Functional Ecology 2004
18, 25–33

The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panamá

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Summary

1. Dwarf stands of the mangrove *Rhizophora mangle* L. are extensive in the Caribbean. We fertilized dwarf trees in Almirante Bay, Bocas del Toro Province, north-eastern Panamá with nitrogen (N) and phosphorus (P) to determine (1) if growth limitations are due to nutrient deficiency; and (2) what morphological and/or physiological factors underlie nutrient limitations to growth.

2. Shoot growth was 10-fold when fertilized with P and twofold with N fertilization, indicating that stunted growth of these mangroves is partially due to nutrient deficiency.

3. Growth enhancements caused by N or P enrichment could not be attributed to increases in photosynthesis on a leaf area basis, although photosynthetic nutrient-use efficiency was improved. The most dramatic effect was on stem hydraulic conductance, which was increased sixfold by P and 2·5-fold with N enrichment. Fertilization with P enhanced leaf and stem P concentrations and reduced C : N ratio, but did not alter leaf damage by herbivores.

4. Our findings indicate that addition of N and P significantly alter tree growth and internal nutrient dynamics of mangroves at Bocas del Toro, but also that the magnitude, pattern and mechanisms of change will be differentially affected by each nutrient.

Key-words: Bocas del Toro, fertilization experiment, herbivory, *Rhizophora mangle*

Functional Ecology (2004) **18**, 25–33

Introduction

Mangrove forests are unique ecosystems that occupy the land–sea margin in the tropics and subtropics. They provide a wide range of ecological services that protect the coast from erosion, buffer adjacent marine ecosystems (often coral reefs) from terrestrial inputs, and are nursery grounds for important commercial fish species and habitats for migratory birds (Ewel, Twilley & Ong 1998). Mangrove forests are among the most threatened tropical environments: they are being cleared at an accelerating rate for aquaculture and other human uses, and are being exposed to nutrient enrichment from aquaculture, agriculture and urban development (Valiela, Bowen & York 2001).

On the Caribbean coast of the Republic of Panamá there is an extensive mangrove forest within Almirante Bay and the Chiriquí Lagoon. These two bays encompass a vast network of islands and mainland peninsulas fringed by mangroves, mangrove overwash islands, seagrass beds and patch coral reefs. The mangrove forest is estimated to cover ≈28 km^2 (D'Croz 1993), approximately half the mangrove area on the Caribbean coast of Panamá. The rainfall of the region is high (3–5 m annually), and several large rivers flow into the lagoon contributing sediments from adjacent forested and agricultural lands.

Much of the forest is low-stature, scrub forest which is a common feature of Caribbean mangroves (Cintrón *et al*. 1978; Lugo 1997; Spalding, Balsco & Field 1997). The cause of their small stature (<1·5 m tall) has been a matter of debate (Lugo & Snedaker 1974; Cintrón *et al*. 1978; Lin & Sternberg 1992; Feller 1995). In some forests, particularly where forests are composed of *Avicennia germinans* or *Laguncularia racemosa*, high soil salinity is often the factor correlating with small forest stature (Cintrón *et al*. 1978). In Belizean *Rhizophora*

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forests in oceanic settings with little or no terrigenous input, low phosphorus (P) and nitrogen (N) concentrations limit the growth of trees (Feller 1995; Feller *et al*. 1999; Feller *et al*. 2002; McKee *et al*. 2002). Low P concentrations also limit growth in Florida (Koch 1997). It is not known whether nutrient limitations on growth are widespread in mangrove forests, even in ecosystems contiguous with terrestrial forest and with potentially high levels of terrigenous inputs.

Dwarf *Rhizophora mangle* L. trees growing within 50 m of tropical forest in Almirante Bay, Bocas del Toro, on the Caribbean coast of Panamá, were fertilized with either N or P. Our main objectives were to determine (1) if growth limitations are due to nutrient deficiency and if so, which nutrient (N or P) is the most limiting; (2) what morphological and/or physiological factors underlie nutrient limitations to growth; and (3) how nutrient enrichment might alter forest structure and function. We measured demographic growth responses, photosynthetic gas exchange and hydraulic conductance, leaf tissue nutrient concentrations, and leaf herbivory. The results provide insight into the mechanisms limiting growth of red mangrove and how nutrient enrichment may alter the structure and function of dwarf forests in the extensive mangrove forest of Almirante Bay, Panamá.

Materials and methods

SITE DESCRIPTION

The Bocas del Toro archipelago (9°21′N, 82°15′W) in eastern Panamá is a vast network of islands and mainland peninsulas fringed by mangroves, mangrove overwash islands and patch coral reefs (Guzmán & Guevara 1999). The mangrove forests are dominated by *R. mangle*, growing on peat that is ≈6 m deep above a fossil coral reef (Phillips, Rouse & Bustin 1997). Peat retrieved from 4·75 m depth on the Almirante peninsula was found to be ≈2000 years old (Phillips & Bustin 1996). Surface peat layers (<1 m depth) contain ≈70% organic matter and are composed primarily of refractory mangrove roots (K.L.M., unpublished data). The rainfall is high (3–5 m annually), and the mangroves are adjacent to tropical rainforest. Hurricanes are rare in the area as Bocas de Toro lies outside the hurricane belt, although flooding from heavy rainfall generated by hurricanes passing to the north is common. Earthquakes are episodic (Phillips, Bustin & Lowe 1994; Phillips & Bustin 1996) and are likely to be the major non-human disturbance regime influencing these forests.

