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Response of *Spartina patens* to Dissolved Inorganic Nutrient Additions in the Field

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ABSTRACT

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Salt marshes provide a buffer between the terrestrial landscape and estuaries and may be important in preventing the movement of land-derived nutrients into coastal waters. We examined the response of *S. patens* in the field to additions of dissolved inorganic nitrogen (N) and phosphorus (P) and found significant ($p < 0.05$) positive N effects on aboveground biomass, leaf chlorophyll, tissue nutrient concentrations, and induction of fluorescence kinetics of chlorophyll *a*. Mean endomycorrhizal colonization among treatments was 22%, and fungal colonization ranged from 2% to 61% in the plots. We found no significant effect of N or P on endomycorrhizal colonization, but there was a significant inverse relationship ($r = -0.66$, $p = 0.005$) between the belowground biomass and fungal colonization. This study showed that *S. patens* could sequester 44-100% of the added N and 82-100% of the added P in its leaves, roots, and rhizomes. However, it is unclear how long-term nutrient overenrichment and the resulting changes in the *S. patens*-microbe-sediment system might alter the marsh buffering capacity.

ADDITIONAL INDEX WORDS: *Eutrophication, endomycorrhizae, photosynthesis, fluorescence, performance index.*



INTRODUCTION

With escalating human activities in coastal watersheds, land-derived nutrient loads to salt marshes are increasing (McKINNEY *et al.*, 2001; MORRIS and BRADLEY, 1999; VALIELA *et al.*, 1997, 2000). Some studies have demonstrated the importance of tidal fringing marshes, those located between the uplands and estuaries, in intercepting and transforming groundwater nitrate before it enters coastal waters (TOBIAS *et al.*, 2001a-c). Most tidal salt marsh studies have demonstrated that *Spartina* is nitrogen (N) limited (*e.g.*, SULLIVAN and DAIBER, 1974; VALIELA, TEAL, and SASS, 1975), although phosphorus (P) limitation can occur secondarily (SUNDARESHWAR *et al.*, 2003; VAN WIJNEN and BAKKER, 1999). The effects of elevated nutrients on *Spartina patens*, the high-marsh-dominant plant in many tidal marshes, have been less studied than such effects on the low-marsh-dominant plant, *Spartina alterniflora*. In contrast

with the positive effect of elevated nutrients on the aboveground and rhizome biomass, one study suggested an inhibitory effect of nutrients on the root biomass of *S. patens* (VALIELA, TEAL, and PERSON, 1976).

Spartina patens harbors fungal symbionts, called vesicular arbuscular mycorrhizae, in its roots (COOKE, BUTLER, and MADOLE, 1993). Endomycorrhizal fungi in terrestrial systems have been shown to facilitate the acquisition of P and N in soils as well as increase photosynthesis and productivity (ALLEN, 1991; SMITH and READ, 1997). *Spartina patens* in a natural stand was shown to have greater endomycorrhizal colonization and plant growth than a stand in a restored site (COOKE and LEFOR, 1990). It has been proposed that the fungal symbionts of *S. patens* facilitate P uptake and also stimulate heterotrophic N fixation in the sediments by promoting root exudation of labile organics to the rhizosphere (BURKE, 2001; BURKE, HAMERLYNCK, and HAHN, 2002). Therefore, in the usually N-limited environment of the salt marsh, the endomycorrhizal *S. patens* would

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have the advantage of harboring a symbiont that directly facilitates P uptake by roots and indirectly promotes increased N availability (via N fixation) to roots. Our study is the first to examine the effect of inorganic nutrient additions on the endomycorrhizal colonization of *S. patens* roots under natural conditions.

The effect of elevated nutrients on the photosynthetic performance of *S. patens* is not well studied, but some physiological responses to nitrogen stress have been reported for *Spartina* species (EWING *et al.*, 1995, 1997; MORRIS, 1982). Physiological indicators have been used to detect the response of terrestrial crop plants to nutrient stress for decades. Furthermore, the use of physiological indicators to detect stress in *S. patens* due to salinity changes or toxicant exposure has been investigated (EWING *et al.*, 1995, 1997; MENDELSOHN, MCKEE, and KONG, 2001).

Here, we report fluorescence induction kinetics, photosynthetic rates, and chlorophyll concentrations for nutrient-treated and control plots in a *S. patens* marsh located in the Narragansett Bay National Estuarine Research Reserve, Rhode Island. In addition, above- and belowground biomass, percent N and P in plant tissues, shoot density and length, and the extent of endomycorrhizal colonization are measured among treatments. We expected that N additions would increase photosynthetic performance and aboveground biomass, but would inhibit belowground biomass. Second, we hypothesized that the addition of P would have an adverse effect on the endomycorrhizal colonization because fungal symbionts usually facilitate P uptake. Under elevated P conditions, mycorrhizal *S. patens* would no longer be dependent on the fungal symbionts for P acquisition. Furthermore, we expected that P additions might have an adverse effect on photosynthetic performance due to the disruption of the endomycorrhizal relationship and its influence on N acquisition. Finally, we discuss the *S. patens*-microbe-sediment system as a sink for elevated nutrients.

METHODS

Study Site

The study site was located in a *S. patens* marsh in the Nags Creek area (41°37.546' N, 71°19.223' W) in the Narragansett Bay National Estuarine Research Reserve on Prudence Island, Rhode Island (Figure 1). Sixteen 1-m² plots were randomly located in vegetated patches at least 3 m apart,

and a 2 × 2 factorial design was employed with N and P as the treatments ($n = 4$ plots per treatment). Dissolved Ca(NO₃)₂ and P₂O₅ were sprinkled during low tide on the sediment surface twice per month for the growing season, May–August, but monthly thereafter. Fertilizer application rates were 2 g N/m² and 0.2 g P/m², with a total addition of 80 g N/m² and 8 g P/m² for the entire experiment, which ran from mid-May 2000 to early September 2002. On an annual basis, the nutrient load was 32.0 g N/m²/y and 3.2 g P/m²/y.

Shoot Density, Length, Biomass, and Tissue Nutrients

Using nondestructive measures, the shoot density and length were measured in late July 2001 and in early September 2002. Live *S. patens* shoots in a 0.1-m² quadrat were counted in the field to determine density, and the length of 10 randomly selected shoots was recorded to determine mean height. In September (2002) during peak biomass, the aboveground biomass was harvested in the quadrat, and live and dead shoots were dried to a constant weight at 60°C. Annual aboveground production was estimated as the mass of the live shoots plus standing dead. Belowground biomass was also sampled in early September 2002 by taking a sediment core (4.6-cm diameter, 10-cm length). Most of the roots and rhizomes of *S. patens* are in the top 5 cm (VALIELA, TEAL, and PERSSON, 1976); therefore, a sample to a depth of 10 cm should yield most of the belowground biomass. Root and rhizomes were separated from the soil using a hydropneumatic root washer (Gillison's Variety Fabrication, Inc., MI) and oven dried to a constant weight at 60°C to estimate belowground biomass and annual belowground production. Roots and rhizomes that appeared living and were not decomposed were included as the current year's growth. Herbivory, leaf loss, and decomposition processes were not accounted for in the estimates of annual above- and belowground production.

