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## Morphological plasticity of *Sagittaria lancifolia* in response to phosphorus

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### Abstract

*Sagittaria lancifolia* leaf morphology varies in the Everglades of southern Florida, USA, from plants with broadly ovate laminae to plants with linear laminae. To understand the morphological response of *S. lancifolia* to increased nutrients, we (1) grew *S. lancifolia* plants in solutions with either 1 mM or 10  $\mu$ M P and measured morphological responses, as well as tissue N:P content and (2) grew field-collected plants that had a range of leaf morphologies in the greenhouse and measured morphological and tissue N:P responses. Plants in high P (HP), as compared to low P (LP), were larger (biomass 48.3 g<sub>HP</sub> versus 16.9 g<sub>LP</sub>), produced more leaves (16.9<sub>HP</sub> versus 10.7<sub>LP</sub>) and inflorescences (2.9<sub>HP</sub> versus 0.3<sub>LP</sub>), and branched more (shoots 3.8<sub>HP</sub> versus 1.1<sub>LP</sub>). Biomass allocation to leaves and inflorescences was greater in HP as compared to LP plants (leaves 61%<sub>HP</sub> versus 37%<sub>LP</sub>; inflorescences 15%<sub>HP</sub> versus 2%<sub>LP</sub>), while plants in LP allocated relatively more biomass to roots and rhizomes than plants in HP (roots 8%<sub>HP</sub> versus 25%<sub>LP</sub>; rhizomes 15%<sub>HP</sub> versus 35%<sub>LP</sub>). Leaf morphology changed with P-addition, so that plants in high P produced leaves with longer leaf bases (352 mm<sub>HP</sub> versus 219 mm<sub>LP</sub>), longer and wider blades (290 mm  $\times$  49 mm<sub>HP</sub> versus 176 mm  $\times$  9 mm<sub>LP</sub>), and a larger leaf area (99 cm<sup>2</sup><sub>HP</sub> versus 13 cm<sup>2</sup><sub>LP</sub>) than plants in LP, whereas plants in LP produced leaves with longer petioles (277 mm<sub>HP</sub> versus 346 mm<sub>LP</sub>). Leaf N:P ratios decreased in HP relative to initial ratios (43<sub>initial</sub> to 17<sub>final</sub>) and increased in LP (45<sub>initial</sub> to 86<sub>final</sub>). The direction of change in lamina width of field-collected plants grown under greenhouse conditions depended on the original leaf morphology—plants with narrow blades in the field produced wider laminae in the greenhouse, while plants with wide blades in the field produced narrower laminae. These plastic changes in leaf morphology mimic the differences in leaf morphology between *S. lancifolia* subsp. *media* and *S. lancifolia* subsp. *lancifolia*. Our results

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show that *S. lancifolia* responds rapidly to changes in environmental nutrient status, especially in lamina morphology. Blade shape thus provides a field indicator of soil/pore water nutrient status: narrow blades indicate nutrient limitation, whereas broader blades indicate nutrient enrichment.

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## 1. Introduction

Emergent aquatic plants are noted for their phenotypic plasticity, especially in relation to leaf morphology (Sculthorpe, 1967; Lee and Richards, 1991). This morphological variability is associated with the aquatic habitat and is often interpreted as a response to growing from a submerged to emergent condition. Many species of *Sagittaria* produce both submerged and emergent leaves that differ morphologically, but emergent leaves can also vary widely in leaf shape (Wooten, 1970, 1986; Kaul, 1991). This variation has been most commonly associated with different water levels (Wooten, 1970, 1986; Kaul, 1991), although Wooten (1973) also found differences in soil nutrients and pH that distinguished the environments of ecotypes of the *S. graminea* complex.

The bulltongue arrowhead, *Sagittaria lancifolia* L., lacks the sagittate emergent leaves from which the genus gets its name, having instead linear to ovate or elliptic aerial foliage with blades 0.7–16 cm wide (Haynes and Hellquist, 2000). The shape of the emergent leaves is one character that has been used to distinguish subspecies *lancifolia* (= *S. angustifolia* Lindley, leaves linear to ovate) from subspecies *media* (Micheli) Bogin (leaves lanceolate to broadly ovate) (Haynes and Hellquist, 2000). In the wetlands of southern Florida, where *S. lancifolia* is the common species of *Sagittaria*, laminae of this species vary from linear to broadly ovate, and the blade shape can vary over relatively short distances (Stober et al., 2001). In a study of morphological variation of field-collected plants, we found that morphological differences could be correlated with several environmental parameters but were most strongly related to phosphorus (P) levels in the soil; plants at sites with higher soil P had wider leaf blades (Stober et al., 2001). Because P is the limiting nutrient in the Everglades ecosystem (McCormick et al., 2001; Noe et al., 2001), *S. lancifolia* leaf morphology is a potential indicator of P enrichment in the field. We experimentally studied variation in *S. lancifolia* morphology in response to different levels of P by (1) growing plants under different P levels when other nutrients were not limiting (P-addition experiment) and (2) growing field-collected plants that differed in morphology under uniform greenhouse conditions (common garden experiment).