The mangrove forest is characterized by a typical tree height gradient across the intertidal zone (I.C.F., C.E.L. and K.L.M., unpublished data from permanent plots). Trees fringing the ocean are larger (≈4 m tall), giving way to vast interior stands of dwarf trees (≤1 m tall). Trees closer to the terrestrial boundary are taller (≈4–12 m in height). A fertilization experiment was established on Isla San Cristóbal in the dwarf forests, where average tree density is ≈1800 stems per 0·01 ha (basal area ≈7 m^2 ha^{-1}, I.C.F., C.E.L. and K.L.M., unpublished data).

Substrates of the site were characterized by using the methods of McKee, Mendelssohn & Hester (1988). The soil was highly organic (68 ± 1% loss on ignition) with a low bulk density (0·15 ± 0·01 g cm^{-3}). Pore-water salinity averaged 31 ± 1 g kg^{-1}, which was slightly higher than that in the seaward fringe (27 ± 1 g kg^{-1}) and the landward transition zone (13 ± 6 g kg^{-1}). The sediment was moderately reducing (E_h range = +6 mV at 1 cm depth to –80 mV at 30 cm depth), and interstitial sulfide concentrations were 0·92 ± 0·17 mM. Pore-water pH was near neutrality (6·66 ± 0·05), and typical of other mangrove forests in the Caribbean region (McKee 1995). Pore-water concentrations of NH_4-N and PO_4-P, respectively, were low, 4·45 ± 1·04 and 6·25 ± 0·33 μM, but were not substantially different from that in the seaward fringe (6·13 ± 3·16 and 8·31 ± 0·72 μM) or landward transition zone (6·21 ± 0·89 and 5·44 ± 0·25 μM). Extractable P in the dwarf stand averaged 30 ± 5 μg g^{-1} (compared to 55 ± 11 in fringe and 22 ± 10 in landward transition), and total P was 332 ± 31 μg g^{-1} (compared to 315 ± 152 in fringe and 349 ± 39 in landward transition). Thus, although there were some differences between the dwarf forest and adjacent stands of taller trees, no single soil factor appeared to be sufficiently extreme to explain the stunted growth.

EXPERIMENTAL DESIGN

Twenty-four trees were randomly selected in an area ≈150 × 100 m in the dwarf zone on Isla San Cristóbal in July 1996, and the following treatments were randomly assigned. Eight trees were fertilized with N as urea (45 : 0 : 0) by coring an 8 cm diameter and 30 cm deep hole in the sediment at two locations either side of single trees, inserting into each hole 150 g urea encased in dialysis tubing (total dose 300 g per tree), and resealing it with the peat core, as described by Feller (1995). Eight trees were fertilized with P using 300 g triple super-phosphate, P_2O_5 (0 : 45 : 0) in dialysis tubing. Eight control trees (C) were similarly cored but not fertilized. These treatments were applied in July 1996, May 1997 and December 1998.

PLANT GROWTH AND HERBIVORY

As a bioassay of the effects of nutrient treatment on plant growth, we monitored the number and length of shoots on five, initially unbranched, shoots (first order) in sunlit positions in the outer part of the canopy of each tree in July 1996, May 1997 and December 1998. To distinguish the growth produced over each interval, we labelled the leaves in the apical position on each of these shoots at each sampling period. Shoot length and number of new leaves were measured from the previously marked apical position to the base of

the current apical bud along the main axis and any shoots. Demographic growth analysis was used to determine effects of nutrient enrichment on plant growth rates (McGraw & Garbutt 1990a; McGraw & Garbutt 1990b; Feller 1995). Demographic absolute growth rates (DAGR) were calculated for monthly increases in shoot length for periods 1 (July 1996–May 1997) and 2 (May 1997–December 1998), using the formula:

$$(\text{shoot length}_{\text{time1}} - \text{shoot length}_{\text{time0}})/(\text{time}_1 - \text{time}_0) = \text{DAGR (cm month}^{-1})$$

Demographic relative growth rates (DRGR) were calculated for monthly rates of leaf production for intervals 1 and 2, using the formula:

$$[\ln(\text{number of leaves})_{\text{time1}} - \ln(\text{number of leaves})_{\text{time0}}]/(\text{time}_1 - \text{time}_0) = \text{DRGR (leaves month}^{-1})$$

Primary consumption of leaf tissue that accrued during the entire life spans of leaves was determined by measuring herbivore damage on 10 senescing leaves harvested from basal stem positions on each of the fertilized trees. Images of individual leaves were recorded with a digital camera (Nikon Coolpix 990, Nikon Corp., Tokyo, Japan). The fraction of leaf area damaged was determined from the digitized images using SIGMASCAN PRO4 (SPSS Science, Chicago, IL, USA).