Dried shoots and the belowground fraction were ground using a Wiley Mill and analyzed for total N and carbon (C) content on a Carlo Erba NA 1500 NCS elemental analyzer. Standard methods (CHAMBERS and FOURQUREAN, 1991; PARSONS, MAITA, and LALLI, 1984) for the determination of P in the plant tissues were used, in which the dried, ground plant tissue was ashed at 450°C, then digested in 0.2 N HCL at 60°C, and

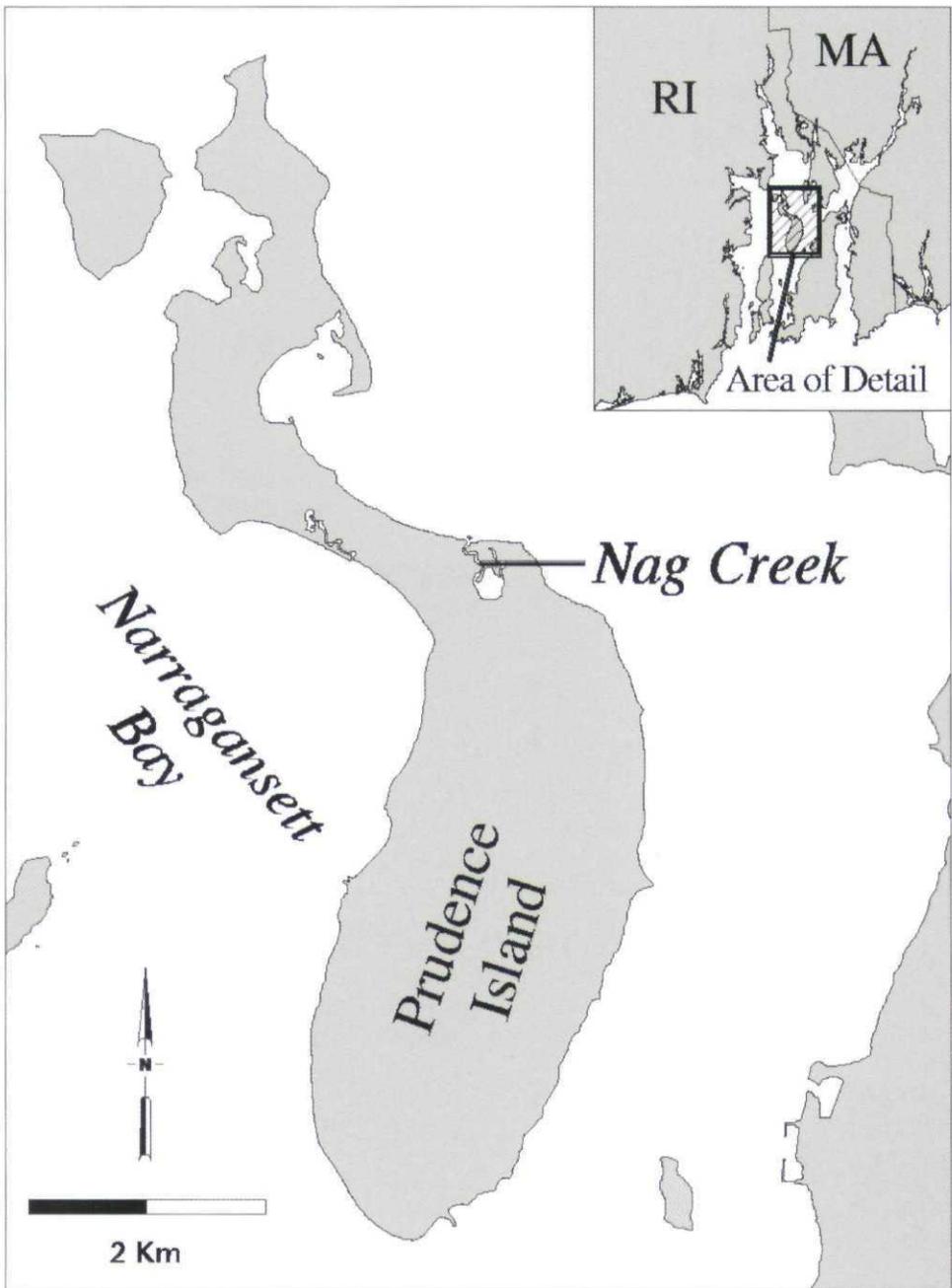


Figure 1. Location of the nutrient addition experiment at Nag Creek ($41^{\circ}37.546' N$, $71^{\circ}19.223' W$), part of the Narragansett Bay National Estuarine Research Reserve, Rhode Island.

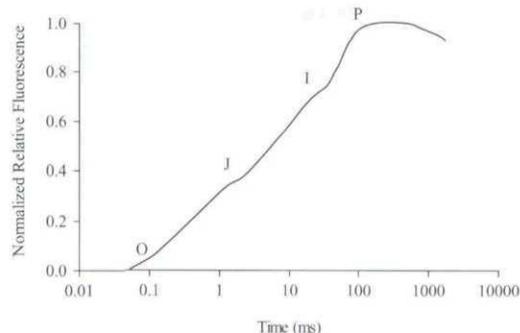


Figure 2. Sample fluorescence transient for *Spartina patens*. Data expressed as normalized relative fluorescence. See text for explanation of O, J, I and P steps.

the supernatant analyzed for P using the molybdate blue method. Plant tissue-nutrient concentrations were used to calculate molar N:P, C:N, and C:P ratios.

Leaf Physiological Metrics

To assess the physiological status of the plants, we measured the light-harvesting efficiency of photosystem II (PSII) reaction centers with a Plant Efficiency Analyzer (Hanzateck, UK). All fluorescence measurements were made with 2 s of excitation light, about 3,000 $\mu\text{mol}/\text{m}^2/\text{s}$ of red light (peak at 660 nm), on clusters of about six young leaves while still attached to the plant. In early August 2001, small sediment cores (cut-off 60-ml syringes) with plants intact were taken from the field and fluorescence of the *S. patens* leaves was measured in the laboratory. In mid-June 2002, the fluorescence measurements were made in the field. All measurements were performed on leaves that had been dark adapted for at least 20 min.

A healthy, dark-adapted plant exposed to saturating light contains fluorescence levels ranging between F_0 (the minimum) and F_M (the maximum). The course of induced fluorescence is referred to as an O-J-I-P transient (STRASSER and STRASSER, 1995; STRASSER, SRIVASTAVA, and GONVINDGEE, 1995; STRASSER, SRIVASTAVA, and TSMILLI-MICHAEL, 2000) (Figure 2). Steps (i.e., O-J, J-I, and I-P) are believed to result at least partly from heterogeneous PSII reaction centers. The O-J step represents reaction centers that cannot reduce quinone-B (Q_B), and the J-I and I-P steps represent groups of reaction centers that reduce

Q_B quickly and more slowly, respectively (LEBKEUCHER *et al.*, 1999). We use this explanation for the steps in the fluorescence transient to derive relative pools of non- (Q_B) -reducing (O-J step), fast (Q_B) -reducing (J-I step), and slow (Q_B) -reducing (I-P step) reaction centers in the leaves.

The following fluorescence data were used for subsequent calculations for the derivation of the photosynthetic metrics: (1) the fluorescence value at 50 μs , considered as F_0 when all reaction centers of PSII are open; (2) the maximum fluorescence value, F_M , assuming that the level of excitation light was high enough to close all the reaction centers; (3) the fluorescence values at 300 μs (F_{300}), 2 ms (J point), denoted as F_J , and 30 ms (I point) (Figure 2).

Besides the standard F_V/F_M calculation (where $F_V = F_M - F_0$) for estimating the maximum quantum efficiency, a fluorescence performance index for assessing plant vitality was calculated (SRIVASTAVA *et al.*, 1999). The performance index is the ratio of expressions for photosynthetic activity to expressions for absorbed energy lost in electron transport (CLARK *et al.*, 2000). The equation for the performance index is

$$PI_{\text{ABS}} = [(F_V/F_M)(V_J/M_0)][F_V/F_0][(1 - V_J)/V_J]$$

where PI_{ABS} is the performance index per unit of energy absorbed, M_0 is the slope at the origin of the relative variable fluorescence [$4(F_{300} - F_0)/(F_M - F_0)$], and V_J is the relative variable fluorescence at point J (Figure 2) [$(F_J - F_0)/(F_M - F_0)$]. This index refers to the relative performance of photochemical events.

