



Nutrient Limitation in Two Everglades Tree Species Planted on Constructed Tree Islands

Suresh C. Subedi · Michael S. Ross · Leonard J. Scinto

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Abstract The Everglades is a low-nutrient ecosystem occupied by marsh plant species adapted to low availability of phosphorus. Recently, however, tree islands that are scattered throughout the marsh have been recognized as biogeochemical hotspots. The goal of this study was to determine the general patterns of response by common tree species when conditions limiting to optimal growth were improved by fertilization in an experimentally constructed and managed Everglades wetland. Thirty-six trees of two species, *Annona glabra* and *Chrysobalanus icaco*, were randomly selected on two peat- and two limestone-based islands. Each tree was treated with one of three nutrient regimes: Nitrogen (N), Phosphorus (P), or Control (no addition of nutrients). Positive highly significant P-treatment effects on leaf total P and leaf N:P were observed in both species in comparison to Control trees, but neither species exhibited a similar response to N-fertilization. However, among the two species, only *A. glabra* responded to P-fertilization with increased growth. Both fertilized and unfertilized trees of each species exhibited a highly significant growth response to hydrological condition, with growth enhanced on less persistently flooded sites. Our experimental results identify a clear difference in species growth responses to substrate type in the two species, but do not support the idea that a single critical N:P ratio can be used to indicate nutrient limitation for all wetland trees.

Keywords Everglades tree islands · Fertilization experiment · Nutrient limitation · Leaf N:P · $\delta^{13}\text{C}$ · $\delta^{15}\text{N}$

Introduction

The Everglades is a low-nutrient system, particularly with regard to the availability of phosphorus (Noe et al. 2001; Wetzel et al. 2005). However, within this vast wetland, tree islands have been recognized as biogeochemical hotspots (Wetzel et al. 2005; Ross et al. 2006; Ross and Sah 2010; Espinar et al. 2011). In the heads of the tree islands, under tropical hardwood forest (hammock) cover, soil total phosphorus (P) concentration is extraordinarily high in comparison to the surrounding marsh soils (Wetzel et al. 2008; Ross and Sah 2010). In contrast, soil total nitrogen (N) is reported to be highest in flooded areas and decreases towards the higher elevations (Jayachandran et al. 2004). Several P accumulation mechanisms, including deposition of aerosols, precipitation, movement in groundwater through transpiration stream, deposition of guano by birds and animal feces, creation of middens full of phosphorus-rich bones by pre-Columbian humans, and bedrock mineralization (Ross et al. 2006; Givnish et al. 2008; Graf et al. 2008) have been demonstrated, but their relative importance is currently debated. In contrast, the total nitrogen in tree island soils primarily depends on soil organic matter content and its interaction with hydrology (Jayachandran et al. 2004). Differences in hydroperiod and nutrient accumulation with the elevation in the tree islands may lead to relative N-limitation at upslope positions, and P-limitation downslope.

Biomass production and nutrient dynamics on the tree island may also be influenced by geomorphology, as the interaction of underlying substrate with hydrology is a fundamental driver of variation in nutrient availability among sites. There are two distinct categories of tree islands found in the Everglades based on underlying substratum: those composed entirely of peat and those that developed on shallow soils above limestone bedrock (hereafter referred

S. C. Subedi (✉) · M. S. Ross · L. J. Scinto
Florida International University,
Miami, FL, USA
e-mail: ssube001@fiu.edu

as peat and limestone islands) (Gleason and Stone 1994; Sklar and van der Valk 2002).

Tree islands are one of the many Everglades communities affected by hydrology, which affects biogeochemical cycling and nutrient availability for specific tree species. Here, we recognize background nutrient limitation by an increase in the growth of individual plants when supplied with a limiting nutrient (Koerselman and Meuleman 1996; Tanner et al. 1998; Ferdie and Fourqurean 2004). This type of nutrient limitation is recognized by applying a particular fertilizer and observing whether it significantly increases above-ground biomass and plant tissue concentrations compared with a control (Feller 1995; McKee et al. 2002; Güsewell et al. 2003). Plant response varies depending on the nutrient availability in different soil environments. Plant species generally react to an increase in the supply of the limiting nutrient, but not the non-limiting ones (Chapin 1980; Vitousek et al. 1995; Verhoeven et al. 1996; van Duren and Pegtel 2000; Fisher et al. 2006).

Nitrogen (N) and phosphorus (P) are the most frequent limiting nutrients for plant growth. Under conditions of relatively high P and low N availability, plants are expected to take up more N and less P (Koerselman and Meuleman 1996). In this case, due to the luxury consumption of P, i.e. the absorption of phosphorus in excess of the immediate plant growth requirements, the N:P ratio in plant tissue will be relatively low. In contrast, there will be a higher N:P ratio in plant tissue under conditions of relatively high N supply and low P supply. Thus, each plant species may have a critical N:P ratio that can be used to determine whether growth of the species is N-limited or P-limited in a given environment (Koerselman and Meuleman 1996). Further insight on the physiological controls of growth may be provided by the stable isotopic ratios of plant biomass, especially $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Farquhar 1993; Montoya and McCarthy 1995; Inglett and Reddy 2006). Individuals may vary substantially in isotopic ratio across sites due to variation in physiological fractionation associated with stressful conditions (Lin and Sternberg 1992; Fry et al. 2000). For instance, a pattern of decreasing leaf $\delta^{13}\text{C}$ with increasing tree height, and increasing $\delta^{15}\text{N}$ under N-limited condition have been reported in various studies (Lin and Sternberg 1992; McKee et al. 2002; Inglett and Reddy 2006).