PHOTOSYNTHESIS AND HYDRAULIC CONDUCTANCE

Photosynthetic gas exchange was measured in December 1998 on a cloudless morning with a portable gas-exchange system (LiCor 6400, LiCor Corp., Lincoln, NE, USA), and the leaves collected afterwards for mineral analysis. Measurements were made on three youngest fully developed leaves (usually the penapical leaf pair) per tree using natural light, under ambient temperature, humidity and CO_2 concentrations. Rates of photosynthetic electron transport were measured with a chlorophyll fluorescence system (mini-PAM, H. Walz, Effecltrich, Germany) on a cloudless day in January 2001. After measurements of photosynthesis, leaves were detached and their area measured (LI 3000 A leaf-area meter attached to LI 3050 transparent belt conveyer, LiCor). Leaves were dried to a constant weight at 70 °C and ground to a fine powder. Concentrations of total carbon (C) and N were determined with a CHN Analyzer (Perkin-Elmer 2400, Perkin Elmer, Norwalk, CT, USA) at the Smithsonian Environmental Research Center, Edgewater, MD. Phosphorus concentration was determined using an inductively coupled plasma spectrophotometer (ICP) by Analytical Services, Pennsylvania State University, State College, PA, USA.

Hydraulic conductivity (k_h) was measured in January 2001 on stem segments harvested from branches of trees directly next to the fertilized tree that had their aerial roots within the fertilized area, and showed a growth response similar to experimental trees. Branches were collected in the morning and immediately transported to the laboratory in closed plastic bags. The proximal 5 cm of the stem was discarded, and re-cut stem segments of about 10 cm length and 1 cm diameter were immersed in distilled water until analysis (up to 2 h later). Measurements of stem hydraulic conductance were made in an apparatus similar to that described by Sperry, Tyree & Donnelly (1988a) configured to accommodate six samples. Stem segments were installed so that the direction of the water flux was opposite to the *in situ* direction of flow. We applied a gravimetric water pressure of about 0·05 MPa for 30 min, and collected the water flowing from each stem segment. The volume or weight of the collected water was then measured in a volumetric cylinder (accuracy ±1 ml), or if the volume was less than 2 ml it was determined gravimetrically on a scale to 0·1 mg. Flux measurements were started immediately after installation of the stem segments. Flow rates generally increased slightly (flushing out of emboli) or decreased ('clogging') between measurement cycles. We therefore carried out at least three consecutive measurements for each segment until the flow rate was constant. For each stem segment, we took the highest measured flow rate for further calculations.

We measured the leaf area downstream of each stem segment, and calculated leaf specific conductivity (k_L) by dividing k_h by the leaf area supplied by the segment. The diameters of both ends of the stem segments were measured with a caliper (to 0·1 mm), and the smaller diameter (which could be the proximal or distal end) was used to calculate specific conductivity (k_S) by dividing k_h by the cross-sectional area of the stem segment. Pressure drop was calculated as the transpiration rate per leaf area (from gas-exchange measurements) divided by leaf specific conductance. After measurements, stems were dried and ground for nutrient analysis.

NUTRIENT CONCENTRATIONS IN LEAVES AND STEMS, AND INTERNAL NUTRIENT CYCLING

Leaves and stems for analyses were harvested in December 1998 (after physiological measurements) and January 1999. By that time all the leaves on the targeted trees had been produced under the influence of the experimental treatment. From a sunlit position in the top of the canopy, we collected fresh, fully mature green leaves (hereafter referred to as green leaves) from a penapical stem position. We also collected fully senescent (yellow) leaves with a well developed abscission layer (hereafter referred to as senescent leaves) from a basal position on first-order branches. Senescent leaves were taken directly from the trees to eliminate nutrient loss via leaching and leaf loss by tidal flushing, which happen when litter drops to the forest floor in this mangrove wetland. We assumed that yellow leaves that could be removed from a stem with only

slight pressure represented the senescent leaf litter. For each leaf, area was determined. Leaf samples were dried at 70 °C in a convection oven and ground in a Wiley Mill to pass through a 40 (0·38 mm) mesh screen. Concentrations of total C, N and mineral nutrients were determined as described above.

Resorption efficiency was calculated for each experimental tree as the percentage of N or P recovered from senescing leaves before leaf fall (Chapin & Van Cleve 1989):

$$\text{Resorption efficiency} = \{[\text{N or P (mg cm}^{-2})_{\text{green leaves}} - \text{N or P (mg cm}^{-2})_{\text{senescent leaves}}]/\text{N or P (mg cm}^{-2})_{\text{green leaves}}\} \times 100$$

DATA ANALYSIS

Data were analysed by ANOVA using the statistical computing packages DATA DESK 6·1 (Data Descriptions, NY, USA) and SYSTAT 8·0 (SPSS Science, Chicago, IL, USA). For analysis of DAGR and DRGR, a repeated-measures analysis was performed using data from both time intervals. Fertilization treatment was considered a fixed effect in the ANOVA models. Levels of leaf damage due to herbivory were arcsine-square root transformed prior to analysis to normalize the variance of the data. Pairwise tests of individual means were carried out using Fisher's least significant difference *post hoc* hypothesis test. Residual plots were inspected to assess the suitability of alternative ANOVA models. Regression of rates of photosynthetic C gain over variation in leaf temperature and stomatal conductance were performed in SIGMAPLOT 6·1 (SPSS Science) using an exponential function.