As an indicator of the photosynthetic rate, the uptake rate of CO_2 was measured using a portable, flow-through infrared gas analyzer-IRGA (model LCA4; ADC Bioscientific, UK). Light was ambient sunlight and was measured simultaneously using the light sensor attached to the leaf chamber of the IRGA unit. Measurements were made midmorning to minimize effects from photoinhibition. All CO_2 rates were normalized to grams dry weight (gdw) of plant tissue within the leaf chamber (0.1–0.2 g dry wt) and are expressed as $\text{nmol CO}_2/\text{gdw}/\text{s}$.

In early September 2000, at the end of the first growing season of the experiment, leaves for chlorophyll *a* and *b* analyses were collected from each of the 16 plots. In early August 2001 and mid-June 2002, the same time as the measurement of leaf fluorescence, leaves for chlorophyll *a* and *b* analyses were again collected, wrapped in aluminum foil, and stored on ice until frozen (-80°C) upon

return to the laboratory. Only the green parts of the leaves were used to measure chlorophyll *a* and *b* using cold acetone extraction and sonication (PARSONS, MAITA, and LALLI, 1984). The green leaves were cut into 1–2 mm sections and ground fine with a mortar and pestle. HPLC (high-performance liquid chromatography) was used to measure the pigments using Millennium 32 chromatography manager software.

Endomycorrhizal Colonization and Sugar Content of the Roots

In early August 2001, one vegetated sediment core (4.6-cm diameter, 10-cm length) from each plot was collected, extruded, and washed as described earlier for the determination of the belowground biomass. One subsample of live roots was stored in 95% ethanol for later determination of glucose and sucrose using a standard ethanol extraction and normal-phase HPLC with a refractive index detector (RICHMOND *et al.*, 1981). A second subsample of the live roots was preserved in a formalin–ethyl alcohol–acetic acid solution for histological analyses of endomycorrhizae (PHILLIPS and HAYMAN, 1970). Roots were cut into 2-cm segments, heated in 10% KOH, acidified, and stained with trypan blue as described by standard methods (GIANINAZZI and GIANINAZZI-PEARSON, 1992; PHILLIPS and HAYMAN, 1970). At least 50 segments for each plot were examined with light microscopy, and endomycorrhizal colonization was recorded as present in the root segment, if fungal vesicles and/or arbuscules were observed.

Statistical Analyses

To test for the main effects of N, P, and for N × P interactions, two-way analysis of variance (ANOVA) was used. Pearson correlation analyses were used to test for relationships of endomycorrhizal colonization with the biomass metrics as well as to test for relationships of plant performance (PI_{ABS}) with leaf chlorophyll and plant biomass. Significance is reported as $p < 0.05$.

RESULTS

There was a significant nitrogen effect on the total aboveground biomass, the range of which was 584–1,009 $g/m^2/y$ (Figure 3A, Table 1), but there was no significant N or P effect on the belowground biomass (Figure 3B). The belowground biomass, which was in the range 4,783–6,961 $g/m^2/y$,

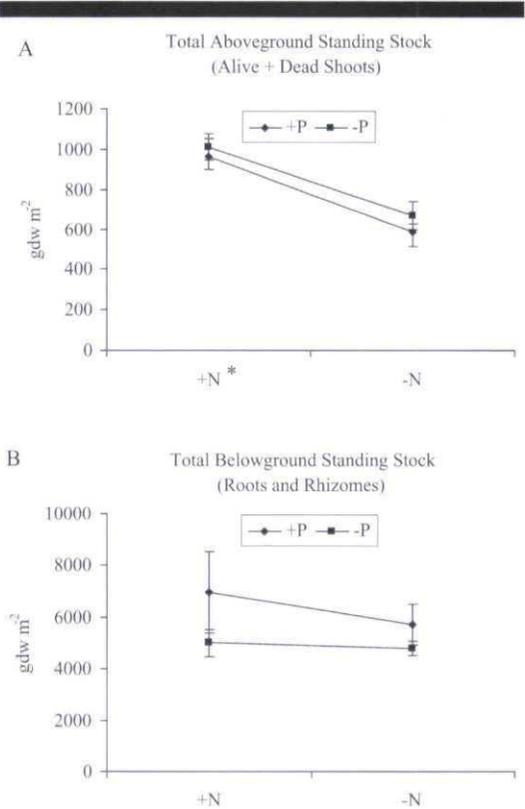


Figure 3. (A) Above- and (B) belowground biomass (mean \pm SE) of the fertilized treatments and control group measured in early September 2002. (* $p < 0.05$; SE = standard error of the mean.)

accounted for 83–91% of the total biomass. Although there was no significant difference in root glucose and sucrose storage among the treatments because of high variability among plots within a treatment, on average, there appeared to be less sugars stored in the roots of the fertilized plots than in the controls (Table 1). There was a positive N effect on shoot lengths, with significantly greater shoot lengths in July 2001 and a trend ($p = 0.06$) of increased shoot lengths in early September 2002. There was no significant effect of N on shoot density (Table 1).

The N additions had a significant positive effect on the plant performance index (PI_{ABS}) in both August 2001 and June 2002 (Table 2, Figure 4). Although not statistically significant, a trend ($p = 0.09$) of reduced PI_{ABS} in the P treatments was observed in June 2002, which was supported by a negative P effect ($p = 0.06$) on the relative fraction

Table 1. Measures (mean \pm SE) above- and belowground biomass, shoot density and lengths, endomycorrhizal colonization, root sugars; SE = standard error of the mean. (ns = not statistically significant.)

	Significance (Probability)						
	+N+P	+N	+P	Control	N Effect	P Effect	N \times P Interaction
Aboveground biomass for 2002							
Live shoots (g/m)	586 (70.5)	647 (31.6)	341 (18.0)	394 (55.7)	*	ns	ns
Standing dead (g/m)	379 (39.3)	363 (52.2)	244 (35.1)	273 (19.5)	*	ns	ns
Total dead and live shoots (g/m ² /y)	965 (86.3)	1,009 (64.2)	584 (41.5)	667 (69.1)	*	ns	ns
2001 shoot length (cm)	47.1 (4.5)	42.8 (2.0)	37.5 (2.9)	34.7 (1.0)	*	ns	ns
2002 shoot length (cm)	40.5 (4.3)	38.4 (2.2)	31.2 (3.4)	34.0 (2.8)	ns	ns	ns
2001 live shoot density (m ⁻²)	4,310 (817)	5,160 (336)	3,890 (194)	3,735 (378)	ns	ns	ns
2002 live shoot density (m ⁻²)	5,835 (838)	5,955 (612)	4,800 (1,122)	4,563 (401)	ns	ns	ns
2002 total roots and rhizomes (g/m ² /y)	6,961 (1,556.1)	5,003 (523.8)	5,708 (774.0)	4,783 (268.9)	ns	ns	ns
Percent fungal colonization	21.1 (11.6)	24.0 (13.0)	20.0 (10.1)	24.2 (3.7)	ns	ns	ns
root sucrose (mg/gdw)	6.54 (1.96)	5.52 (2.04)	3.40 (1.52)	15.48 (6.56)	ns	ns	ns
root glucose (mg/gdw)	1.11 (0.40)	1.62 (0.44)	1.18 (0.52)	2.45 (1.00)	ns	ns	ns

* $p < 0.05$.