To address the topic of tree island nutrient availability experimentally, we designed an experiment to determine if N or P limitation is responsible for tree growth limitation in two major types of tree island (limestone and peat) and across a hydrological gradient. We specifically tested the following hypotheses/predictions: (i) trees on peat islands will respond most strongly to an experimental increase in the supply of phosphorus, while trees on limestone islands will respond to increase in the supply of nitrogen; and (ii) leaf N:P will decrease with added P at the base of the islands

and increase with added N at the higher elevation of the islands.

Methods

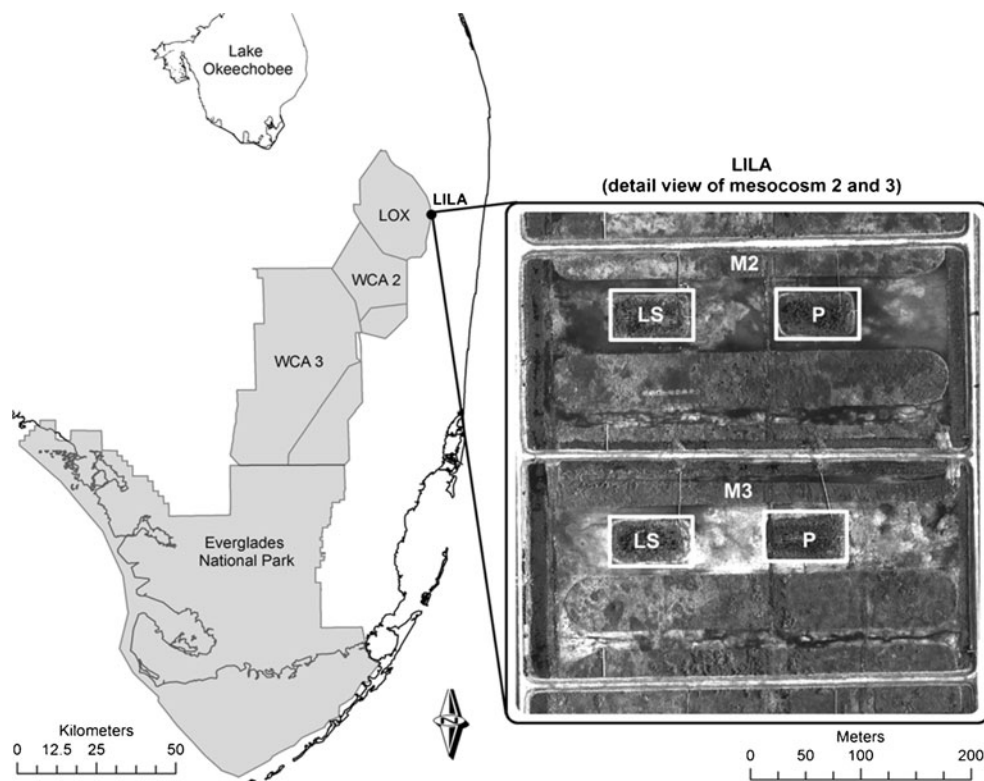
Study Site

The study took place from July 2009 to September 2010 at the Loxahatchee Impoundment Landscape Assessment (LILA) site at the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LOX) in Boynton Beach, Florida. The LILA facility (lat 26 °17.999'N, long 80°13.979'W; WGS 84) was constructed in 2002–2003 through a partnership between the South Florida Water Management District (SFWMD), U. S. Army Corps of Engineers and U.S. Fish and Wildlife Service. It serves as a landscape-scale physical model of the Everglades that allows researchers to conduct experiments in a semi-controlled environment. LILA consists of four identical 8 ha macrocosms (M1–4) (Fig. 1). In each macrocosm, one limestone and one peat island were constructed. Limestone tree islands represent the large islands organized around outcrops in the southern Everglades, whereas peat tree islands represent pop-up islands in the northern Everglades. Maximum elevation of each 73 × 43 m island was 0.90 m above the surrounding slough surface. Peat islands were built entirely from organic surface soil while limestone islands were constructed above a base of locally mined limestone occupying the 49 m × 14 m central portion of the island; the limestone core was covered with 0.3 m of peat soil (van der Valk et al. 2007; Stoffella et al. 2010). Therefore, the fringes of the limestone islands beyond the central region were composed of the same organic soil material as the peat islands. Islands were divided into four quadrants, and in each, seedlings of 10 tree species common to Everglades tree islands were planted at a different density (spacing of 1.0, 1.67, 2.33 and 3.0 m) during March of 2006 and 2007. In each of these density treatments, species were randomly assigned positions to ensure good representation along the hydrologic gradient (Stoffella et al. 2010).

Experimental Design

LILA's M2 and M3 macrocosms provided the setting for the experiment. Eighteen trees of each of two species, *Annona glabra* and *Chrysobalanus icaco*, were selected randomly on each island for a total of 36 trees per island, 72 trees per macrocosm, and 144 trees overall. Each tree received one of three nutrient treatments: Nitrogen (N), Phosphorus (P), or no nutrient enrichment (Control), resulting in six replicates per species-treatment combination per island. To avoid competition among individual trees at this early stage of

Fig. 1 A map showing location of study area with LILA macrocosms (M2 and M3) that mimic the Everglades. Each macrocosm comprises of two tree islands with an underlying geology of either peat (P) or limestone (LS). LOX, WC2 and WC3 refer for Loxahatchee National Wildlife Refuge, water conservation area 2 and 3 respectively



development, all the sample trees were selected from low density plots (spacing of 3 m, 2.33 m and 1.67 m).