## 2. Materials and methods

### 2.1. Field collections

Plants of *S. lancifolia* were collected as part of the 1999 US-EPA REMAP South Florida survey (Stober et al., 2001). The REMAP study sampled 240 sites throughout southern

Florida wetlands in a stratified random-sampling design from 12 to 19 May 1999, at the end of the dry season, and from 30 September to 7 October 1999, during the wet season (Stober et al., 2001). Five to twenty plants of *S. lancifolia* were collected at each of 90 sites where it was present and brought back to the Florida International University (FIU) campus. The three most recently matured leaves, as determined by relative position, length, and texture, were measured for leaf base, petiole and lamina length, and lamina width; all other leaves and roots were removed. Rhizome diameter, length, and fresh weight were also recorded. In order to standardize initial plant leaf area in plantings, leaves were trimmed to 20 cm total length as measured from the rhizome; thus, only portions of the leaf base of three leaves remained on each plant. Plants used in the common garden experiment came from the May collection. These plants were planted in greenhouse-potting soil in 10 cm plastic pots; in order to keep the pots saturated, they were set in tubs filled with water in the FIU greenhouse. Plants used in the nutrient experiment were collected in the September/October sample. These plants were held in open, water-filled, liter plastic bags set in tubs in the FIU greenhouse.

## 2.2. P-addition experiment

Plants used in this experiment were collected during the wet season sampling period and measured as described above. Each rhizome was grown in a plastic bag filled with tap water in the greenhouse. We chose the 27 sites with the smallest average lamina widths and selected a single plant from each site for use in the experiment. Sites selected were distributed throughout the southern Florida Everglades. Median water pH at the original sites was 7.7 (range: 6.7–8.4), soil total P was  $228 \pm 136$  mg/kg, and soil ash-free dry weight was  $61 \pm 25\%$  (subset of data from Stober et al., 2001).

On 16 February 2000, the 27 plants were transplanted from the plastic bags to individual 16.3 L plastic containers in the greenhouse, each of which held 10 L sand that had been washed with 0.2 N HCl and rinsed in de-ionized water to a constant pH. Each container also held 8.7 L of de-ionized water. Prior to planting, leaves that emerged while the plants were growing in the plastic bags were counted, measured and cut to uniform length (5 cm leaf base remaining); roots were removed and rhizomes were washed, weighed and their length was measured. The cut parts of the leaves were dried in an 80 °C oven and analyzed for CNP in the FIU Southeast Environmental Research Center (SERC) analytic lab. Total P determination followed the Solorzano and Sharp method (1980). Total C and N were analyzed with a Carlo-Erba NA-1500 CN Analyzer (Haak–Buchler Instruments, Saddlebrook, NJ).

On 22 February 2000, each pot was drained and filled with 8.7 L of a solution of 0.50 mM  $K_2SO_4$ , 0.98 mM  $MgSO_4$ , 1.0 mM  $CaSO_4$ , 4.50 mM  $NH_4NO_3$ , 2  $\mu M$   $MnSO_4$ , 0.02 mM  $H_3BO_3$ , 2  $\mu M$   $ZnSO_4$ , 0.2  $\mu M$   $CuSO_4$ , 0.08 mM KCl, 0.4  $\mu M$   $Na_2MoO_4$ , and 0.87 mL chelated Liquid Iron (Southern Agricultural Insecticides Inc., Palmetto, FL). Fourteen pots (randomly assigned to high-P treatment) included 0.47 mM  $NaH_2PO_4$  and 0.53 mM  $NaHPO_4$ , whereas the remaining thirteen pots (assigned to low-P treatment) included 4.7  $\mu M$   $NaH_2PO_4$  and 5.3  $\mu M$   $NaHPO_4$ . The high-P treatment thus had 1000  $\mu M$  P and a molar N:P ratio of 9, whereas the low-P treatment had 10  $\mu M$  P and a molar N:P ratio of 900. Initial solution pH was 7.9 (range: 7.1–8.5) in the high-P treatment and 7.8

(range: 7.0–8.4) in the low-P treatment. Every 2 weeks the solution was drained from each pot and replaced with fresh solution.

To assess the nutrient availability of plants during the experiment and to see how this changed during each two-week period, we subsampled water from three randomly chosen pots in each treatment at two times during the experiment: (1) early, shortly after the experiment was initiated (2 and 14 days) and (2) late, near the end of the experiment (56 and 63 days). The late-sampled pots did not include any that had been sampled earlier. At each sampling time, we measured the nutrient conditions of the water twice: 24 h after fresh nutrient solution had been added to the pots, which allowed nutrient conditions to stabilize following the addition of fresh nutrient solution, and immediately before fresh nutrient solution was to be added.

The positions of pots in the greenhouse were randomized weekly throughout the experiment. Plants were destructively harvested after 12 weeks on 17 May 2000. The number of inflorescences and branches produced by each plant was counted. On the original stem in each pot, the total number of leaves matured on the main shoot by each plant since the experiment began was recorded, as well as the position (leaf number) of the most recently matured leaf. Leaf-base length, petiole length, lamina length and lamina width were measured to the nearest mm for the seventh leaf matured on the main shoot since the beginning of the experiment and for the most recently matured leaf. Laminae were removed and leaf area measured using a Delta-T leaf area meter (Delta-T Devices Ltd., Cambridge, England).

At harvest, roots, rhizomes, leaves, and inflorescences were separated. Rhizome length and diameter were measured and the rhizomes were weighed on an electronic balance (precision 0.01 g). Plant material was dried to a constant weight in 80 °C drying ovens and weighed on the balance. The three most recently matured leaves were dried separately and analyzed for CNP as described above.

### 2.3. *Common garden experiment*

Plants from the dry season collection were used in the common garden experiment. After planting, plants in pots were set in plastic dishpans filled with tap water. These plants were grown in the greenhouse from June 1999 until May 2000. Lamina length and lamina width, and petiole length and width were measured on the three most recently matured leaves of all apices in all pots in September 1999, and again in March 2000. Plants were fertilized by adding 15 mL of 15:9:12 (N:P:K) slow-release fertilizer to each pot in the winter months. For analysis, data from the three measured leaves in a pot were averaged. The final morphological data set included 250 plants collected from 46 sites.