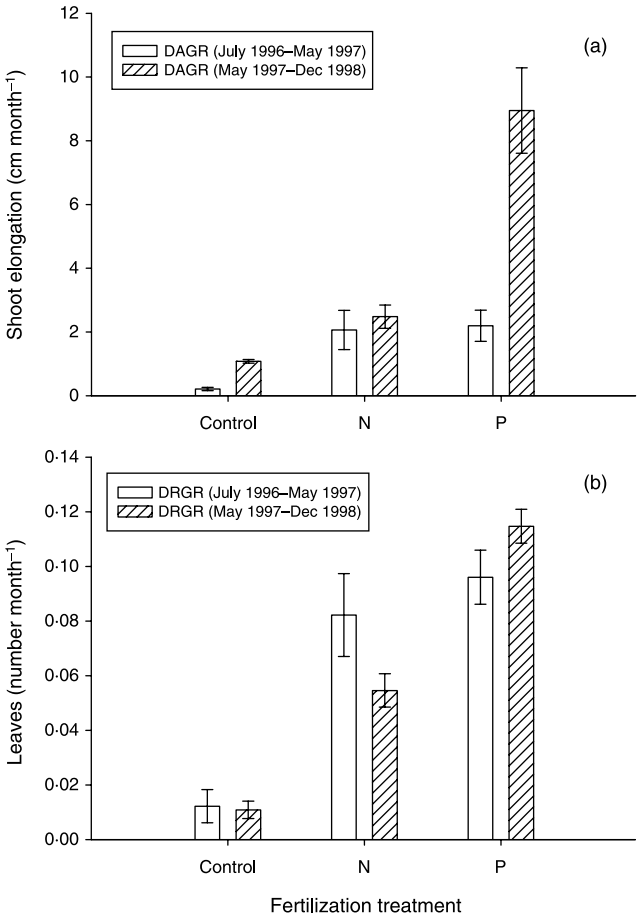

Fig. 1. Effects of fertilization with N or P on rates of shoot elongation (DAGR, a) and number of new shoots initiated (DRGR, b) in a dwarf stand of *Rhizophora mangle* mangroves growing in peat substrate in Bocas del Toro, Republic of Panamá. Growth rates were measured over two intervals: July 1996–May 1997, directly after the fertilization treatments were applied (open bars); and May 1997–December 1998 (hatched bars).

Results

PLANT GROWTH

During the first 10 months after fertilization, shoot elongation (Fig. 1a) and changes in the number of shoots (Fig. 1b) showed significant and similar increases when trees were fertilized with N or P. Shoot growth rates were generally greater in the second interval (18 months later) compared to the first interval (main effect of time, $F_{1,18} = 66{\cdot}64$, $P < 0{\cdot}0001$). During the second measurement interval, shoot elongation and numbers of shoots were enhanced in trees fertilized with N, but even more so in trees fertilized with P (time–treatment interaction, $F_{2,18} = 35{\cdot}76$, $P < 0{\cdot}0001$) where rates were close to 10-fold greater than those observed in the controls.

INTERNAL NUTRIENT CYCLING AND HERBIVORY

Fertilization with P enhanced leaf P concentrations on a dry mass basis (Table 1, $F_{1,18} = 4{\cdot}771$, $P < 0{\cdot}022$), while reducing leaf N concentrations ($F_{1,18} = 8{\cdot}369$, $P < 0{\cdot}003$). Fertilization with N did not lead to significant enhancements in N concentrations per mass of leaf tissues, or changes in C : N or N : P ratios, but instead reduced the concentration of P within leaves on a dry mass (Table 1) and leaf area basis (Table 2). Fertilization with P resulted in increases in C : N ratio and declines in N : P ratio within leaf tissue (Table 1). Concentrations of N and P in stem tissues showed similar changes to those in leaf tissues with fertilization (Table 3). Fertilization with P doubled P concentrations in stems compared to controls, and resulted in a large reduction in the N : P ratio of tissue. Fertilization with N slightly enhanced N concentrations and the N : P ratio of the tissue compared to controls.

Despite significant differences in leaf tissue quality, the fraction of leaf area removed by primary consumption was not significantly affected by fertilization treatments (Table 1). Total leaf lifetime damage was ≈10% of total leaf area. Percentage leaf damage was weakly but significantly ($F_{1,18} = 5{\cdot}053$, $P < 0{\cdot}038$) positively correlated with the C : N ratio of leaf tissue ($R^2 = 0{\cdot}24$).

In control leaves, ≈50% of leaf N and 80% of leaf P was resorbed by the plant before leaf senescence (Table 1). Resorption efficiency during senescence was not significantly influenced by fertilization with N. In contrast, fertilization with P reduced the resorption of P to 65%.