A total of 108 trees planted in March 2007 were fertilized with one of the two nutrient enrichments (excluding the 36 Control trees) six times during the year from July 2009 to June 2010. N enrichment was added in the form of urea (45-0-0), and P enrichment was added as orthophosphate, Na_2HPO_4 , (0-45-0). Nutrient enrichments were applied as a solution of dry pellet fertilizer dissolved in water. An allometric biomass equation was used to calculate the total amount of biomass increase expected annually in each species population, based on data from previous years (Stoffella et al. 2010). Background soil nutrient concentrations were determined by collecting soil samples from 0 to 10 cm depth under the eighteen sample trees from each island for each species at the beginning of the experiment. The results showed no significant variation between tree islands for soil TP (mean 0.11 mg g^{-1}); however, mean soil nitrogen was significantly higher in peat islands (12 mg g^{-1}) than limestone islands (7 mg g^{-1}).

Since data on tissue nutrient concentration (mg g^{-1}) was available for each species, we used those data as references to estimate the total amount of nutrient that an individual will accumulate annually for each species. We applied fertilizer at a cumulative rate that would equal three times the amount of N and P an individual tree normally incorporates into live tissue during an annual cycle (about 72 g of N or P per tree). A total

of six doses of N, P or water (Controls) were applied to each tree, at approximately bimonthly intervals. Each P-treated tree received about 500 ml nutrient solution, each N-treated tree received about 300 ml of nutrient solutions and each Control tree received 300 ml of tap water. To apply the nutrient enrichments, two 30 cm deep holes were cored into the substrate within the canopy shadow of each tree. A cap with holes was fixed to the bottom of a 1.3 m long PVC pipe (0.75 inch diameter) to release the nutrient solution into the surrounding substrate. The pipe was inserted into the hole, leaving 1.0 m exposed above the substrate. The top of the pipe was capped after nutrient delivery. Holes were cored and pipes installed for all trees including Control trees following identical procedures to ensure homogeneous experimental conditions.

Total height (HT), crown length (C_L), crown width (C_W), height to crown base (HC), and basal diameter (BD) of each tree were measured at the beginning and end of the experiment. The final measurements were taken 5 months after the last nutrient dose to ensure that plants had enough time to respond to nutrient application. Crown volume (CV) was calculated as $CV = \pi (C_L/2) (C_W/2) (HT - HC)$ (Sah et al. 2004).

Tissue Nutrient Analysis

At the end of the experiment, a sample of 5–7 young, fully expanded leaves were collected from each tree, and brought

immediately to the laboratory for nutrient analysis. We analyzed leaf TN and TP and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ content for all individuals of both species.

Leaf samples were dried at 65 °C until constant weight was reached. Leaves were ground to a fine powder, and 1–2 mg subsamples were combusted in an elemental analyzer (Carlo Erba) coupled to an isotope ratio mass spectrometer (IRMS Delta Plus, Finnigan Mat, San Jose, CA, USA) operating in the continuous flow mode. From these analyses, both isotope ratio ($\delta^{13}\text{C}$; $\delta^{15}\text{N}$) and elemental content (%C; %N) were obtained. Data are expressed in “delta” notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as:

$$\delta = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$
, where R_{sample} and R_{standard} are the ratio $^{13}\text{C}:^{12}\text{C}$ or the ratio $^{15}\text{N}:^{14}\text{N}$ of the sample and standard, respectively, and R_{standard} for carbon and nitrogen are those of PDB (Pee Dee Belemnite) and air, respectively. All results were normalized to mg g^{-1} dry weight concentrations.

Total phosphorus was analyzed colorimetrically according to the standard method for orthophosphate P (EPA method 365.1). After oxidation by dry combustion using MgSO_4 and HCl (Solórzano and Sharp 1980), products were stored at 4 °C and analyzed within 48 h of digestion.

Hydrology

A continuous record of surface water level was available from the LILA facility over the experimental period. Daily surface water level was monitored at the western (intake) and eastern (outflow) ends of each macrocosm. Surface water level at each tree island was estimated from a linear interpolation between water levels at the two ends of the macrocosm.

Tree island elevations were established by 1) surveying with an auto-level (3 mm accuracy) from vertical control benchmarks established by the SFWMD in each macrocosm to a temporary benchmark established in the center of each island, 2) surveying from the temporary benchmark to the base of approximately 150 newly planted trees of known horizontal location, 3) developing a contour plot of elevation from these data through ARC-GIS 9.2, and 4) applying the Spatial Analyst extension in ARC-GIS to determine the elevation of each tree. Relative elevation (RE) of each tree was calculated as the position of each tree above or below the mean tree island surface water level over the experiment. For example, 20 cm RE means the soil surface at the tree base was located 20 cm above mean surface water, while a location at –20 cm RE was 20 cm below mean surface water level and therefore on flooded soil.

Statistical Analysis

The effects of nutrient treatment, substrate, and relative elevation on annual tree growth and leaf chemistry were analyzed for both species. A two-way ANOVA was

performed to examine the effects of nutrient treatment and substrate on leaf chemical composition (TN, TP, N:P, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and growth response for each species. Since, limestone cores were only present in the central region of the islands (49 m × 14 m), only individuals in this region of all the tree islands were considered for the purpose of substrate comparisons.

A linear regression model was used to examine the effects of relative elevation on species growth and the five leaf chemistry variables (TN, TP, N:P, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Since sediments at the fringes of the limestone islands were of peat material, we considered only peat islands in this analysis in order to avoid the potential confounding effects of substrate variation. Results were considered statistically significant at p-values less than 0.05. Prior to using ANOVA, assumptions of normality and homogeneity of variance were tested by the Shapiro-Wilkes and Levene’s tests, respectively. All analyses were done in STATISTICA (Version 7.1, StatSoft Inc., Tulsa, OK, USA).