In order to document changes in CNP in these plants and compare these to changes in morphology, in May 2000 the three most recently matured leaves from a subset of the greenhouse grown plants were harvested and processed for CNP analysis, as described above.

### 2.4. *Statistical analysis*

Data for the P-addition experiment were analyzed with SAS v. 9 (SAS Institute Inc., Cary, NC), using one-way analysis of variance (ANOVA) for normally distributed data or

Kruskal–Wallis (KW) tests for non-normal data to assess differences between treatments. Differences between leaves or nutrients on the same plants were compared using paired *t*-tests of means.

Data for the common garden experiment were also analyzed with SAS v. 9. The data taken from multiple leaves on a plant were averaged to obtain a single measurement for that plant. Differences between morphological parameters for the field-collected plants and plants after 4 and 10 months in the greenhouse were calculated, then linear regressions were used to explore the correlations of these differences to original leaf measurements. Regression analysis was also used to examine N to P content and the relation of %N, %P, and N:P ratio to leaf morphology. Changes in C, N, and P content were examined with paired *t*-tests. Kruskal–Wallis (KW) tests were used for data that were not normally distributed.

### 3. Results

#### 3.1. P-addition experiment

Both early and late water samples were within the range of desired concentrations (Table 1). Twenty-four hours after solutions were changed, the N:P ratio was approximately hundred times higher in the high-P treatment (Table 1). Levels of P decreased over time (day 14 and day 63 samples, Table 1) in the high-P treatment, while the reduction was much less in the low-P treatment. N:P ratios in the solutions increased prior to changing nutrient solutions both for high- and low-P treatments, but the increase was greater for LP.

Plants used for the high- and low-P treatments did not differ significantly in their leaf morphology when they were collected (Table 2), nor did they differ significantly in lamina length (LL) or lamina width (LW) at the time the experiment was initiated ( $LL_{\text{High P}} = 40 \pm 12$  mm,  $LL_{\text{Low P}} = 41 \pm 10$  mm;  $LW_{\text{High P}} = 3 \pm 2$  mm,  $LW_{\text{Low P}} = 3 \pm 1$  mm;  $P > 0.05$  for both LL and LW, KW test). Plants produced leaves during the 4 months that they were in plastic bags in the greenhouse, using stored reserves in the rhizome, so the fresh weight per centimeter of rhizome (RFW) decreased over this time from  $17.9 \pm 12.6$  g/cm of rhizome for field-collected plants to  $6.2 \pm 2.3$  g/cm for plants at the beginning of the experiment. There were not significant differences in RFW between plants put in the two treatments either at collection ( $P = 0.31$ , KW test) or at treatment initiation ( $P = 0.38$ , KW test).

After 12 weeks of treatment, *S. lancifolia* plants in HP had greater total biomass than plants in LP (high P =  $48.3 \pm 28.2$  g, low P =  $16.9 \pm 10.9$  g (mean  $\pm$  S.D.);  $P = 0.0003$ ,

Table 1  
Total N and P in water samples from the experimental pots

Sample	Day	N ( $\mu\text{M}$ )		P ( $\mu\text{M}$ )		N:P (molar)	
		High P	Low P	High P	Low P	High P	Low P
Early	2	406 $\pm$ 8	413 $\pm$ 4	997 $\pm$ 48	7 $\pm$ 3	0.41 $\pm$ 0.01	67.83 $\pm$ 27.52
	14	381 $\pm$ 6	384 $\pm$ 3	583 $\pm$ 29	3 $\pm$ 1	0.65 $\pm$ 0.03	155.61 $\pm$ 68.41
Late	56	639 $\pm$ 26	593 $\pm$ 125	1283 $\pm$ 213	12 $\pm$ 1	0.51 $\pm$ 0.11	49.61 $\pm$ 7.63
	63	551 $\pm$ 24	668 $\pm$ 44	906 $\pm$ 91	7 $\pm$ 4	0.61 $\pm$ 0.04	138.61 $\pm$ 90.81

Mean  $\pm$  1 S.D.;  $N = 3$  for each value.

Table 2

Measurements on leaves of field-collected plants and of the seventh leaf and the most recently matured (MRM) leaf produced 12 weeks after initiation of experimental P treatment

	Field leaves <sup>a</sup>		Seventh leaf <sup>b</sup>		MRM leaf <sup>b</sup>	
	High P	Low P	High P	Low P	High P	Low P
Total leaf length (mm)	1005 ± 296	1137 ± 198	661 ± 120a	676 ± 83a	920 ± 78a	741 ± 87b
Leaf base length (mm)	364 ± 149	409 ± 123	231 ± 42a	196 ± 35b	352 ± 48a	219 ± 40b
Petiole length (mm)	521 ± 129	593 ± 134	243 ± 42a	310 ± 30b	277 ± 31a	346 ± 42b
Lamina length (mm)	114 ± 56	108 ± 46	188 ± 67a	169 ± 32a	290 ± 31a	176 ± 31b
Lamina width (mm)	6 ± 4	5 ± 3	26 ± 20a	9 ± 4b	49 ± 29a	9 ± 5b
Leaf area (cm <sup>2</sup> )	–	–	41 ± 31a	12 ± 6b	99 ± 59a	13 ± 7b

Numbers are mean ± 1 S.D.;  $N_{\text{High P}} = 13$  and  $N_{\text{Low P}} = 14$ . Numbers in a row within a leaf position with different letters are significantly different at  $P \leq 0.05$ , ANOVA or KW tests; no parts of leaves on field-collected plants differed significantly between treatments ( $P > 0.05$ , all measurements).