Table 2. Photosynthetic response and leaf chlorophyll measurements (mean \pm SE) for the fertilized treatments and control. The performance index (PI_{ABS}) is the ratio of expressions for photosynthetic activity to expressions for absorbed energy lost in electron transport; Q_B = Quinone-B. (ns = not statistically significant.)

	Significance (Probability)						
	+N+P	+N	+P	Control	N Effect	P Effect	N \times P Interaction
Performance index,	1.1	1.2	0.8	0.8	*	ns	ns
P_{ABS} (August 2001)	(0.08)	(0.18)	(0.13)	(0.18)			
Performance index,	1.6	2.0	1.1	1.2	*	ns	ns
P_{ABS} (June 2002)	(0.23)	(0.13)	(0.06)	(0.20)			
Non- Q_B -reducing	0.375	0.355	0.441	0.421	*	ns	ns
reaction centers	(0.013)	(0.008)	(0.007)	(0.023)			
Slow (Q_B)-reducing	0.293	0.312	0.258	0.283	*	ns	ns
reaction centers	(0.014)	(0.004)	(0.010)	(0.012)			
Fast (Q_B)-reducing	0.332	0.333	0.301	0.296	*	ns	ns
reaction centers	(0.008)	(0.006)	(0.005)	(0.013)			
Chlorophyll a	1,712.0	1,724.9	1,137.4	1,407.7	*	ns	ns
($\mu\text{g/g}$)	(175.3)	(54.5)	(99.9)	(158.9)			
Chlorophyll b	549.9	530.8	346.7	414.7	*	ns	ns
($\mu\text{g/g}$)	(72.0)	(17.9)	(32.6)	(45.4)			
F_v/F_M	0.699	0.720	0.688	0.696	ns	ns	ns
	(0.014)	(0.006)	(0.009)	(0.010)			
CO_2 uptake	36.74	37.93	32.44	26.60	ns	ns	ns
($\text{nmol CO}_2/\text{gdw}$)	(7.09)	(5.19)	(5.80)	(2.02)			

All values for 2002 unless otherwise indicated.

* $p < 0.05$.

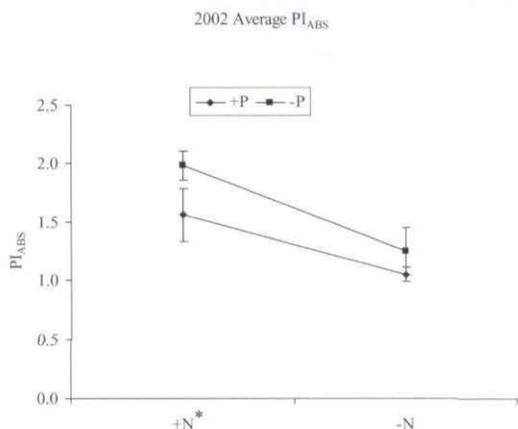


Figure 4. Measures (mean \pm SE) of the leaf performance index (PI_{ABS}) of the fertilized treatments and the control group in mid-June 2002. PI_{ABS} is the ratio of expressions for photosynthetic activity to expressions for absorbed energy lost in electron transport. (* $p < 0.05$; SE = standard error of the mean.)

of the slow (Q_B)-reducing reaction centers. In contrast, there was a significant positive N effect on both the slow and fast (Q_B)-reducing reaction centers (Table 2). As a consequence of the relative increase in the slow and fast (Q_B)-reducing reaction centers in the N-treated plots, the fraction of reaction centers not able to transport electrons beyond quinone A (*i.e.*, the non- (Q_B) -reducing reaction centers) was significantly smaller in the +N treatments (Table 2).

There was a significant positive N effect on chlorophyll *a* and *b* in mid-June 2002 (Figure 5A, B; Table 2). However, there was no significant N or P effect on chlorophyll *a* in the late summers of 2000 and 2001. Using the 2002 chlorophyll data, we found significant positive relationships of PI_{ABS} with the leaf chlorophyll ($r = 0.75$, $p = 0.0009$) and with aboveground biomass (Figure 6). There was no significant effect of N or P on the F_v/F_m ratio or photosynthetic rate as measured by CO_2 uptake per gram leaf tissue (Table 2). However, if photosynthesis is described on a plot basis, there is a significant N effect because of the significant positive N effect on the aboveground biomass.

Although we found no significant effect of N or P on the endomycorrhizal fungi in the roots (Table 1), there was a significant inverse relationship ($r = -0.66$, $p = 0.005$) between the (log-transformed) belowground biomass and percent endomycorrhizal colonization (Figure 7). Among the three

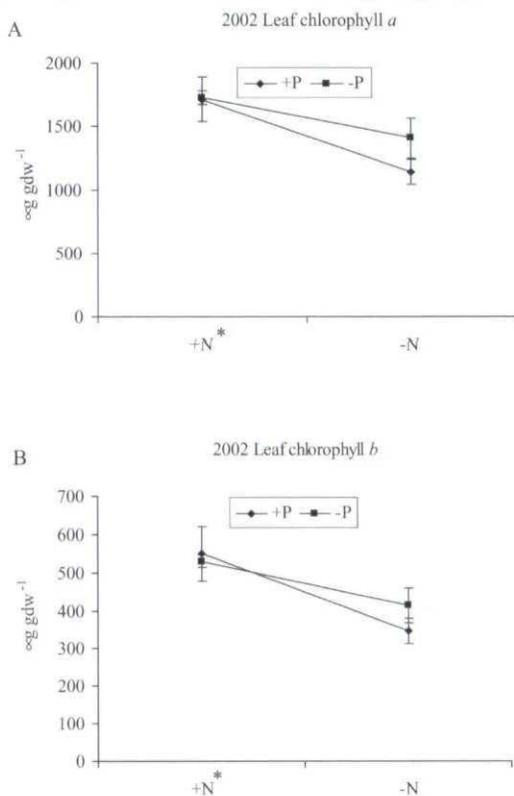


Figure 5. Leaf chlorophyll (mean \pm SE) sampled in mid-June 2002 from the fertilized treatments and the control group: (A) chlorophyll *a*; and (B) chlorophyll *b*. (* $p < 0.05$; SE = standard error of the mean.)

fertilized treatments, there was high variability in the endomycorrhizal colonization among plots, but noticeably less variability in the fungal colonization in the control plots (Table 1). Mean endomycorrhizal colonization among the treatments was 22%, and fungal colonization ranged from 2% to 61% in the experimental plots.

The N-treated plots had a significantly positive effect on the %N in the leaves and belowground fraction, but a significantly negative effect on the %P in the leaves (Table 3). The molar N:P ratios in the leaves were low (< 11) for all treatments, and there was a significant ($p < 0.05$) negative effect of P and positive effect of N on the N:P ratios (Figure 8A, Table 4). There was no significant P effect on the N:P ratio in the belowground fraction (Figure 8B, Table 4).

The molar C:N ratios of the live and standing dead leaves and the belowground fraction were

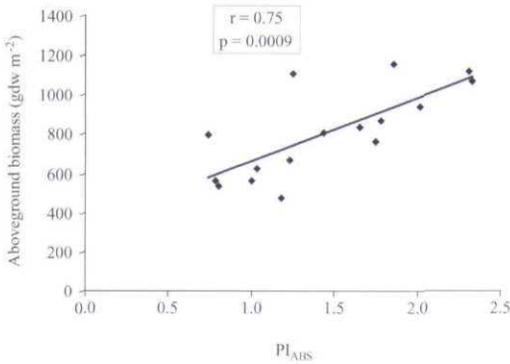


Figure 6. The relationship between the total aboveground biomass and plant performance as measured by PI_{ABS} (see the text for a detailed description of PI_{ABS}).

significantly reduced in the N-treated plots (Table 4). The addition of N had a significant positive effect on the molar C:P ratios in the aboveground plant fraction, whereas in the P-treated plots, the C:P ratio was significantly reduced (Table 4). There was no significant effect of N or P on the C:P ratios of the standing dead leaves or the belowground plant fractions (Table 4). Generally, the C:N ratios were greater in the standing dead leaves than the live leaves or belowground fraction. The C:P ratios were lowest in the live leaves and of similar magnitude in the standing dead leaves and the belowground fraction (Table 4).