Results

Effects of Nutrient Treatment and Substrate on Plant Growth

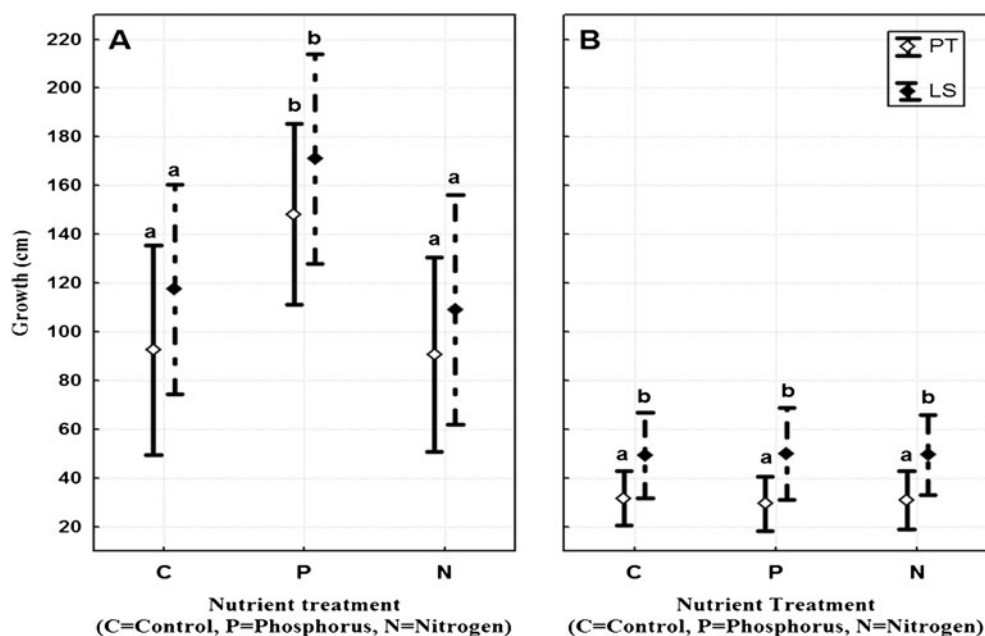
In both species, all measures of growth (change in crown volume, height, or basal diameter) responded similarly to nutrient treatments, therefore only the height growth response is presented. The two species differed in their growth responses to nutrient treatment. *A. glabra* height growth increased significantly in response to P fertilization (Fig. 2, Table 1), but differences in substrate type elicited no response in this species, and no interaction between substrate and nutrient treatment was detected (Table 2). In contrast, *C. icaco* height growth did not respond to nutrient treatment at all but did exhibit a significant response to substrate type; trees grew taller on limestone islands than peat islands regardless of nutrient treatment (Table 1; Fig. 2).

Treatment Effects on Foliar Nutrient Concentration and Leaf N:P Ratio

The effect of nutrient treatment on leaf TN was not significant for either species (Tables 1 and 2). However, a substrate effect on leaf TN was detected in both species. Leaf TN was significantly higher in trees of limestone islands than peat islands; no significant interaction between substrate and nutrient treatment was detected for either species (Table 2).

In both species, leaf TP increased significantly in response to P fertilization but there was almost no change in leaf TP with N-fertilization (Table 1). The effects of substrate on leaf TP and Substrate × Nutrient interaction were

Fig. 2 Multiple comparisons of growth response between nutrient treatments for a) *A. glabra* and b) *C. icaco* in limestone and peat substrates. Each vertical bar represents 95 % confidence interval. Nutrient treatments are C=Control, P=P-treatment, and N=N-treatment. Treatments whose labels include the same letter do not differ ($p < 0.05$)



not significant in *A. glabra*. However, ANOVA indicated a significant Substrate x Nutrient interaction on TP in *C. icaco* (Table 2).

The patterns described above resulted in P-treated trees having significantly lower N:P ratios than those from Control and N-fertilized ones (Table 1, Fig. 3). Furthermore, leaf N:P in N-fertilized and Control trees were almost always observed to be greater than 16, while P-fertilized trees had N:P ratios < 16 in both species (Fig. 3). Substrate effects on leaf N:P were not detected in either species. No interaction (substrate x nutrient treatment) effect on leaf N:P was detected in *A. glabra*, but a significant interaction (substrate x nutrient treatment) was detected in *C. icaco* (Table 2).

The two species differed in how leaf $\delta^{13}\text{C}$ responded to the nutrient treatments. *A. glabra* leaf $\delta^{13}\text{C}$ increased significantly in response to P-fertilization but substrate type had no effect (Table 1). In contrast, neither nutrient treatment nor substrate type affected $\delta^{13}\text{C}$ in *C. icaco*. Interaction effects on leaf $\delta^{13}\text{C}$ were non-significant in both species. Leaf $\delta^{13}\text{C}$ ranged from -29.5 to -26.9 for *A. glabra*, and -31.41 to -28.64 for *C. icaco*. Neither nutrient treatment nor substrate effects on leaf $\delta^{15}\text{N}$ were detected in either species (Tables 1 and 2).