<sup>a</sup> Data for field plants were averages of the three most recently matured leaves on each plant.

<sup>b</sup> Data for the seventh and MRM leaf were measurements of a single leaf per plant.

KW test). Plants in HP allocated relatively more biomass to leaf and inflorescence production than plants in LP, whereas plants in LP allocated more biomass to root and rhizome production (Fig. 1). Part of the increase in biomass resulted from more vigorous branching in HP (Table 3). Plants in HP produced more leaves on the main apex, and expanded more inflorescences than plants in LP (Table 3). The primary rhizome was longer, wider, and had a greater fresh weight per centimeter in the high-P treatment than the low-P treatment (Table 3).

Leaves differed significantly in their morphology between treatments. The seventh leaf to mature had a longer leaf base and wider lamina with larger leaf area in the high-P treatment than the seventh leaf on plants growing in LP (Table 2). Lamina length did not differ significantly between treatments, and petioles in LP were longer than those in HP (Table 2). The differences between treatment in petiole length and lamina plus leaf-base length offset each other, so that total leaf length was not significantly different between treatments for the seventh leaf (Table 2).

Plants in HP grew faster and produced more leaves than plants in LP; after 12 weeks of treatment, high-P plants had an average of 17 leaves, whereas low-P plants had only 11 (Table 3). The morphology of these recently matured leaves differed markedly between treatments, and the difference was similar to how the seventh matured leaves differed, i.e., the leaf base was longer and lamina wider in HP, while the petiole was longer in LP. Lamina length, lamina area and total leaf length were significantly greater in HP than in LP in these later-produced leaves (Table 2).

Lamina length, lamina width and leaf area did not increase significantly between the seventh leaf and the most recently matured leaf on plants in LP (Table 2;  $P > 0.40$  for all parameters, paired  $t$ -tests), while all three parameters increased on plants in HP (Table 2;  $P < 0.01$ , paired  $t$ -tests). Leaf-base length and petiole length increased significantly between the seventh leaf and the later-produced leaves in both treatments (Table 2;  $P < 0.02$  for all parameters, paired  $t$ -tests).

At the beginning of the experiment, leaves in the two treatments had similar C, N, and P concentrations and thus had similar molar N:P ratios (Fig. 2;  $P > 0.05$  for all, KW tests). After 12 weeks of growth, leaves had significantly higher P content in the high-P treatment

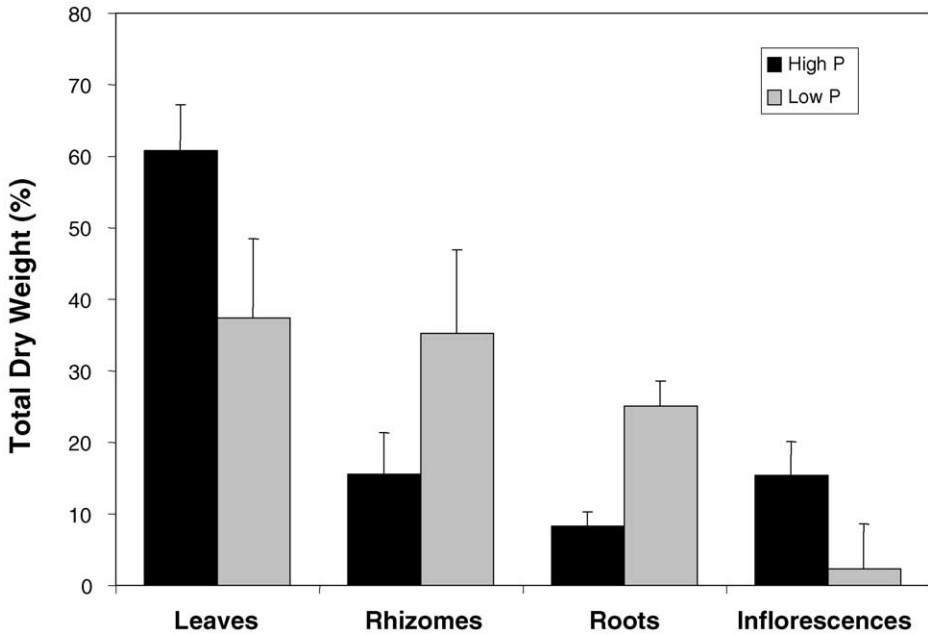


Fig. 1. Relative biomass allocation of *Sagittaria lancifolia* to leaves, rhizomes, roots and inflorescences under high- and low-P treatments.  $N = 13$  for high-P and 14 for low-P treatments.

(Fig. 2;  $P < 0.0001$ , KW test), but percent N did not differ between treatments (Fig. 2;  $P = 0.08$ , KW test). Percent C was marginally greater in the high-P treatment as compared to the low-P treatment, but the difference was not large (Fig. 2;  $P = 0.05$ , KW test). The higher P content of plants in the high-P treatment resulted in a significantly lower N:P ratio when compared to the plants in LP (Fig. 2;  $P < 0.0001$ , KW test). Variation in the N:P ratio was also low in the high-P plants and much higher in the low-P plants (Fig. 2).