Table 1. Leaf nutrient concentrations, nutrient resorption efficiency and proportion of leaves damaged by herbivory for *Rhizophora mangle* growing under different fertilization treatments

			Treatment		
Variable	*F*	*P*	Control	Nitrogen	Phosphorus
Nitrogen (mg g^{-1})	8·369	0·0030	$12·1 \pm 0·03^{a}$	$12·9 \pm 0·03^{a}$	$11·0 \pm 0·4^{b}$
Phosphorus (mg g^{-1})	4·771	0·0220	$0·70 \pm 0·05^{ab}$	$0·58 \pm 0·02^{a}$	$0·76 \pm 0·05^{b}$
Nitrogen : phosphorus	12·34	<0·0001	$17·6 \pm 1·0^{a}$	$22·5 \pm 1·3^{a}$	$14·8 \pm 1·0^{b}$
Carbon : nitrogen	10·95	0·0010	$40·0 \pm 0·9^{a}$	$38·2 \pm 0·5^{a}$	$44·8 \pm 1·4^{b}$
N resorption efficiency (%)		NS	$49·5 \pm 1·8^{a}$	$44·5 \pm 2·3^{a}$	$54·5 \pm 3·0^{a}$
P resorption efficiency (%)	7·239	0·0050	$81·8 \pm 1·4^{a}$	$81·5 \pm 1·3^{a}$	$65·4 \pm 5·1^{b}$
Fraction of leaf area damaged		NS	$0·11 \pm 0·01^{a}$	$0·11 \pm 0·02^{a}$	$0·14 \pm 0·02^{a}$

F value is the ANOVA test for treatment differences. Values with the same superscript do not differ in pairwise tests at $P = 0·05$. All data were log-transformed before analysis. NS, treatment effect not significant.

Table 2. Leaf gas-exchange characteristics and nutrient concentrations for *Rhizophora mangle* growing under different fertilization treatments

			Treatment		
Variable	*F*	*P*	Control	Nitrogen	Phosphorus
Photosynthetic carbon gain (μmol m^{-2} s^{-1})	1·04	NS	$10·1 \pm 0·5^{a}$	$10·3 \pm 0·2^{a}$	$10·9 \pm 0·5^{a}$
Photosynthetic electron transport (μmol m^{-2} s^{-1})	2·17	NS	$88·2 \pm 7·7^{a}$	$80·1 \pm 8·2^{a}$	$96·0 \pm 8·0^{a}$
Transpiration (mmol m^{-2} s^{-1})	2·69	NS	$2·48 \pm 0·16^{a}$	$2·49 \pm 0·06^{a}$	$2·92 \pm 0·20^{a}$
Stomatal conductance (mmol m^{-2} s^{-1})	3·66	0·0479	$0·106 \pm 0·008^{a}$	$0·104 \pm 0·003^{a}$	$0·131 \pm 0·010^{b}$
Specific leaf area (cm^2 g^{-1})	6·74	0·0065	$46·5 \pm 0·8^{a}$	$45·2 \pm 0·5^{a}$	$50·2 \pm 0·7^{b}$
Nitrogen (g m^{-2})	14·34	0·0002	$2·39 \pm 0·06^{a}$	$2·47 \pm 0·06^{a}$	$1·94 \pm 0·05^{b}$
Phosphorus (g m^{-2})	8·37	0·0027	$0·132 \pm 0·004^{ab}$	$0·117 \pm 0·002^{a}$	$0·150 \pm 0·004^{b}$
Photosynthetic nitrogen-use efficiency (μmol g^{-1} s^{-1})	16·81	<0·0001	$4·31 \pm 0·12^{a}$	$4·22 \pm 0·15^{a}$	$5·77 \pm 0·20^{b}$
Photosynthetic phosphorus-use efficiency (μmol g^{-1} s^{-1})	7·31	0·0047	$78·1 \pm 2·0^{a}$	$88·7 \pm 2·3^{b}$	$74·7 \pm 3·0^{a}$

F value is the ANOVA test for treatment differences. Values with the same superscript do not differ in pairwise tests at $P = 0·05$. All data were log-transformed before analysis. NS, treatment effect not significant.

Table 3. Hydraulic conductance and nutrient concentration characteristics of stems of *Rhizophora mangle* growing under different fertilization treatments