Responses with Nutrient Treatment Along a Hydrological Gradient

The effect of RE on tree growth was found to be similar in both species, and across all nutrient treatments. Growth showed a significant positive response to RE in both unfertilized and fertilized trees of both species (Table 3), as they grew taller with a decrease in flooding duration and depth. Among Control and N-fertilized trees of both species, leaf

N:P was unaffected by RE. In P-fertilized trees, leaf N:P ratio was found to increase significantly with increasing RE in *A. glabra* trees ($R^2 = 0.32$, $p < 0.05$); however, *C. icaco* trees did not show such a relationship (Table 3). In *A. glabra*, $\delta^{13}\text{C}$ was found to increase significantly with increased RE in P-fertilized trees ($R^2 = 0.68$, $p < 0.001$) but not in Control or N-fertilized individuals. In contrast, the effect of RE on leaf $\delta^{13}\text{C}$ was not observed in fertilized or unfertilized *C. icaco* trees ($P > 0.05$). Similarly, a significant positive correlation between leaf $\delta^{15}\text{N}$ and RE was only observed in P-fertilized *A. glabra* trees ($R^2 = 0.52$, $p < 0.001$)

Discussion

Plant Response to Nutrient Treatment

Nutrient limited plants are generally expected to respond to fertilization with increases in growth and/or tissue nutrient concentration (Tanner et al. 1998; Tessier and Raynal 2003). In this field experiment, *A. glabra* responded to P-fertilization with increases in both growth and leaf total P, while *C. icaco* responded only by increasing the concentration of total P, and not by increasing growth. Neither species responded to N-fertilization. In most cases, nutrient-limited growth is associated with low concentrations of the limiting nutrient in leaf tissue (Tanner et al. 1998), both of which rise when limiting nutrient is supplied. For instance, several studies have shown increased leaf P content and enhanced shoot growth in seagrass species in response to P-addition (Perez et al. 1991; Fourqurean and Zieman 1992; Ferdie and Fourqurean 2004). However, enhanced growth and nutrient accumulation are not always linked. Tessier and Raynal

Table 1 Responses of two tree island species (SP) *C. icaco* and *A. glabra* for growth (GR, cm), total phosphorus (TP, mg g⁻¹), total nitrogen (TN, mg g⁻¹), leaf N:P, leaf δ¹³C and leaf δ¹⁵N on peat (PT) and limestone (LS) islands. Values are means and standard deviation in parenthesis. N=12 per nutrient treatment (TR)

SP	GR		TP		TN		N:P		δ ¹³ C		δ ¹⁵ N		
	PT	LS	PT	LS	PT	LS	PT	LS	PT	LS	PT	LS	
<i>A. glabra</i>	C	92.5 ^a (5.8)	117.5 ^a (60.0)	1.18 ^a (0.14)	1.23 ^a (0.17)	22.58 ^a (0.61)	23.34 ^a (0.89)	19.70 ^a (1.75)	18.90 ^a (1.89)	-28.15 ^a (0.61)	-28.36 ^a (0.62)	3.36 ^a (2.76)	3.43 ^a (0.88)
	P	148.2 ^b (20.2)	171.0 ^b (26.1)	2.32 ^b (0.57)	1.87 ^b (0.31)	22.79 ^a (0.59)	24.92 ^a (0.95)	11.21 ^b (2.44)	13.56 ^b (2.13)	-27.51 ^a (0.32)	-27.54 ^a (0.41)	3.22 ^a (1.25)	3.53 ^a (1.41)
	N	50.5 ^a (28.3)	109.0 ^a (18.6)	1.41 ^a (0.28)	1.24 ^a (0.20)	24.02 ^a (0.61)	25.70 ^a (1.03)	18.03 ^a (1.58)	20.69 ^a (3.91)	-27.91 ^a (0.73)	-28.29 ^a (0.47)	5.55 ^a (3.07)	4.57 ^a (2.21)
<i>C. icaco</i>	C	31.6 ^a (14.5)	49.2 ^b (23.2)	0.65 ^a (0.19)	0.54 ^a (0.12)	14.40 ^a (0.91)	13.55 ^a (0.98)	23.30 ^a (4.97)	25.80 ^a (5.37)	-29.57 ^a (0.42)	-29.60 ^a (0.45)	2.35 ^a (1.25)	2.21 ^a (1.35)
	P	29.4 ^a (11.8)	50.0 ^b (32.0)	1.73 ^b (0.11)	1.03 ^b (0.36)	13.31 ^a (1.07)	15.15 ^a (1.07)	11.71 ^b (5.88)	15.69 ^b (2.58)	-29.67 ^a (0.49)	-29.90 ^a (0.49)	1.04 ^a (1.48)	2.19 ^a (1.48)
	N	30.8 ^a (9.3)	49.5 ^b (8.9)	0.59 ^a (0.20)	0.77 ^a (0.19)	13.18 ^a (1.20)	15.25 ^a (0.98)	25.20 ^a (6.57)	20.56 ^a (5.37)	-29.80 ^a (0.55)	-29.55 ^a (0.45)	1.65 ^a (1.66)	1.66 ^a (1.35)

Dissimilar letters signify significant differences (at least $p=0.05$ level) due to nutrient treatment and substrate types

(2003) found that P-addition caused all six of the Catskill forest understory species they tested to increase in tissue P-concentration but only one to increase in biomass.

Similar interspecific variation in response was observed in our study, illustrating that not all tree island species respond to nutrient addition in a similar way within the same time period. It may be possible that slow-growing species like *C. icaco* do not respond to short term fertilization with an increase in growth, in order to accumulate nutrients that will be available during subsequent periods of stress (Chapin 1980; Vitousek et al. 1993; Sterner and Elser 2002). Troxler et al. (2005) reported that *C. icaco* was the most efficient user of N and P relative to other co-occurring species in Everglades tree islands. Therefore, it is likely that the observed difference in the response to fertilization by *A. glabra* and *C. icaco* represent ecologically meaningful differences in their short-term growth strategy. Moreover, significantly higher growth responses to combined effects of N + P treatment have been reported by some authors (see Elser et al. 2007), but we were not able to test this hypothesis because of the constraints on the number and distribution of surviving planted individuals for each species.