The sum of the above- and belowground plant sequestration of N and P was in the range 62–101 g N/m² and 8–16 g P/m² among the fertilized treatments and control. Subtracting the mean control levels of sequestered nutrients from the fertilized treatments, we estimated the total excess nutrients sequestered in the plants in the fertilized plots (Table 5). These estimates of excess nutrients sequestered in the plants accounted for 44–100% of added N and 82–100% of added P (Table 6). Most of the excess nutrients were sequestered in the belowground biomass of the plants; 84–89% N for the N-treated plants and 76–79% P for the P-treated plants (Table 5).

DISCUSSION

As expected, N fertilization resulted in significantly increased aboveground biomass and plant performance as measured by PI_{ABS} . The above- and belowground *S. patens* biomass estimates were similar to those found by VALIELA, TEAL, and

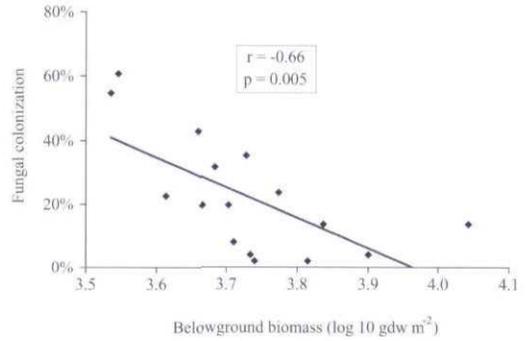


Figure 7. The relationship between endomycorrhizal colonization and total belowground biomass.

PERSOON (1976) in a Massachusetts marsh. Our data suggest that, to detect measurable changes in growth and photosynthetic performance in *S. patens*, it may take about 1 year (e.g., PI_{ABS} and aboveground biomass), while for some less sensitive metrics (e.g., chlorophyll *a* and *b*, belowground biomass, and shoot density), it may take 2 years or more to detect a measurable response to fertilization under field conditions. The performance index (PI_{ABS}) and its associated fractions of non- (Q_B) -reducing and fast and slow (Q_B) -reducing PSII reaction centers were sensitive to sublethal nutrient stress. PI_{ABS} correlated highly with chlorophyll *a* and total aboveground biomass. This is expected because there would be a relationship between chlorophyll *a* concentration and the number of PSII reaction centers in otherwise healthy plants. Additionally, the correlation between PI_{ABS} and chlorophyll *a* suggests that the former could be a good, nondestructive indicator of photosynthetic performance and may even be more sensitive to nutrient stress than chlorophyll *a* concentration because differences in PI_{ABS} between fertilized treatments and the control were found after 1 year. Furthermore, plant performance as measured by PI_{ABS} may be a more sensitive indicator of nutrient stress than either F_v/F_m or leaf photosynthesis because only PI_{ABS} was significantly affected by nutrient addition during the extent of this experiment. This is probably because F_v/F_m only takes into account the minimum and the maximum fluorescence, while PI_{ABS} considers these values as well as fluorescence responses in the intermediate steps of the O–J–I–P transient (STRASSER, SRIVASTAVA, and GONVINDGEE, 1995; STRASSER, SRIVASTAVA, and TSMILLI-MICHAEL,

Table 3. Plant tissue nutrient concentrations (mean \pm SE) for the above- and belowground fractions. (ns = not statistically significant.)

	Significance (Probability)							
	+N+P	+N	+P	Control	N Effect	P Effect	N \times P Interaction	
Aboveground [†] plant fraction	%N	1.085 (0.064)	1.178 (0.066)	0.933 (0.014)	0.930 (0.073)	*	ns	
	%P	0.342 (0.020)	0.242 (0.007)	0.455 (0.067)	0.306 (0.009)	*	*	
	%C	44.2 (0.2)	43.2 (0.4)	43.8 (0.3)	45.8 (1.0)	*	ns	*
	g N/m ²	10.60 (1.49)	11.84 (0.81)	5.45 (0.40)	6.18 (0.69)	*	ns	ns
	g P/m ²	3.35 (0.47)	2.43 (0.14)	2.72 (0.51)	2.05 (0.26)	ns	ns	ns
	%N	0.92 (0.08)	0.87 (0.04)	0.73 (0.02)	0.78 (0.05)	*	ns	ns
Standing dead leaves	%P	0.129 (0.038)	0.294 (0.178)	0.199 (0.045)	0.112 (0.016)	ns	ns	ns
	%C	43.9 (0.8)	44.5 (0.4)	45.0 (0.5)	44.0 (0.7)	ns	ns	ns
	g N/m ²	3.6 (0.6)	3.2 (0.6)	1.8 (0.2)	2.1 (0.1)	*	ns	ns
	g P/m ²	0.451 (0.083)	0.818 (0.361)	0.455 (0.060)	0.315 (0.069)	ns	ns	ns
	%N	1.343 (0.086)	1.275 (0.067)	1.148 (0.049)	1.148 (0.076)	*	ns	ns
	%P	0.199 (0.035)	0.123 (0.030)	0.146 (0.014)	0.140 (0.027)	ns	ns	ns
Belowground plant fraction	%C	44.2 (0.39)	45.6 (0.35)	45.0 (0.44)	45.3 (0.94)	ns	ns	ns
	g N/m ²	90.07 (16.48)	63.80 (7.40)	65.32 (10.72)	55.40 (6.56)	ns	ns	ns
	g P/m ²	12.709 (2.127)	5.699 (0.623)	8.606 (2.054)	6.666 (1.228)	ns	*	ns

* $p < 0.05$.[†] Aboveground: %N, P, and C were determined on live leaves; annual sequestration of N and P was determined using total aboveground estimates of live plus standing dead biomass.

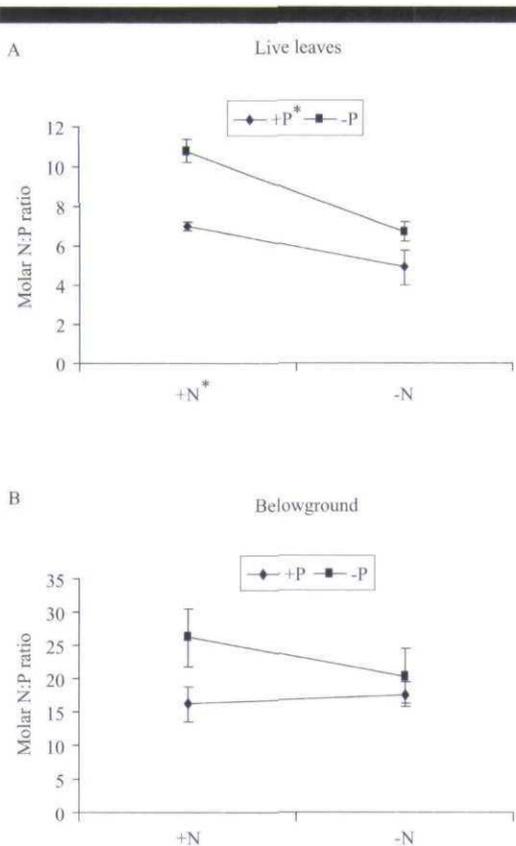


Figure 8. (A) The molar N:P ratio of the live leaves and (B) the belowground fraction (roots plus rhizomes) of the fertilized treatments and the control group sampled in early September 2002. Ratios are means \pm SE. (* $p < 0.05$; SE = standard error of the mean.)