Leaf %N increased 1.9-fold in both high- and low-P treatments (Fig. 2). Phosphorus in leaves increased 4.9-fold in the high-P treatments but only 1.2-fold in the low-P treatment (Fig. 2). The changes in N and P levels after treatment altered the N:P ratio in both high- and low-P plants as compared to their initial N:P ratios. The N:P ratio decreased

Table 3  
Whole plant parameters after 12 weeks of high and low-P treatments

	High P	Low P
Total no. of shoots	$3.8 \pm 1.6a$	$1.1 \pm 0.3b$
Total no. of leaves on the main apex	$16.9 \pm 2.6a$	$10.7 \pm 1.6b$
Total no. inflorescences	$2.9 \pm 1.9a$	$0.3 \pm 0.5b$
Rhizome length (mm)	$56 \pm 15a$	$33 \pm 10b$
Rhizome diameter (mm)	$32 \pm 6a$	$21 \pm 5b$
FW per centimeter rhizome (g/cm)	$4.3 \pm 1.4a$	$2.4 \pm 1.3b$

Values are average  $\pm$  S.D.;  $N_{\text{High P}} = 13$  and  $N_{\text{Low P}} = 14$ . Values in a row that have different letters were significantly different at  $P \leq 0.05$ , ANOVA or KW tests. FW: fresh weight.

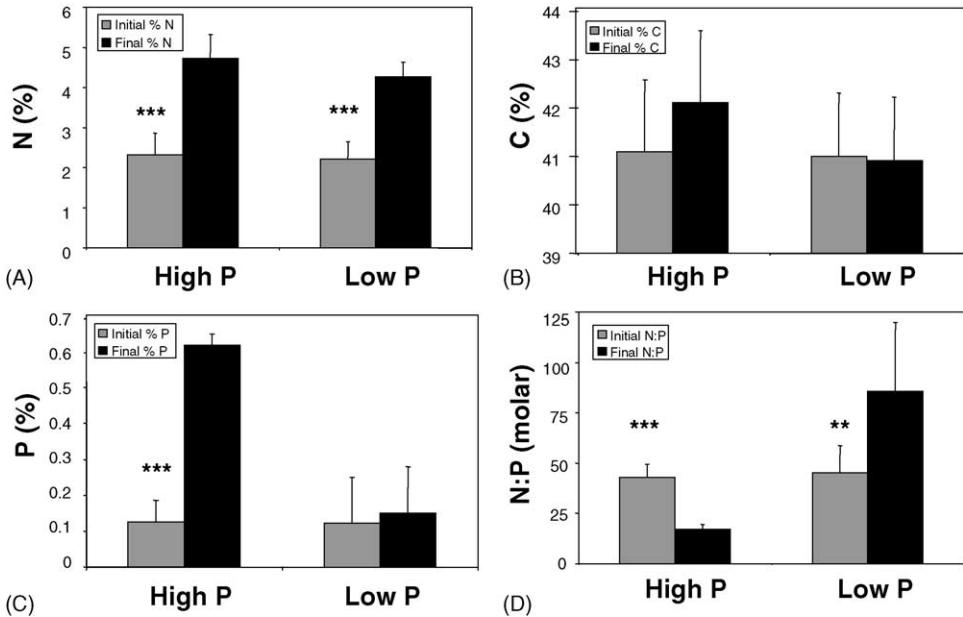


Fig. 2. Percent nitrogen (A), carbon (B), and phosphorus (C) and molar N:P ratio (D) in leaves of *Sagittaria lancifolia* after 12 weeks in high and low P. (\*):  $P < 0.05$ ; (\*\*):  $P < 0.01$ ; (\*\*\*):  $P < 0.001$  for comparisons between initial and final nutrient content (KW tests); bars lacking asterisks are not significantly different.  $N = 12$  for high-P and 14 for low-P treatments.

significantly in the high-P treatment compared to the initial N:P ratio for these plants (Fig. 2). In contrast, this ratio increased dramatically in the low-P treatment as a result of the relatively large increase in N content of the leaves (Fig. 2). When the N:P ratio was graphed against other parameters, such as leaf biomass or lamina width (Fig. 3), the biomass or morphological parameter varied widely in the high-P treatment, while the N:P ratio was relatively constant and low. These parameters were much less variable in the low-P treatment, while the N:P ratio was high and variable (Fig. 3).

### 3.2. Common garden experiment

Leaf morphology of plants grown in the greenhouse changed over time from that of field-collected plants ( $P < 0.0001$  for all parameters, KW tests; Table 4). Variation among plants also changed ( $P < 0.01$  for all parameters, Levene's tests for homogeneity of variance among months; Table 4). When comparing different parts of the leaf, the coefficient of variation was greatest for lamina width in all samples but decreased from 93 to 54% in the first 4 months of the experiment (Table 4).

For the entire sample, average lamina length, lamina width, and petiole width increased over time, while petiole length decreased (Table 4). Responses of individual plants, however, depended on the initial morphology of the field-collected plants. For example, the difference in lamina width between field-collected plants and plants after 4 months in the greenhouse was positively correlated with the original lamina width of the plants and