Substrate Effect on Nutrient Availability

A substrate effect on growth was detected only in *C. icaco*, in which higher growth was observed on the limestone substrate. Just as moisture stress is one of the major factors that determine the survival and growth of plant species, flooding and the variation in species tolerance may influence community composition and dynamics (Stoffella et al. 2010). *A. glabra* has been consistently ranked among the most flood-tolerant of Everglades tree island species, while *C. icaco* is somewhat less flood tolerant (Gunderson et al. 1988; Armentano et al. 2002; Jones et al. 2006; van der Valk et al. 2007). Since the substrate effect in our experiment could only be tested in the center of the islands and at higher elevations, the lower growth response exhibited by *C. icaco* in peat substrate may be a result of soil saturation in the peat soils. Sullivan et al. (2010) observed that the water table was lower on limestone substrate islands at LILA than on peat islands, and responded more abruptly to groundwater draw-down. In tree islands whose surface is higher than the surrounding landscape, peat substrates often maintain water levels several centimeters above the surrounding surface water due to greater soil water holding capacity of organic-rich soils compared to most mineral soils (Mitsch and Gosselink 2007). Conversely, limestone islands have little capacity to retain water and generally drain rapidly, thereby maintaining aerobic conditions within the rooting zone of the plants (Stoffella et al. 2010).

Drainage conditions also affect nutrient availability. The higher leaf TN observed on limestone islands in both

Table 2 Summary of two-way ANOVAs performed on growth (cm), leaf total nitrogen (TN), leaf total phosphorus (TP), leaf nitrogen phosphorus ratio (N:P), leaf $\delta^{13}\text{C}$ ($\delta^{13}\text{C}$) and leaf $\delta^{15}\text{N}$ ($\delta^{15}\text{N}$) of *A.**glabra* and *C. icaco* trees by Nutrients, Nut (N, P, Control); Substrate, Sub (Peat, Limestone). Values are F-statistics; total sample size (N)=48 trees per species

Species	Source	DF	Growth	TN	TP	N:P	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>A. glabra</i>	Nut	2	5.33**	1.96	21.36***	39.96***	6.21**	2.56
	Sub	1	1.69	4.87*	2.27	3.47	1.41	0.08
	Nut X Sub	2	0.01	0.04	1.39	2.12	0.33	0.33
	Error	43						
<i>C. icaco</i>	Nut	2	0.51	0.17	14.01***	8.85***	0.97	0.54
	Sub	1	9.75**	6.08*	2.54	0.07	0.69	0.55
	Nut X Sub	2	0.19	1.66	3.87*	1.28	0.62	0.61
	Error	43						

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

species indicates that N availability was lower in the peat substrate. Higher water retention in peat islands may periodically create an anoxic or hypoxic environment, reducing mineralization rates of organic nitrogen, and thereby reducing its availability. In contrast, the more mesic conditions that exist on limestone substrate may have accelerated mineralization and nitrification, making N more readily available (Shure et al. 1981).

Critical N:P Ratio and Nutrient Limitation

The nutrient ratios of plant tissue have become widely used as an alternative approach to the analysis of nutrient limitation (Güsewell 2004) that avoids more laborious and time consuming fertilization experiments (Verhoeven et al. 1996; Bedford et al. 1999; Olde Venterink et al. 2001; Güsewell

2004). Koerselman and Meuleman (1996) reviewed data on fertilization studies in a variety of European freshwater wetlands and proposed the following critical N:P mass ratios: below 14 indicated N limitation, above 16 indicated P limitation and between 14 and 16 indicated co-limitation. Güsewell et al. (2003) suggested that biomass N:P ratios do reflect the relative availability of N and P to plants and may indicate the degree of N or P deficiency experienced by a plant population more reliably and economically than fertilization experiments. Bedford et al. (1999) reviewed the extensive literature on nutrient stoichiometry in plant tissues and surface soils to draw conclusions about nutrient limitation in temperate North American wetlands. They found that, on the basis of N:P ratios in live tissues, marshes dominated by vascular herbaceous species were predominantly N limited, while other wetland types and growth forms were P-limited. Güsewell

Fig. 3 Scatterplot showing effect of nutrient treatment on N:P ratios for *A. glabra* (AG) and *C. icaco* (CI). Nutrient treatments are C=Control, P=P-treatment, and N=N-treatment. Broken lines at the center of the graph showing N:P critical ratios i.e. >16 and <14