2000). Other researchers have reported that F_v/F_m is not sensitive to sublethal stress in *S. patens* (BURKE, HAMERLYNCH, and HAHN, 2002; MENDELSSOHN, MCKEE, and KONG, 2001).

Under elevated N, it appears that *S. patens* allocates C and energy to increasing aboveground biomass at the expense of belowground biomass. One strategy that *S. patens* might use to acquire sufficient P to increase photosynthetic performance under high N conditions is to stimulate endomycorrhizal colonization in its existing roots, in contrast with the strategy of increasing root biomass to mine for more P. The observation of the significant inverse relationship of endomycorrhizal colonization with belowground biomass combined with the trend of increased aboveground:belowground biomass ratios in the +N treatments (two-way ANOVA, $p = 0.07$) infers that this strategy

may have been employed by some plants. Furthermore, VALIELA, TEAL, and PERSSON, (1976) reported depressed root growth by *S. patens* under elevated nutrients; however, they did not examine the roots for endomycorrhizal colonization.

Percent P in the leaves of the controls was about twice that found in the belowground fraction, suggesting translocation of P from the roots to the leaves during the growing season (POMEROY *et al.*, 1969; REIMOLD, 1972). Molar N:P ratios were generally low (~ 7) in the leaves, suggesting P storage and N limitation (KOERSELMAN and MEULEMAN, 1996). On the other hand, the belowground fraction of the controls had high molar N:P ratios (20 ± 4), suggesting P-limitation, possibly because of the translocation of P from the roots to the leaves. Under high N conditions, the P stored in the leaves could be used to enhance photosynthesis. This mechanism might explain, in part, the depressed %P in the leaves and the increased molar N:P ratio in the +N group as compared with the control group. However, increased N storage in the leaves under high N conditions would also increase the molar N:P ratio. The increased N storage in the leaves of the N-treated plots was also evident in the standing dead leaves (i.e., the %N), which suggests that the +N treatments would have more labile litter that could fuel remineralization processes in the sediment.

Increasing aboveground biomass under high N conditions might promote the release of labile organic exudates by roots, perhaps mediated by endomycorrhizae in *S. patens*, which fuel the processes of N fixation (BURKE, 2001; BURKE, HAMERLYNCK, and HAHN, 2002; SUNDARESHWAR *et al.*, 2003) and denitrification (SHERR and PAYNE, 1978). By log transforming the aboveground data presented here and the denitrification data previously reported for this same fertilization experiment (WIGAND *et al.*, 2004), we observed a significant relationship between denitrification enzyme activity (DEA) and the aboveground biomass ($r = 0.66$, $p = 0.007$). Significantly greater DEA was measured in the +N treatments, and there was no detectable P effect on DEA (WIGAND *et al.*, 2004).

Under elevated P conditions, the molar N:P ratios in the shoots were significantly reduced relative to the controls, suggesting N limitation. Although not statistically significant, we observed an inhibitory effect of P on plant photosynthetic performance as measured by reduced PI_{ABS} ($p = 0.09$) and slow (Q_B)-reducing reaction centers ($p = 0.06$). However, we did not detect a significant P effect

Table 4. Molar N:P, C:N, and C:P ratios (mean \pm SE) for the above- and belowground plant fraction. (ns = not statistically significant.)

	Molar Ratios	+N+P	+N	+P	Control	Significance (Probability)		
						N Effect	P Effect	N \times P Interaction
Aboveground plant fraction	N:P	7.0 (0.2)	10.8 (0.6)	4.9 (0.9)	6.7 (0.5)	*	*	ns
	C:N	48.0 (2.9)	43.1 (2.2)	54.9 (1.3)	58.7 (5.3)	*	ns	ns
	C:P	336.8 (20.3)	462.7 (16.3)	268.7 (46.8)	388.7 (17.6)	*	*	ns
Standing dead leaves	N:P	19.9 (4.9)	15.2 (5.8)	9.1 (1.5)	16.7 (3.5)	ns	ns	ns
	C:N	56.9 (5.6)	61.4 (5.2)	71.8 (2.6)	67.0 (3.7)	*	ns	ns
	C:P	1,075.1 (243.9)	861.2 (289.6)	655.8 (110.7)	1,080.5 (160.3)	ns	ns	ns
Belowground plant fraction	N:P	16.2 (2.6)	26.2 (4.4)	17.6 (1.9)	20.3 (4.1)	ns	ns	ns
	C:N	38.8 (2.3)	42.0 (2.1)	46.6 (1.9)	46.7 (3.5)	*	ns	ns
	C:P	634.1 (115.7)	1,095.0 (191.2)	819.1 (78.7)	912.8 (137.1)	ns	ns	ns

* $p < 0.05$.

Table 5. Excess nutrient sequestration in plant tissues and potential losses due to denitrification activity in the fertilized treatments. (na = not applicable.)

		Fertilized Treatments			
		+N+P		+N	+P
		g N/m ² /y	g P/m ² /y	g N/m ² /y	g P/m ² /y
<i>S. patens</i> sequestration	Aboveground nutrient sequestered	10.6	3.3	11.8	2.7
	Belowground nutrient sequestered	90.1	12.7	63.8	8.6
	Total nutrients sequestered	100.7	16.1	75.6	11.3
	Control total nutrient sequestered	61.6	8.7	61.6	8.7
	Excess total nutrients sequestered	39.1	7.3	14.0	2.6
	Potential denitrification losses	DEA in N-fertilized plots*	8.1	na	17.3
	Control DEA	2.5	na	2.5	na
	Excess DEA	5.6	na	14.8	na

* DEA = denitrification enzyme activity and was first reported in WIGAND *et al.*, 2004.

on the above- or belowground biomass of *S. patens* because of high variability among plots within treatment groups.

The molar C:N ratios (43–59) of the live *S. patens* leaves reported in our study are slightly greater than the range of 37–41 reported by VALIELA (1995). At the protected research reserve in the present study, the %N in the leaves was generally low in all of the plots (0.9–1.2%), which might ac-

count for the generally high C:N ratios. In contrast, in leaves of *S. patens* collected throughout Narragansett Bay ($n = 10$ marshes, not including the present site) in May and June 1999, the %N ranged from 1.7% to 3.0%, with high N percentages in shoots at marsh sites with high watershed nitrogen loads (Wigand, unpublished data). In the present study, standing dead leaves had greater molar C:N ratios than did live leaves or the below-

Table 6. Estimation of the added inorganic N that is either sequestered in the plants or potentially lost to denitrification activity. (na = not applicable.)