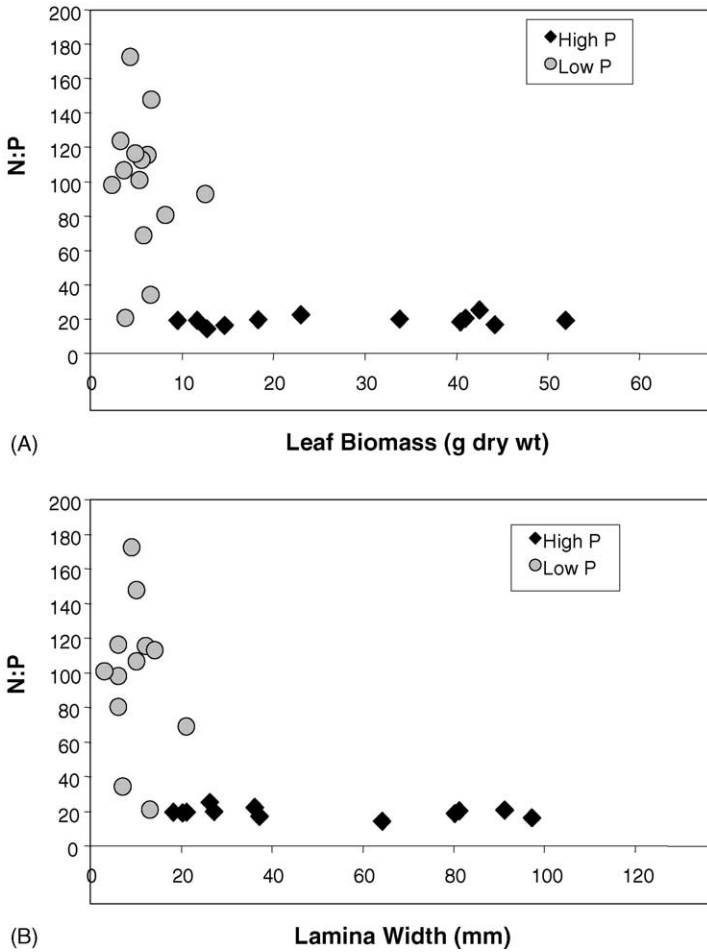
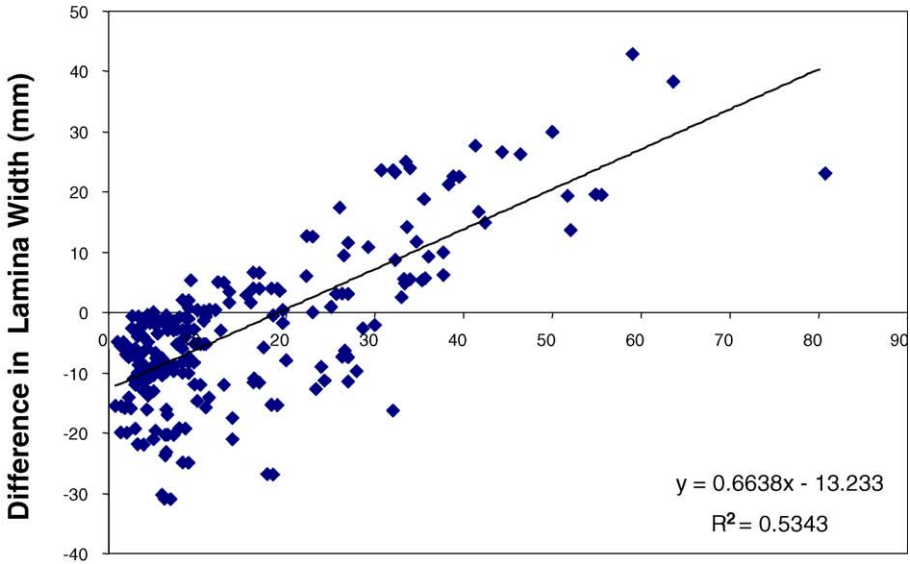


Fig. 3. Total leaf biomass (A) and lamina width (B) vs. N:P ratio after 12 weeks in high- and low-P treatments.  $N = 12$  for high-P and 14 for low-P treatments.

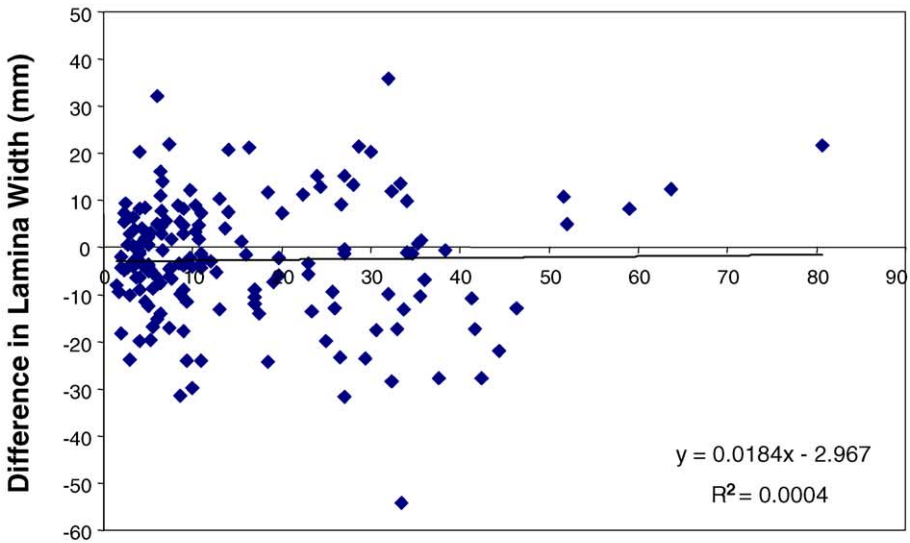
Table 4  
Change in leaves of *Sagittaria lancifolia* on plants grown in the greenhouse

	May 1999	September 1999	March 2000
Lamina length (mm)	128 ± 49 (38%)a	147 ± 38 (26%)b	221 ± 43 (19%)c
Lamina width (mm)	15 ± 14 (93%)a	18 ± 10 (54%)b	21 ± 11 (55%)b
Petiole length (mm)	313 ± 113 (36%)a	318 ± 62 (20%)a	247 ± 43 (18%)b
Petiole width (mm)	4.0 ± 1.6 (26%)a	3.8 ± 0.8 (20%)a	4.7 ± 0.6 (13%)b

Data from 250 plants collected from 46 sites (May 1999), and measured after 4 (September 1999) and 10 (March 2000) months in the glasshouse. Numbers are average ± S.D. (coefficient of variation). Row values followed by different letters were significantly different in pair-wise comparisons at  $P < 0.05$ .