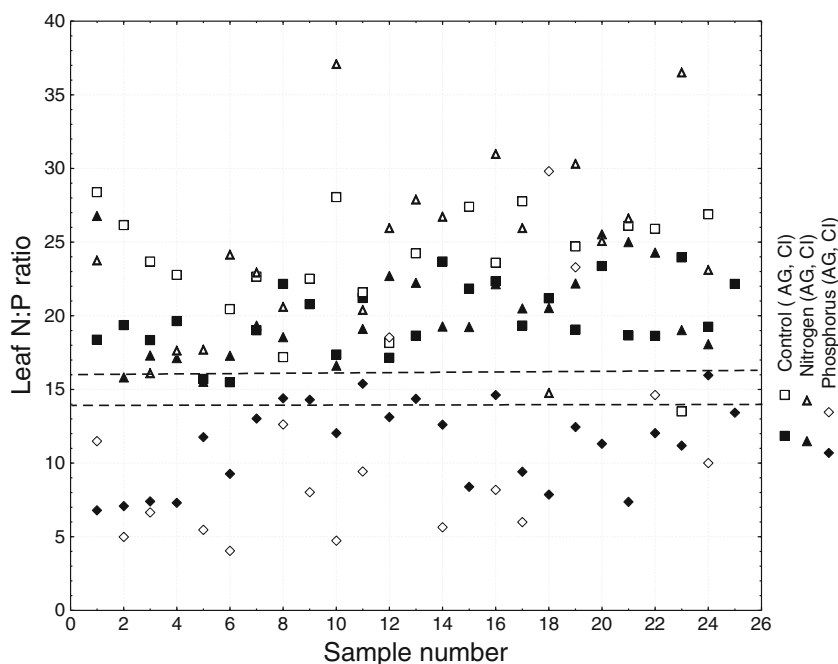


Table 3 Summary of correlation between relative elevation (RE) on growth response, leaf TP, leaf TN leaf $\delta^{15}\text{N}$, leaf $\delta^{13}\text{C}$, N:P of *A. glabra* and *C. icaco* trees by nutrient treatments (Control, Nitrogenand Phosphorous). Values are R^2 and Pearson's correlation coefficient in parenthesis. $N=36$ trees per species

Species	Nutrient treatment	Growth	TN	TP	N:P	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>A. glabra</i>	Control	0.40* (0.63)	0.52** (0.72)	0.13 (0.36)	0.03 (0.01)	0.05 (0.07)	0.10 (0.31)
	Nitrogen	0.53** (0.73)	0.02 (0.15)	0.11 (0.33)	0.22 (-0.47)	0.13 (0.36)	0.24 (0.49)
	Phosphorus	0.62* (0.53)	0.55** (0.74)	0.09 (0.01)	0.32* (0.57)	0.68*** (0.82)	0.52** (0.72)
<i>C. icaco</i>	Control	0.53** (0.73)	0.54* (0.73)	0.16 (0.40)	0.02 (0.15)	0.35 (0.59)	0.14 (-0.12)
	Nitrogen	0.37* (0.61)	0.08 (0.29)	0.21 (0.45)	0.01 (-0.05)	0.02 (-0.17)	0.03 (-0.19)
	Phosphorus	0.86** (0.84)	0.74** (0.86)	0.06 (-0.24)	0.19 (0.44)	0.22 (0.47)	0.01 (0.05)

* $P\leq 0.05$; ** $P\leq 0.01$; *** $P\leq 0.001$

and Koerselman (2002) again reviewed data from field fertilization experiments, and suggested a lowering of the critical N:P ratios for N-limitation to 13:1.

Threshold values given by Koerselman and Meuleman (1996) have been broadly used for various plant growth forms. Since, their critical N:P ratios were based on herbaceous vegetation, the broad applicability of these values has been questioned by some researchers (e.g., Tessier and Raynal 2003; Soudzilovskaia et al. 2005). In order to use this tool for management and monitoring purposes, there is clearly a need for experimental testing to define the critical N:P ratios for tree species. The present study provides valuable information on critical foliar N:P ratio for Everglades tree species on the basis of a fertilization experiments. In both test species, leaf N:P ratio was >16 in Control trees which gives an indication of P limitation on the tree islands they inhabit. Saha et al. (2009) reported high average annual leaf N:P ratios for hammock (45) and pine-land (42) species in the Everglades. Wang et al. (2010) observed that foliar N:P in tree island communities in seasonally flooded prairie averaged 21:1, while foliar N:P ratios in well-drained tree island communities embedded in long hydroperiod marsh averaged 10:1. They concluded that plants in prairie tree islands were P limited, while tree islands in the semi-permanently flooded marsh had an adequate supply of P relative to N.

P-limitation in Control trees was suggested by high N:P ratios (>16) which decreased with P enrichment to below 16, while remaining the same with N-enrichment i.e. N:P >16 . This implies that trees were first limited by P, and when the limiting nutrient was supplied in excess, plants became limited by N. According to von Liebig's law of minimum, individual plant species can be characterized by a fixed order in their nutrient requirements, such that only one nutrient actually limits growth at any one time. If there is a continuing application of the initially-limiting nutrient, the result is that growth limitation eventually switches to another nutrient. However, even though both species do respond to P-addition in terms of leaf N:P ratio, *C. icaco* did not

respond in growth. Since the relationship between nutrient availability and growth is the criterion for evaluating the extent of nutrient limitation (Aerts and Chapin 2000), N:P ratios cannot provide an infallible substitute, as biomass production might also be limited by elements other than N or P (van Duren and Pegtel 2000). It is also likely that leaf N:P ratio is not a strong indicator of growth for plants in high nutrient substrates (McJannet et al. 1995). In plants adapted to phosphorus-poor environments, luxury consumption during periods of high resource availability sometimes lead to the accumulation of inorganic P in vacuoles (Güsewell 2004). Thus, our data do not support the idea that a single N:P can be used to indicate nutrient limitation for all wetland trees.