	Treatments			
	+N+P		+N	+P
	g N/m ² /y	g P/m ² /y	g N/m ² /y	g P/m ² /y
Annual nutrient addition	32.0	3.2	32.0	3.2
Excess nutrient sequestered in <i>S. patens</i> (plant sink)	39.1	7.3	14.0	2.6
Excess DEA*	5.6	na	14.8	na
Total plant and denitrification sink	44.7	7.3	28.8	2.6
Difference of plant sink from nutrient addition	-7.1	-4.1	18.0	0.6
Difference of total sink from nutrient addition	-12.7	-4.1	3.2	0.6
Percentage sequestered by <i>S. patens</i>	>100	>100	43.8	81.7
Percentage unaccounted for by plant sink	0	0	56.3	18.3
Percentage nutrient unaccounted for by total sink	0	0	9.9	18.3

* DEA = denitrification enzyme activity; see Table 5.

ground fraction. The higher molar C:N ratio of the standing dead leaves may be attributed to a number of causes, including the translocation of N to the roots or leaching of N to the water during senescence, decomposition processes, or grazing of the dead leaves by snails and crabs (CURRIN, NEWELL, and PAERL, 1995; HOPKINSON and SCHUBAUER, 1984; NEWELL, FALLON, and MILLER, 1989; WHITE and HOWES, 1994). One explanation for the lower molar C:P ratios in the live leaves compared with the standing dead leaves and belowground fraction might be the P pumping mechanism proposed for leaves of *Spartina* (POMEROY *et al.*, 1969; REIMOLD, 1972). Phosphorus might be pumped from the roots and stored in the leaves, resulting in a high C:P ratio in the roots but a lower ratio in the leaves. Furthermore, during leaf senescence, P might be released from the leaves to the overlying water, resulting in a high C:P ratio in the standing dead leaves. In addition, decomposition and grazing of the standing dead leaves might also release P, resulting in a high C:P ratio (POMEROY *et al.*, 1969).

To place in perspective the magnitude of the annual N dose (32 g N/m²/y) added in the present experiment, we related it to the estimate of the natural actively cycling N pools (34–35 g N/m²/y) reported for a Massachusetts salt marsh (WHITE and HOWES, 1994) and the annual N uptake by roots of *S. alterniflora* (35 g N/m²/y) reported for a Georgia marsh (HOPKINSON and SCHUBAUER, 1984). If the magnitude of the N pools in this Rhode Island salt marsh is similar to the Massachusetts and Georgia marshes, N additions in the present study may have nearly doubled the available N for plant assimilation. Plants (above- and belowground biomass) sequestered 44–100% of the added inorganic dissolved N (Table 6). Part of the unaccounted N may have been lost to denitrification processes. The potential denitrification values at this site (Tables 5 and 6) are within the range of denitrification rates reported for other coastal salt marsh systems (WHITE and HOWES, 1994). Assays of denitrifier biomass are a good index of denitrification potential because they integrate multiple factors that influence denitrification activity (GROFFMAN, 1987, 1994). Accounting for potential denitrification losses (WIGAND *et al.*, 2004), it appears that 90–100% of the added dissolved inorganic N could be sequestered and/or transformed by this *S. patens* marsh (Table 6).

For the +N+P treatment, the plants alone have the potential to sequester all of the added nutri-

ents (Table 6). It appears that greater N sequestration occurred in the +N+P-treated plants than can be accounted for by only the added nutrients in the experiment. It is possible that the +N+P additions stimulated decomposition processes of organic matter and peat in the sediments that provided additional N that could further enrich the plant tissues (MORRIS and BRADLEY, 1999). For the +N group, potential denitrification and plant sequestration in excess of control levels can account for about 90% of the added N. The remaining 10% of the added N is unaccounted for and might be attributed to burial of N in the sediments or export of N from the system (*e.g.*, foliar leaching, leaf export, and tidal exchange). In the +P group, about 82% of the added P could potentially be sequestered in the plant tissues and 18% of the P is unaccounted for. In this same Prudence Island fertilization experiment, J. CAFFREY (personal communication) measured a significantly higher flux of dissolved inorganic P from the P-treated plots. Additionally, some of the missing P could be accounted for by P burial in the sediment, foliar leaching, leaf export, and tidal exchange (POMEROY *et al.*, 1969).

CHALMERS (1979) attributed <5% of the loss of added organic N to sequestration in the aboveground *S. alterniflora* tissue and ~50% to sediment uptake, which would include roots and rhizomes. Therefore, the marsh sequestration rates of CHALMERS (1979) are in the same range as estimated in the present study. Furthermore, Chalmers suggested that as much as 44% of organic sewage added to the marsh might be flushed by tides and that very little loss could be attributed to denitrification because of sewage inhibition of this process at the site (SHERR and PAYNE, 1981). In the present study, we added dissolved inorganic nutrients and conducted the experiment in the high marsh, which is less flushed by the tides than the low marsh. WHITE and HOWES (1994) suggested that adding dissolved inorganic N in contrast with organic N (*e.g.*, sewage) would allow for quick and almost complete short-term uptake or transformation of N by the plant-sediment system, including losses due to high denitrification rates.

The results of our study suggest that the addition of dissolved inorganic N increases *S. patens* photosynthetic performance and aboveground biomass. Although only observed as a trend in this study because of high variability among plots in treatment groups, dissolved inorganic P additions are likely to

inhibit *S. patens* photosynthetic performance. It appears that limiting nutrients (e.g., N, P, labile C) drive many of the observed relationships in the *S. patens*-microbe-sediment system, causing some interdependent relationships and other competitive ones. While this study suggests that some *S. patens* marshes have the potential to sequester and/or transform a large percentage of dissolved inorganic nutrient loads, it is unclear how long-term nutrient overenrichment and the resulting changes in the *S. patens*-microbe-sediment system might alter this buffering capacity.

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LITERATURE CITED

- ALLEN, M.F., 1991. *The Ecology of Mycorrhizae*. Cambridge: Cambridge University Press, 184p.
- BURKE, D.J., 2001. The Interaction Between the Grass *Spartina patens*, N-fixing Bacteria and Vesicular Arbuscular Mycorrhizae in a Northeastern Salt Marsh. New Brunswick, New Jersey, Rutgers University, Ph.D. thesis, 146p.
- BURKE, D.J.; HAMERLYNCK, E.P., and HAHN, D., 2002. Effect of arbuscular mycorrhizae on soil microbial populations and associated plant performance of the salt marsh grass *Spartina patens*. *Plant and Soil*, 239, 141-154.
- CHALMERS, A.G., 1979. The effects of fertilization on nitrogen distribution in *Spartina alterniflora* marsh. *Estuarine and Coastal Marine Science*, 8, 327-337.
- CHAMBERS, R.M. and FOURQUREAN, J.W., 1991. Alternative criteria for assessing nutrient limitation of a wetland macrophyte (*Peltandra virginica* (L.) Kunth). *Aquatic Botany*, 40, 305-320.
- CLARK, A.J.; LANDOLT, W.; BUCHER, J.B., and STRASSER, R.J., 2000. How wind affects the photosynthetic performance of trees: quantified with chlorophyll *a* fluorescence and open-top chambers. *Photosynthetica*, 38, 349-360.
- COOKE, J.C.; BUTLER, R., and MADOLE, G., 1993. Some observations on the vertical distribution of vesicular arbuscular mycorrhizae in the roots of salt marsh grasses growing in saturated soils. *Mycologia*, 84, 547-550.
- COOKE, J.C. and LEFOR, W. W., 1990. Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, CT, USA. *Environmental Management*, 14, 131-137.
- CURRIN, C.A.; NEWELL, S.Y., and PAERL, H.W., 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Marine Ecology Progress Series*, 121, 99-116.
- EWING, K.; MCKEE, K.L., and MENDELSSOHN, I.A., 1997. A field comparison of indicators of sublethal stress in the salt-marsh grass *Spartina patens*. *Estuaries*, 20, 48-65.
- EWING, K.; MCKEE, K.L.; MENDELSSOHN, I.A., and HESTER, M.W., 1995. A comparison of indicators of sublethal salinity stress in the salt marsh grass *Spartina patens*. *Environmental and Experimental Botany*, 35, 331-343.
- GIANINAZZI, S. and GIANINAZZI-PEARSON, V., 1992. Cytology, histochemistry and immunocytochemistry as tools for studying structure and function in endomycorrhiza. In: NORRIS, J.R., READ, D.J., and VARMA, A.K., (eds.), *Techniques for Mycorrhizal Research*. New York: Academic Press, pp. 569-599.
- GROFFMAN, P.M., 1987. Nitrification and denitrification in soil: a comparison of enzyme assay, incubation and enumeration methods. *Plant and Soil*, 97, 445-450.
- GROFFMAN, P.M., 1994. Denitrification in freshwater wetlands. *Current Topics in Wetland Biogeochemistry*, 1, 15-35.
- HOPKINSON, C. S. and SCHUBAUER, J. P., 1984. Static and dynamic aspects of nitrogen cycling in the salt marsh graminoid *Spartina alterniflora*. *Ecology*, 65, 961-969.
- KOERSELMAN, W. and MEULEMAN, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33, 1441-1450.
- LEBKUECHER, J.G.; HALDEMAN, K.A.; HARRIS, C.E.; HOLZ, S.L.; JOUDAH, S.A., and MINTON, D.A., 1999. Development of photosystem-II activity during irra-