(A)



(B)

Fig. 4. Regression of difference in lamina width after 4 months (A) and from 4 to 10 months (B) in the greenhouse to lamina width of the same field-collected plant.  $N = 256$ .

Table 5

Carbon, nitrogen and phosphorus content of leaves on field-collected plants (initial) and the same plants after growing in the greenhouse for 12 months (final)

	Initial	Final
Percentage TN	2.79 ± 0.66a	2.25 ± 0.44b
Percentage TC	41.05 ± 1.20a	42.41 ± 1.34b
Percentage P	0.15 ± 0.08a	0.29 ± 0.09b
N:P (molar)	47 ± 15a	18 ± 5b

Mean ± S.D.; *N* = 69. Row values followed by the different letters are significantly different in paired *t*-tests of the means ( $P \leq 0.05$ ).

explained 53% of the variation among plants in blade difference (Fig. 4A). Thus, plants that had narrower laminae in the field ( $8.6 \pm 6.8$  mm; range: 1.5–32 mm width) tended to have broader blades, i.e. a negative difference in blade width (Fig. 4A), after 4 months in the greenhouse, while plants that had broader laminae in the field ( $31 \pm 14.8$  mm; range: 9–81 mm width) tended to have narrower blades in the greenhouse, i.e. a positive difference in blade width (Fig. 4A). The difference in blade width between 4 and 10 months, however, was unrelated to initial blade width in the field (Fig. 4B). Similarly, original measurements for lamina length, petiole length, and petiole width were correlated to differences in these measurements after 4 months in the greenhouse but not after 10 months (data not shown).

The amount of P and C in leaves increased in plants in the greenhouse, while the amount of N decreased (Table 5,  $P < 0.0001$  in paired *t*-tests). The increased P content and reduced

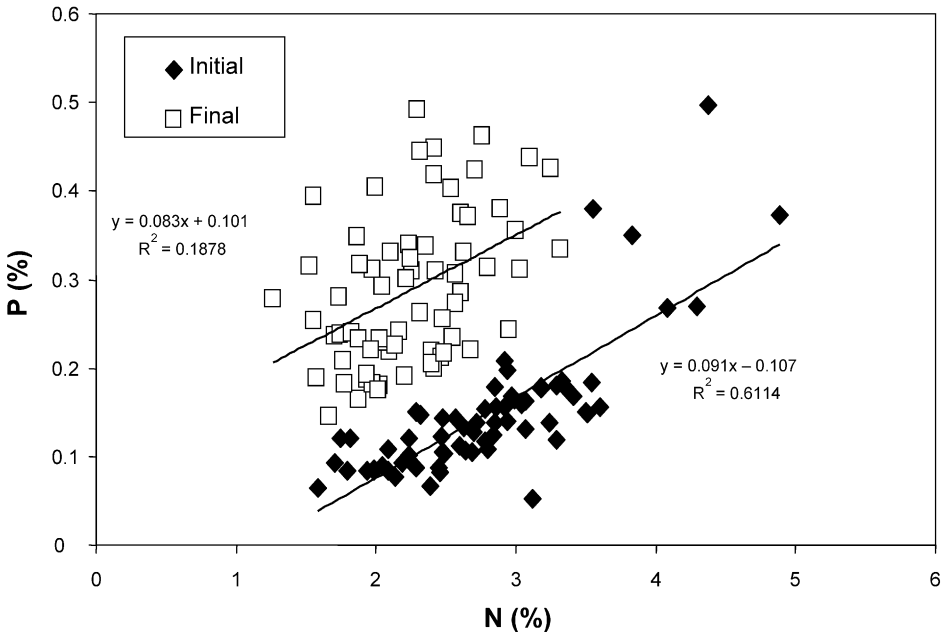


Fig. 5. Regression of %N vs. %P in leaf blades of field-collected plants (initial) and the same plants after 12 months growth in the greenhouse (final). *N* = 69.

N content resulted in a lower N:P ratio for plants after 12 months in the greenhouse (Table 5;  $P < 0.0001$  in paired  $t$ -tests). Changes in N and P content were weakly negatively correlated to initial lamina width, such that leaves that had wide laminae tended to have smaller or negative changes in N and P content than leaves that had narrow laminae ( $R_N^2 = 0.11$ ,  $R_P^2 = 0.18$ ). There was not a significant correlation, however, between N:P, %N, or %P and any of the morphological parameters for the subset of plants on which these were measured (data not shown). Nitrogen content was more strongly correlated to P content in field-collected plants than in the same plants after 12 months in the greenhouse (Fig. 5;  $R^2 = 0.61_{\text{initial}}$  versus  $0.19_{\text{final}}$ ).

#### 4. Discussion

*S. lancifolia* morphology varies widely in the natural environments of southern Florida, from plants that have distinctly laminate-petiolate leaves through plants that have lanceolate laminae to plants with leaves that appear linear (Stober et al., 2001). Our experimental results indicate that this variation can be reproduced on a single plant, i.e., it is a plastic response, and that it can be produced by changes in nutrient levels. The data presented here show that morphology of *S. lancifolia* plants changed both in the experimental P-addition treatment and after growth under uniform conditions in a greenhouse. In the P-addition experiment, leaf blades increased in width, while under common garden conditions, plants either increased or decreased blade width, depending on original blade width.