			Treatment		
Variable	*F*	*P*	Control	Nitrogen	Phosphorus
Nodes per stem length (nodes m^{-1})	583·22	<0·0001	267 ± 5^{a}	260 ± 8^{a}	20 ± 2^{b}
Leaf area per stem (cm^2)	4·950	0·0194	477 ± 94^{a}	527 ± 82^{a}	885 ± 113^{b}
Stem conductivity, k_h ($\times 10^{-6}$ kg m^{-1} s^{-1} MPa^{-1})	18·664	<0·0001	$7·2 \pm 2·2^{a}$	$17·2 \pm 4·5^{b}$	$44·5 \pm 9·6^{c}$
Leaf specific conductivity, k_L ($\times 10^{-4}$ kg m^{-1} s^{-1} MPa^{-1})	12·943	0·0004	$1·6 \pm 0·3^{a}$	$3·0 \pm 0·4^{b}$	$4·7 \pm 0·7^{b}$
k_h/stem cross-section area (kg m^{-1} s^{-1} MPa^{-1})	29·186	<0·0001	$0·11 \pm 0·03^{a}$	$0·27 \pm 0·07^{b}$	$0·90 \pm 0·17^{c}$
Pressure drop (MPa m^{-1})	8·166	0·0035	$0·20 \pm 0·06^{a}$	$0·09 \pm 0·01^{b}$	$0·07 \pm 0·01^{b}$
Nitrogen (mg g^{-1})	3·707	0·0461	$4·91 \pm 0·23^{ab}$	$5·41 \pm 0·41^{a}$	$3·88 \pm 0·53^{b}$
Phosphorus (mg g^{-1})	53·293	<0·0001	$0·236 \pm 0·010^{a}$	$0·214 \pm 0·010^{a}$	$0·520 \pm 0·050^{b}$
Nitrogen : phosphorus	43·91	<0·0001	$20·3 \pm 1·3^{a}$	$25·3 \pm 1·6^{b}$	$6·8 \pm 0·8^{c}$

F value is the ANOVA test for treatment differences. Values with the same superscript do not differ in pairwise tests at $P = 0·05$. All data were log-transformed before analysis.

PHOTOSYNTHESIS AND HYDRAULIC CONDUCTANCE

In order to understand the physiological processes underlying enhanced growth in fertilized trees, we assessed photosynthetic gas exchange and hydraulic conductivity of stems. Fertilization with N and P did not significantly enhance rates of photosynthetic carbon gain (Table 2). Photosynthetic electron transport was not enhanced significantly by fertilization with P (Table 2). Rates of photosynthetic carbon gain were weakly correlated with leaf P concentrations ($R^2 = 0·311$, $P = 0·004$; data not shown), but were not correlated with leaf N concentrations. Photosynthetic rates declined with increasing leaf temperature over the morning of measurement (Fig. 2a, $R^2 = 0·16$, $P =$

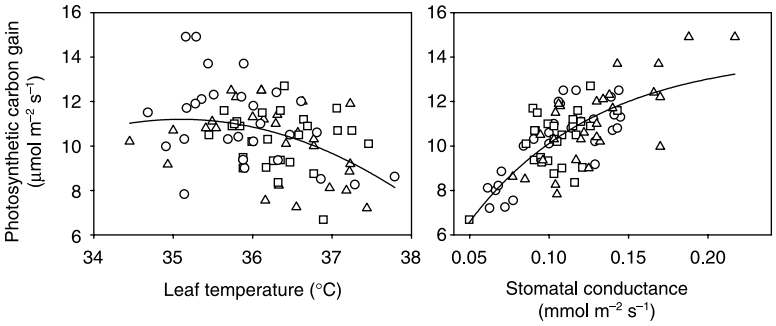

Fig. 2. Relationship between photosynthetic carbon gain and leaf temperature (a) and stomatal conductance (b) in a dwarf stand of *Rhizophora mangle* mangroves growing in peat substrate in Bocas del Toro, Republic of Panamá. Trees were fertilized with phosphorus (△); nitrogen (□); or were not fertilized (○).

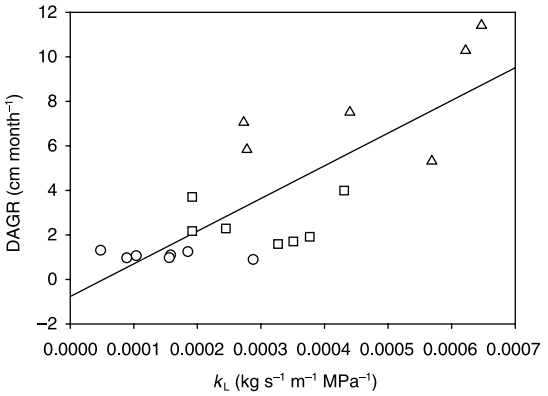

Fig. 3. Relationship between the hydraulic conductivity of stems expressed per unit leaf area (k_L) and shoot elongation rate (DAGR) measured between May 1997 and December 1998 in a dwarf stand of *Rhizophora mangle* mangroves growing in peat substrate in Bocas del Toro, Republic of Panamá. Trees were fertilized with phosphorus (△); nitrogen (□); or were not fertilized (○). Regression: DAGR = −0·768 + 14 686 × k_L, R^2 = 0·60.

0·0007). Photosynthetic rates were correlated with stomatal conductance (Fig. 2b, R^2 = 0·53, P < 0·0001).

Fertilization with P did increase mean stomatal conductance, and specific leaf area (SLA), and altered nutrient-use efficiencies of photosynthetic C gain (Table 2). Fertilization with P enhanced photosynthetic nitrogen-use efficiency, while N additions enhanced photosynthetic phosphorus-use efficiency (Table 2).