- diance of etiolated *Helianthus* (Asteraceae) seedlings. *American Journal of Botany*, 86, 1087–1092.
- McKINNEY, R.A.; NELSON, W.G.; CHARPENTIER, M.A., and WIGAND, C., 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. *Ecological Applications*, 11, 203–214.
- MENDELSSOHN, I.A.; MCKEE, K.L., and KONG, T., 2001. A comparison of physiological indicators of sublethal cadmium stress in wetland plants. *Environmental and Experimental Botany*, 46, 263–275.
- MORRIS, J.T., 1982. A model of growth responses by *Spartina alterniflora* to nitrogen limitation. *Journal of Ecology*, 70, 25–42.
- MORRIS, J.T. and BRADLEY, P. M., 1999. Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnology and Oceanography*, 44, 699–702.
- NEWELL, S.Y.; FALLON, R.D., and MILLER, J.D., 1989. Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass, *Spartina alterniflora*. *Marine Biology*, 101, 471–481.
- PARSONS, T.R.; MAITA, Y., and LALLI, C.M., 1984. *A Manual of Chemical and Biological Methods for Sea-water Analysis*. Oxford: Pergamon Press, 173p.
- PHILLIPS, K.M. and HAYMAN, D. S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55, 158–160.
- POMEROY, L.R.; JOHANNES, R.E.; ODUM, E.P., and ROFFMAN, B., 1969. The phosphorus and zinc cycles and productivity of a salt marsh. In: NELSON, D.J. and EVANS, F.C. (eds.), *Symposium on Radioecology*. Washington, D.C., US Atomic Energy commission, CONF-67503, pp. 412–419.
- REIMOLD, R., 1972. The movement of phosphorus through the salt marsh cord grass, *Spartina alterniflora* Loisel. *Limnology and Oceanography*, 17, 606–611.
- RICHMOND, M.L.; BRANDAO, S.C.C.; GRAY, J.I.; MARKAKIS, P., and STINE, C.M., 1981. Analysis of simple sugars and sorbitol in fruit by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 29, 4–7.
- SHERR, B.F. and PAYNE, W.J., 1978. Effect of the *Spartina alterniflora* root-rhizome system on salt marsh soil denitrifying bacteria. *Applied and Environmental Microbiology*, 35, 724–729.
- SHERR, B.F. and PAYNE, W.J., 1981. The effect of sewage sludge on salt-marsh denitrifying bacteria. *Estuaries*, 4, 146–149.
- SMITH, S.E. and READ, D.J., 1997. *Mycorrhizal Symbiosis*. San Diego: Academic Press.
- SRIVASTAVA, A.; STRASSER, R.J., and GONVINDJEE, 1999. Greening of peas: parallel measurements of 77 K emission spectra, OJIP chlorophyll *a* fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. *Photosynthetica*, 37, 365–392.
- STRASSER, R.J.; SRIVASTAVA, A., and GONVINDJEE, 1995. Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology*, 61, 32–42.
- STRASSER, R.J.; SRIVASTAVA, A., and TSMILLI-MICHAEL, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: YUNUS, M., PATHRE, U., and MOHANTY, P. (eds.), *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. London: Taylor and Francis, pp. 445–483.
- STRASSER, B.J. and STRASSER, R.J., 1995. Measuring fast fluorescence transients to address environmental questions: The JIP-Test. In: MATHIS, P. (ed.), *Photosynthesis: From Light to Biosphere*. London: Kluwer Academic, pp. 977–980.
- SULLIVAN, M.J. and DAIBER, F.C., 1974. Response in production of cord grass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. *Chesapeake Science*, 15(2), 121–123.
- SUNDARESHWAR, P.V.; MORRIS, J.T.; KOEPLER, E.K., and FORNWALT, B., 2003. Phosphorus limitation of coastal ecosystem processes. *Science*, 299, 563–565.
- TOBIAS, C.R.; ANDERSON, I.C.; CANUEL, E.A., and MACKO, S.A., 2001a. Nitrogen cycling through a fringing marsh-aquifer ecotone. *Marine Ecology Progress Series*, 210, 25–39.
- TOBIAS, C.R.; HARVEY, J.W., and ANDERSON, I.C., 2001b. Quantifying groundwater discharge through riparian wetlands to estuaries: seasonal variability, methods comparison, and implications for wetland-estuary exchange. *Limnology and Oceanography*, 46, 604–615.
- TOBIAS, C.R.; MACKO, S.A.; ANDERSON, I.C.; CANUEL, E.A., and HARVEY, J.W., 2001c. Tracking the fate of a high concentration groundwater plume through a fringing marsh: a combined groundwater tracer and in situ isotope enrichment study. *Limnology and Oceanography*, 46, 1977–1989.
- VALIELA, I., 1995. *Marine Ecological Processes*, 2nd edition. New York: Springer-Verlag.
- VALIELA, I.; COLE, M.L.; MCCLELLAND, J.; HAUXWELL, J.; CEBRIAN, J., and JOYE, S.B., 2000. Role of salt marshes as part of coastal landscapes. In: WEINSTEIN, M.P. and KREEGER, D.A. (eds.), *Concepts and Controversies in Tidal Marsh Ecology*. New York: Kluwer, pp. 23–38.
- VALIELA, I.; COLLINS, G.; KREMER, J.; LAJTHA, K.; GEIST, M.; SEELY, B.; BRAWLEY, J., and SHAM, C.H., 1997. Nitrogen loading from coastal watersheds to receiving estuaries: new method and application. *Ecological Applications*, 7, 358–380.
- VALIELA, I.; TEAL, J.M., and PERSSON, N.Y., 1976. Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. *Limnology and Oceanography*, 21, 245–252.
- VALIELA, I.; TEAL, J.M., and SASS, W.J., 1975. Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge. *Journal of Applied Ecology*, 12, 973–981.
- VAN WIJNEN, H.J. and BAKKER, J.P., 1999. Nitrogen and phosphorus limitation in a coastal barrier salt marsh: the implications for vegetation succession. *Journal of Ecology*, 87, 265–272.
- WHITE, D.S. and HOWES, B.L., 1994. Long-term N-15 nitrogen retention in the vegetated sediments of a New England salt marsh. *Limnology and Oceanography*, 39, 1878–1892.
- WIGAND, C.; MCKINNEY, R.; CHINTALA, M.; CHARPENTIER, M., and GROFFMAN, P., 2004. Denitrification enzyme activity of fringe salt marshes in New England (USA). *Journal of Environmental Quality*, 33, 1144–1151.

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