Nutrient Treatment Effect on Leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Carbon-stable isotopic ratio of plant tissues is often used as an indicator of gas exchange in the leaf, which is largely controlled by the limitation on carbon dioxide uptake via stomatal diffusion (Farquhar et al. 1982; Farquhar and Richards 1984). Generally, stomatal closure has been associated with reduced carbon isotope discrimination in plants exposed to stress (Guy and Wample 1984; McKee et al. 2002). Our results showed a seemingly opposite response: a significant decrease in ^{13}C discrimination in P-fertilized *A. glabra* with increasing in growth response, suggesting a stimulation of physiological processes, including perhaps photosynthesis. Serret et al. (2008) hypothesized that an observed increase in $\delta^{13}\text{C}$ of fertilized plants could result from a decrease in the ratio of intercellular to ambient CO_2 concentrations caused by higher stomatal limitation of photosynthesis, higher carboxylation capacity of photosynthetic tissues or both. Some short term in vitro studies have shown that P limitation can reduce the rate of photosynthesis (Rao and Terry 1995). Inglett and Reddy (2006) reported that leaf $\delta^{13}\text{C}$ and photosynthesis in *Typha* increased with P availability (proximity to delivery points of high P-water). They argued that the co-occurrence of high leaf $\delta^{13}\text{C}$ and elevated photosynthetic rates at a high nutrient site resulted from a

limitation in stomatal conductivity. Similarly, in the present study, trees with greater growth response to P-fertilization also exhibited greater $\delta^{13}\text{C}$ and the less discrimination against ^{13}C with P-treatment. This result was contrary to our initial hypothesis, but could, as in the studies cited above, be the result of greater photosynthesis (greater growth) rate that exceed the limited stomatal capacity in *A. glabra*. It is not surprising to see the non-significant pattern observed in $\delta^{13}\text{C}$ of P-fertilized *C. icaco* trees, as neither growth nor $\delta^{13}\text{C}$ were affected by nutrient treatments. However, direct photosynthetic measurements (CO_2 assimilation and stomatal conductance rates) of trees could readily define whether limited stomatal capacity can represent a bottleneck in *A. glabra* when conditions for photosynthesis are optimal.

In N-limited systems, plant ^{15}N discrimination is negligible because of the high N demand relative to N availability (Evans 2001). In contrast, if N is abundant, higher ^{15}N fractionation is expected as plant demand is low relative to N supply. With P addition and an increase in the availability of phosphorus, plants often increase their $\delta^{15}\text{N}$ as the result of increased N demand and reduced discrimination against the heavier isotope during N uptake (Evans 2001; McKee et al. 2002; Clarkson et al. 2005; Inglett et al. 2007). Instead, our results showed that direct P fertilization to two non-legume tree species elicited no increase in ^{15}N . A likely explanation could be that the amount of nitrogen in the soil was sufficient to balance the added P during the short study duration. However, other processes may affect the foliar $\delta^{15}\text{N}$ signatures of species, such as mycorrhizal status, nutrient partitioning among species and the sources of soil N or P they utilize (Beyschlag et al. 2009).

Nutrient Limitation Along the Hydrological Gradient

Previous studies from tree islands have demonstrated that moisture has a significant effect on nutrient availability (Jayachandran et al. 2004; Ross et al. 2006; Hanan and Ross 2010). Ross et al. (2006) reported a decreasing trend in leaf N:P ratios from flooded marsh through a well-drained hardwood hammock, suggesting phosphorus limitation of growth with increasing flooding frequency in the Everglades. Therefore, our results are not consistent with their study as no significant change in leaf N:P of Control trees was observed along the RE gradient. However, P-treated trees of both species (but not Control and N-treated trees) exhibited a positive correlation between RE and leaf N:P; thus, maximum N:P ratio was found at the highest elevation and the lowest N:P was found at lowest elevation when P-availability was augmented. Since P-treated trees had N:P ratios lower than 16 in both species, trees at lower elevations (flooded zone) are likely to be N-limited after P-addition. This is further supported by the significant higher increase in $\delta^{15}\text{N}$ with the decrease in RE in P-fertilized *A. glabra*.

The higher $\delta^{15}\text{N}$ corresponding with P-fertilization indicates that an increase in N-demand associated with the increase in P availability reduced discrimination against ^{15}N .

Flood-related stress such as anoxia in lower elevation trees can negatively affect plant growth and inhibit nutrient uptake (Lin and Sternberg 2007). The low N:P corresponding to low growth response to P-treatment at lower elevation could be due to flooding stress, which is also reflected in leaf $\delta^{13}\text{C}$ in *A. glabra*.

Why is LILA P-Limited?

Our results suggested that improved P supply was the principal reason for increased growth in LILA tree islands, which is not in general agreement with other tree island studies in the Everglades. Various authors reported much higher soil phosphorous content at the head or center of islands than along their flanks (Ross et al. 2006, Troxler et al. 2005, Wetzel et al. 2009). Ross and Sah (2010) reported mean soil total phosphorus 42.1 mg g^{-1} for 76 tree islands in long-hydroperiod Everglades' marshes. Similarly, Jayachandran et al. (2001) reported up to 30 mg g^{-1} in tree island hammocks. However, the background soil nutrient content clearly shows that LILA tree island soils have very low P content (0.11 mg g^{-1}), which is uncharacteristic of Everglades' tree islands. LILA islands extended well above the marsh surface, but have not been in place long enough to concentrate P to the extent reported of tropical hardwood hammocks in the interior Everglades. In Everglades tree islands, three external sources were hypothesized to contribute to the high P content in soil (Jayachandran et al. 2004; Wetzel et al. 2005; Ross et al. 2006): 1) dissolved P, carried toward the tree islands because of higher evapotranspiration, and sequestered there; 2) animal (especially bird) excreta (Givnish et al. 2008); and 3) Native American inhabitants, through the disposal of bones of fish and other animal food sources that they brought to the islands (Coultas et al. 2008; Graf et al. 2008). Being a very young (~6 years old) with 3 years old trees, all of these mechanisms in LILA tree islands would be at preliminary stages of influence.

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