Hydraulic conductance of stems was greatly affected by fertilization (Table 3). Patterns of fertilizer enhancement of hydraulic conductance closely matched those observed for plant growth (Fig. 3). Fertilization with P decreased the number of nodes per stem length by an order of magnitude. Stem conductivity was increased sixfold when fertilized with P, and 2·5 times when fertilized with N. Specific leaf conductivity (k_L, where stem conductance is corrected for enhanced leaf area of fertilized plants) of P-fertilized trees was three times greater than of controls, while fertilization with N doubled leaf specific conductance.

Discussion

NUTRIENT LIMITATIONS TO GROWTH

Growth enhancement of trees fertilized with N and P supports the hypothesis that the low stature of a large proportion of the extensive scrub mangrove forests of Bocas del Toro is, at least partly, due to nutrient limitations on growth. Responses to P fertilization were particularly strong, and similar to those observed in dwarf mangroves in the oceanic setting of Belize (Feller 1995; Feller *et al*. 1999; Feller *et al*. 2002; McKee *et al*. 2002).

Low external availability of nutrients cannot fully explain the stunted growth of mangroves in this ecosystem because nutrient availability (as indicated by pore-water N and P concentrations and extractable P) in adjacent and other more productive stands is equal or lower. This observation may indicate that nutrients are limiting because of interaction with another factor present in the dwarf habitat that affects nutrient uptake or plant nutrient demand (McKee *et al*. 2002), as has been suggested for salt marshes (Mendelssohn 1979). Internal requirements for N and P may differ depending on which stress factors (e.g. anoxia, or high concentrations of hydrogen sulfide) are interacting with low nutrient availability. Experimental manipulation of other environmental stressors, in addition to low nutrient availability, are required to understand the underlying mechanisms for growth limitations at this site.

Differences in response of trees to fertilization over the two measurement intervals were similar to those observed in dwarf mangroves fertilized in Belize (Feller 1995). Growth was initially enhanced by N and P, then later spectacularly by P (Fig. 1; the small enhancement due to N fertilization persisted). This finding suggests that the limitations to growth imposed by N deficiency can almost immediately be overcome, while those due to P limitation are more severe. The slowness of the response to P compared to N could be due to: (1) uptake rates are slow due to relative immobility of phosphate in soil/sediments compared to ammonium (Nye & Tinker 1977) and the necessity that tree roots grow into the fertilizer patch to increase their P uptake (McKee 2001); (2) replacement of tissues constructed under previously limiting nutrient conditions is necessary to increase growth (Wilson 2000); (3) threshold concentrations of P have to be achieved before growth enhancements can be fully expressed; and/or (4) greater internal requirement for P *vs* N relative to external availability (McKee *et al*. 2002).

Additions of N and P influenced tree growth in different ways. Fertilization with N resulted in enhanced production of new leaves, while fertilization with P increased the number of leaves and the rate of shoot elongation. High rates of shoot elongation were correlated with decreases in numbers of nodes and increases in hydraulic conductivity of stems (Fig. 3). The high variation in hydraulic conductance of stems were in the range of those measured for *R. mangle* by Sperry,

Tyree & Donnelly (1988b) and Melcher *et al*. (2001), and reflect the highly variable xylem anatomy of this species (Sun & Suzuki 2001). These data suggest that both N and P availability limit the production and/or activity of meristems, while the large number of nodes per length in control plants compared to P-fertilized plants (Table 3) suggests that availability of P strongly limits cell expansion rates (Grossman & Takahashi 2001), which probably affects the length and width of xylem vessels and the porosity of their pit membranes, properties that determine their hydraulic conductance (Tyree & Ewers 1996; Comstock & Sperry 2000).

While nutrient limitations on meristem activity have been observed in many other plant species (Wilson 2000; Bonser & Aarssen 2001), enhanced stem elongation with P additions is less commonly reported (but cf Feller 1995; Feller *et al*. 2003). The mechanism by which P deficiencies could reduce cell elongation may be partially through reductions in the number and function of water channels (aquaporins) within the root plasma membrane. Reduced aquaporin function with nutrient deficiencies has been reported in agricultural species (Carvajal, Cooke & Clarkson 1996; Clarkson *et al*. 2000). Thus a low capacity for water transport in dwarf trees could possibly result in insufficient water uptake and a reduction in turgor pressure that is required for cell expansion.

Leaf area removed by herbivores is a significant cost that can result in reduced growth and fitness (Coley & Barone 1996). Leaf area removed by herbivores in Bocas del Toro was low (≈10%) compared to damage caused by wood-boring insects that remove whole branches (Feller 1995), and similar to that observed in other studies in *Rhizophora* mangroves (Farnsworth & Ellison 1991; Feller 1995). Herbivory was not significantly affected by nutrient additions (Table 1) as might be expected with significant increases in C : N ratio of leaf tissue (Bryant *et al*. 1983). Increased C : N ratio (from 33 to 40) also did not affect percentage leaf area damaged (Feller 1995), perhaps suggesting the major leaf herbivores of *R. mangle* in this region are insensitive to C : N ratio over this range. On a whole plant level, however, higher growth rates and leaf area of fertilized plants resulted in greater resource availability for herbivores, and an increase in the total amount of leaf area removed by consumers in fertilized trees. Similar proportional levels of leaf herbivory in fertilized and control trees suggest that nutrient enrichment will not enhance the impact of herbivores on tree growth.