In the nutrient-addition experiment, the morphological response was to P levels when other nutrients were not limiting. Our data show that the response involved the whole plant, including growth rates, allocation of resources to leaves, stems, roots, and vegetative and sexual reproduction, as well as leaf morphology and tissue nutrient levels. Our results mirror data that have been reported for terrestrial plants, in which reduced biomass and leaf area, as well as lower shoot:root were found under P depletion (Plesnicar et al., 1994; Kondracka and Rychter, 1997).

In the common garden experiment, where we measured only leaf morphology, individual plants adjusted their leaf shape to reflect their recent environment. Thus, plants that initially had broad leaves produced leaves with narrower laminae, while plants with narrow leaves broadened their laminae. Plants that initially had broader laminae generally came from Everglades' sites that had higher soil TP, while plants with narrower laminae came from sites with lower TP (Stober et al., 2001).

Although the response to experimental nutrient addition involved the whole plant, changes in leaf morphology were especially dramatic and were most marked by changes in lamina width—specifically, plants in the high-P treatment had wider laminae than plants in low-P. Thus, blade width can be used as an indicator of high nutrient levels. Our experiment was not designed to indicate whether the increase in blade width was specific to P, i.e., a similar experiment that varied N when P levels were high might produce similar results. In environments such as the Florida Everglades, however, where the limiting nutrient is P (Doren et al., 1996; Noe et al., 2001), wide blades on *S. lancifolia* can be interpreted to indicate that P is not limiting.

Lamina width did not respond homogeneously to P levels—i.e., when both N and P were high, lamina width was not uniformly broad but varied between 18 and 97 mm (Fig. 3B). When P levels in the water were low, however, lamina width was more uniform, varying between 3 and 21 mm (Fig. 3B). Thus, P has to be in sufficient supply for leaf blades to expand, but other factors must also affect leaf blade width. Our results suggest, however, that the importance of genetic versus environmental factors in determining differences in leaf width, used to distinguish *S. lancifolia* subsp. *media* from *S. lancifolia* subsp. *lancifolia* (Haynes and Hellquist, 2000), needs to be investigated. These two subspecies have fairly distinct distributions, with subsp. *media* occurring from coastal Louisiana through Mexico and Central America, while subsp. *lancifolia* primarily occurs east from Louisiana throughout Florida to the Caribbean islands and South America (Haynes and Holm-Nielsen, 1994; Haynes and Hellquist, 2000). If the limiting nutrient is different across these regions, the differences in leaf morphology between these presumed subspecies could reflect environmental differences, rather than subspeciation.

The response to increased P was not simply an increase in lamina size—i.e., leaf blades of nutrient-limited plants were not arrested versions of leaves on plants in the high-P treatment. Our data show that leaf length increased over the course of the experiment in both LP and HP, but blade width did not increase in the low-P treatment, i.e., increase in blade width was uncoupled from leaf length increase. The %N in the leaves of both treatments also increased. Thus, blade widening was specific to increased P rather than a consequence of plant growth alone. In addition, different parts of the leaf responded independently. Leaf base-length and lamina length, lamina width and area increased in HP, while petiole length decreased. We have found similar positive correlations between lamina length and lamina width and soil TP but negative correlations of petiole length and soil TP in analyses of field-collected plants (Stober et al., 2001).

*S. lancifolia* plants grew more slowly in the low-P treatment. This reduction in growth rate reduces the generalized demand for P. *S. lancifolia*'s reduction of blade width under P-deficiency, however, may be a specific response to effects of LP on photosynthesis. The reduction of lamina surface area decreases investment in photosynthetic area that cannot be used because lack of P limits photosynthesis (Foyer and Spencer, 1986; Fredeen et al., 1990; Jacob and Lawlor, 1992; Kondracka and Rychter, 1997). The plant avoids production of photosynthetic biomass and instead increases relative allocation of biomass to roots, thus increasing nutrient up-take capacity.

Changes in leaf shape in response to water depth have been reported for other species of *Sagittaria* (Wooten, 1970, 1986; Kaul, 1991). These changes resemble the changes we found in *S. lancifolia* in response to P limitation. Both seedlings and mature ramets of *S. graminea* produced longer, narrower blades in deeper water in a greenhouse experiment (Wooten, 1970), while *S. brevirostra* and *S. calycina* produced larger, broader blades in wet field environments than in dry environments, but shorter and much narrower, less lobed blades in deep-water field environments (Kaul, 1991). Because water depth can affect plant nutrient uptake in numerous ways (e.g., altered root:shoot ratios, increased root and soil anoxia, altered nutrient cycling in the soil), a nutrient limitation response may contribute to the response to water depth in this genus.

N:P ratios have been used as indicators of nutrient limitations at both the community and species level in a variety of ecosystems (Chambers and Fourqurean, 1991; Fourqurean

et al., 1992; Hogberg and Alexander, 1995; McJannet et al., 1995; Koerselman and Meuleman, 1996; Fourqurean et al., 1997; Bedford et al., 1999). *S. lancifolia*'s foliar N:P ratio indicated that plants were P limited at the beginning of our nutrient experiment (*sensu* Koerselman and Meuleman, 1996). Over the 12 weeks of P addition, foliar N:P changed to ratios indicating that plants were no longer P limited in the high-P treatment and were even more P limited in the low-P treatment.

Lamina width in *S. lancifolia* also provides an indication of nutrient limitation. The plants showed a threshold for morphological response in relation to N:P ratios, i.e., if leaf N:P was below a certain level, indicating that P was not limiting, then the lamina could broaden. If leaf N:P indicated that P was limiting, then the blade did not broaden. In the high-P treatment the N:P ratio converged on 20 with low variation around that number, while blade width varied dramatically. In contrast, in the low-P treatment leaf blades were < 20 mm wide and N:P varied dramatically but was generally > 40 (Fig. 3).

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