. *Ecological Engineering* 18: 257–264.

Collier, S.C., C.T. Yarnes, and R.P. Herman. 2003. Mycorrhizal dependency of Chihuahuan Desert plants is influenced by life history strategy and root morphology. *Journal of Arid Environments* 55: 223–229.

Crain, C.M. 2007. Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries and Coasts* 30: 26–34.

Darby, F.A., and R.E. Turner. 2008. Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Marine Ecology Progress Series* 363: 63–70.

Dauer, D.M., J.A. Ranasinghe, and S.B. Weisberg. 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. *Estuaries* 23: 80–96.

Deegan, L.A., J.L. Bowen, D. Drake, J.W. Fleeger, C.T. Friedrichs, K. A. Galvan, J.E. Hobble, C. Hopkinson, D.S. Johnson, J.M. Johnson, L.E. Lemay, E. Miller, B.J. Peterson, C. Picard, S. Sheldon, M. Sutherland, J. Vallino, and R.S. Warren. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecological Applications* 17: S42–S63.

DiTomasso, A., and L.W. Aarssen. 1989. Resource manipulations in natural vegetation: A review. *Vegetatio* 84: 9–29.

Frost, J.W., T. Schleicher, and C. Craft. 2009. Effects of nitrogen and phosphorus additions on primary production and invertebrate densities in a Georgia (USA) tidal freshwater marsh. *Wetlands* 29: 196–203.

Gough, L., J.B. Grace, and K.L. Taylor. 1994. The relationship between species richness and community biomass: The importance of environmental variables. *Oikos* 70: 271–279.

Graham, S.A., and I.A. Mendelssohn. 2010. Multiple levels of nitrogen applied to an oligohaline marsh identify a plant community response sequence to eutrophication. *Marine Ecology Progress Series* 417: 73–82.

Gurevitch, J., S.M. Scheiner, and G.A. Fox. 2006. *The ecology of plants*, 2nd ed. Sunderland: Sinauer.

Gusewell, S. 2004. N:P ratios in terrestrial plants: Variation and functional significance. *The New Phytologist* 164: 243–266.

Gusewell, S., W. Koerselman, and J.T.A. Verhoeven. 2003. Biomass N:P ratios as indicators of nutrient limitation for plant populations in wetlands. *Ecological Applications* 13: 372–384.

Gusewell, S., K.M. Bailey, W.J. Roem, and B.L. Bedford. 2005. Nutrient limitation and botanical diversity in wetlands: Can fertilisation raise species richness? *Oikos* 109: 71–80.

Henry, G.H.R., B. Freedman, and J. Svoboda. 1986. Effects of fertilization on three tundra plant communities of a polar desert oasis. *Canadian Journal of Botany* 64: 2502–2507.

Howarth, R.W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25: 656–676.

Johnson, S., and E. Rejmankova. 2005. Impacts of land use on nutrient distribution and vegetation composition of freshwater wetlands in Northern Belize. *Wetlands* 25: 89–100.

Ket, W.A., J.P. Schubauer-Berigan, and C.B. Craft. 2011. Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh. *Aquatic Botany* 95: 17–23.

Koerselman, W., and A.F.M. Meuleman. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33: 1441–1450.

LeBauer, D.S., and K.K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89: 371–379.

Levine, J.M., J.S. Brewer, and M.D. Bertness. 1998. Nutrients, competition, and plant zonation in a New England salt marsh. *Journal of Ecology* 86: 285–292.

Morse, J.L., J.P. Megonigal, and M.R. Walbridge. 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., T.J. Smith III, J.K. Hoover, and C.C. McIvor. 1984. *The ecology of tidal freshwater marshes of the United States east coast: A community profile*. Washington: U.S. Fish and Wildlife Service.

Peat, H.J., and A.H. Fitter. 1993. The distribution of arbuscular mycorrhizas in the British flora. *The New Phytologist* 125: 845–854.

Peet, R.K., T.R. Wentworth, and P.S. White. 1998. A flexible, multipurpose method for recording vegetation composition and structure. *Castanea* 63: 262–274.

Pennings, S.C., L.E. Stanton, and J.S. Brewer. 2002. Nutrient effects on the composition of salt marsh plant communities along the southern Atlantic and Gulf coasts of the United States. *Estuaries* 25: 1164–1173.

Pennings, S.C., C.M. Clark, E.E. Cleland, S.L. Collins, L. Gough, K.L. Gross, D.G. Milchunas, and K.N. Suding. 2005. Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos* 110: 547–555.

Peterson, J.E., and A.H. Baldwin. 2004. Variation in wetland seed banks across a tidal freshwater landscape. *American Journal of Botany* 91: 1251–1259.

Peterson-Smith, J.P., and A.H. Baldwin. 2006. Constraints on understory vegetation communities of tidal freshwater swamps: A reciprocal transplant experiment. *Journal of the Torrey Botanical Society* 133: 566–576.

Rajaniemi, T.K., and H.L. Reynolds. 2004. Root foraging for patchy resources in eight herbaceous plant species. *Oecologia* 141: 519–525.

Rheinhardt, R. 1992. A multivariate analysis of vegetation patterns in tidal freshwater swamps of lower Chesapeake Bay, U.S.A. *Bulletin of the Torrey Botanical Club* 119: 192–207.

Roumet, C., C. Urcelay, and S. Diaz. 2006. Suites of root traits differ between annual and perennial species growing in the field. *The New Phytologist* 170: 357–368.

Russell, M.J., D.E. Weller, T.E. Jordan, K.J. Sigwart, and K.J. Sullivan. 2008. Net anthropogenic phosphorus inputs: Spatial and temporal variability in the Chesapeake Bay region. *Biogeochemistry* 88: 285–304.

Sharpe, P.J., and A.H. Baldwin. 2009. Patterns of wetland plant species richness across estuarine gradients of Chesapeake Bay. *Wetlands* 29: 225–235.

Silliman, B.R., and M.D. Bertness. 2004. Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology* 18: 1424–1434.

Suding, K.N., S.L. Collins, L. Gough, C. Clark, E.E. Cleland, K.L. Gross, D.G. Milchunas, and S. Pennings. 2005. Funtional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 102: 4387–4392.

Tilman, D. 1985. The resource-ratio hypothese of plant succession. *The American Naturalist* 125: 827–852.

TNC. 1998. *Nanticoke river bioreserve strategic plan*. The Nature Conservancy Maryland and District of Columbia Field Office and Delaware Field Office, Chevy Chase, MD.

Valiela, I., J.M. Teal, and N.Y. Persson. 1976. Production and dynamics of experimentally enriched salt marsh vegetation: Belowground biomass. *Limnology and Oceanography* 21: 245–252.

Valiela, I., J.M. Teal, and W.G. Deuser. 1978. The nature of growth forms in the salt marsh grass *Spartina alterniflora*. *The American Naturalist* 112: 461–470.

van Wijnen, H., and J.P. Bakker. 1999. Nitrogen and phosphorus limitation in a coastal barrier salt marsh: The implications for vegetation succession. *Journal of Ecology* 87: 265–272.

Venterink, H.O., M.J. Wassen, A.W.M. Verkroost, and P.C. de Ruiter. 2003. Species richness–productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology* 84: 2191–2199.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7: 737–750.

Vitousek, P.M., S. Porder, B.Z. Houlton, and O.A. Chadwick. 2010. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications* 20: 5–15.

Whigham, D.F. 2009. Primary production in tidal freshwater wetlands. In *Tidal freshwater wetlands*, ed. A. Barendregt, D.F. Whigham, and A.H. Baldwin, 115–122. Leiden, The Netherlands: Backhuys.