PHOTOSYNTHESIS AND ALLOCATION PATTERNS

Enhancements in growth caused by N and particularly by P fertilization were not associated with enhanced rates of photosynthetic C gain on a leaf area basis (Table 2), as has been observed in other studies (Chalmers 1979; Seeman & Sharkey 1986; Evans 1989; Reich, Walters & Tabone 1989; Dai & Wiegert 1997; Poorter & Evans 1998), although there was a slight positive relationship between leaf P and photosynthesis. Instead, growth was stimulated by allocation of C and nutrients to increased production of leaf area and stem tissue. Low correlations among leaf N concentrations and photosynthetic rates have been observed in desert and arctic species (Lajtha & Whitford 1989; Baddeley, Woodin & Alexander 1994), and in other mangrove species (Lovelock & Feller 2003). Weak relationships among rates of photosynthesis and leaf nutrient concentrations may occur where species have evolved under conditions where nutrient investment in the photosynthetic apparatus is constrained by some other, more important, environmental pressure (Chapin 1991). For mangrove species, the selective pressures resulting in the evolution of low investment in the photosynthetic apparatus may be limited availability of water due to high substrate salinities and anoxia (Ball 1996).

Use of nutrients within the photosynthetic apparatus was significantly influenced by fertilization (Table 2). Fertilization with N increased photosynthetic phosphorus-use efficiency, while fertilization with P increased photosynthetic nitrogen-use efficiency. These complicated patterns reveal that leaf-level photosynthesis is probably co-limited by N and P – by adding N, P can be more fully utilized; and likewise, when P availability is increased, N can be more efficiently utilized. These complex patterns of nutrient utilization when trees are released from N or P limitation are likely to reflect shifting balances among metabolite pool sizes (Grossman & Takahashi 2001). The observation that photosynthetic C gain on a leaf area basis is held relatively constant over a wide range of nutrient availability at this site, even with changes in the efficiency with which nutrients are utilized, supports the hypothesis that maximum rates of photosynthesis are constrained in response to some other environmental pressure (Ball 1996). Rates of photosynthetic C gain observed were similar to those measured in *R. mangle* at other sites (Lin & Sternberg 1992; Lin & Sternberg 1993; Ellison & Farnsworth 1997; Pezeshki, Delaune & Meeder 1997; Sobrado 2000), and lead us to conclude that leaf-level traits are relatively conservative in this species over a wide range of environmental conditions. In contrast, allocation of biomass to different organs and stem elongation is extremely plastic.

Enhanced growth due to changes in resource allocation – essentially due to development of more shoots and greater leaf area, rather than through increased productivity of individual leaves – has been observed after fertilization in other woody species (Lajtha & Whitford 1989; Baddeley, Woodin & Alexander 1994), including mangroves (McKee 1995). In this study the only photosynthetic characteristic significantly altered by fertilization was stomatal conductance (although photosynthetic C gain was significantly correlated with stomatal conductance; Fig. 2), which was higher in P-fertilized trees. Higher stomatal conductance may be

due to higher stem hydraulic conductivity (Brodribb & Field 2000; Nadini & Salleo 2000), and could possibly contribute to enhanced C gain in fertilized plants early in the day before stomatal conductance and hydraulic conductance becomes limiting. Cloudless skies and high temperatures on the day when photosynthetic gas exchange was measured may have obscured the effects of the fertilizer treatment on photosynthetic gas exchange.

The efficiency with which nutrients were resorbed from leaves before senescence was also affected by P fertilization, but not by additions of N (Table 1), further supporting our conclusion that these dwarf mangroves are limited by P. In P-fertilized plants, the amount of P resorbed was reduced from 81 to 65% of green leaf values. This reduction in the efficiency of internal nutrient recycling has been observed in other mangrove studies (Feller *et al*. 1999; Feller *et al*. 2003). High concentrations of P in leaf litter could enhance rates of litter decomposition and reduce nutrient retention within mangroves on a whole-stand level if leaf litter and dissolved P are exported on outgoing tides (Feller *et al*. 2003).

CONCLUSIONS AND IMPLICATIONS

We conclude that the primary nutrient limiting growth of the dwarf scrub mangroves of Bocas del Toro is P, but that growth is also limited to a lesser extent by N. Enhancements in stem elongation and hydraulic conductance with P fertilization possibly indicate that the mechanism by which P fertilization enhances growth is through improved water uptake and transport. Our findings indicate that addition of P and N will significantly increase tree growth and reduce the efficiency of internal nutrient use and conservation; but the magnitude, pattern and mechanisms of change are differentially affected by each nutrient.

Acknowledgements

We thank the Smithsonian Tropical Research Institute (STRI) for support, and the staff of the Bocas del Toro Field Station. Partial support was provided by NSF award DEB 9981535. Any use of trade, product, or firm names is for descriptive purposes only and does not constitute endorsement by the US Government.

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Received 23 February 2003; revised 22 August 2003; accepted 9 September 2003