

Linking physiological processes with mangrove forest structure: phosphorus deficiency limits canopy development, hydraulic conductivity and photosynthetic carbon gain in dwarf *Rhizophora mangle*

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ABSTRACT

Spatial gradients in mangrove tree height in barrier islands of Belize are associated with nutrient deficiency and sustained flooding in the absence of a salinity gradient. While nutrient deficiency is likely to affect many parameters, here we show that addition of phosphorus (P) to dwarf mangroves stimulated increases in diameters of xylem vessels, area of conductive xylem tissue and leaf area index (LAI) of the canopy. These changes in structure were consistent with related changes in function, as addition of P also increased hydraulic conductivity (K_s), stomatal conductance and photosynthetic assimilation rates to the same levels measured in taller trees fringing the seaward margin of the mangrove. Increased xylem vessel size and corresponding enhancements in stem hydraulic conductivity in P fertilized dwarf trees came at the cost of enhanced mid-day loss of hydraulic conductivity and was associated with decreased assimilation rates in the afternoon. Analysis of trait plasticity identifies hydraulic properties of trees as more plastic than those of leaf structural and physiological characteristics, implying that hydraulic properties are key in controlling growth in mangroves. Alleviation of P deficiency, which released trees from hydraulic limitations, reduced the structural and functional distinctions between dwarf and taller fringing tree forms of *Rhizophora mangle*.

Key-words: Belize; fertilization; leaf area index (LAI); photosynthesis; xylem.

INTRODUCTION

Spatial variation in tree height and productivity in mangrove forests are usually associated with complex abiotic

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gradients; salinity, flooding, redox potentials and nutrient availability contributing to observed patterns in forest structure (Lugo & Snedaker 1974; McKee 1993). In Belize, Feller *et al.* (2002) reported a switch from nitrogen (N) limitation to growth of trees in stands (5–6 m height) fringing the water's edge to phosphorus (P) limitation in interior dwarf forests (≤ 1.5 m tall). Some of the hypotheses that have been proposed for this change from N to P limitation are: (1) higher external supply of N relative to P in dwarf forests compared to taller fringe forests; (2) increased waterlogging or other abiotic conditions resulting in soil nutrient transformations that reduce the availability of P relative to N in the dwarf forests; and (3) non-resource factors affecting plant nutrient uptake, assimilation or translocation in the dwarf relative to the fringing forests.

One of the hypotheses put forward to explain the particularly strong growth response to P in the dwarf trees (an approximate 10-fold increase in stem elongation) is that demand for P relative to N increases due to the effects of continuous inundation and sediment anoxia differentially influencing plant requirements for these nutrients (Feller *et al.* 2002; McKee *et al.* 2002). While there are small differences in soil salinity between fringe and dwarf forests (35 g L⁻¹ in the fringe and 40 g L⁻¹ in dwarf forests), the dwarf forests in Belize are permanently flooded, having sediments with lower redox potentials than those in the taller fringing forests (Feller *et al.* 2002; McKee *et al.* 2002). Low oxidation status of roots, coupled with low P status of sediments, may significantly restrict water uptake by roots and water transport to shoots (Carvajal, Cooke & Clarkson 1996). In the dwarf mangroves in Belize, reduction in the capacity to acquire and conduct water could lead to decreased rates of stomatal conductance and photosynthetic carbon gain, with associated nega-

tive impact on growth and canopy development (Tyree & Ewers 1996).

We previously observed an increase in hydraulic conductivity, stem extension and leaf growth when dwarf mangroves were fertilized in Panamá (Lovelock *et al.* 2004). Our data suggested that under some environmental conditions, possibly those of extreme nutrient limitation, addition of nutrients can enhance water supply to leaves and hydraulic conductivity possibly by enhancing root growth and/or improving some aspects of the water conducting pathway. In the current study, the hypothesis that hydraulic conductivity and size of xylem vessels are enhanced in response to fertilization in a highly P limited setting was tested in *Rhizophora mangle* forest at Twin Cays, Belize. This forest has been exposed to 8 years of biannual fertilization with N and P. Our results were also contrasted with those from adjacent plants in taller fringing forests that were not P limited. In addition, because we also expect reductions in the relative allocation of roots to leaves with nutrient enrichment (Givnish 1986), we test the hypothesis that nutrient enrichment results in greater vulnerability to cavitation and loss of hydraulic conductivity when evaporative demand is high (Ewers, Oren & Sperry 2000).

Hydraulic conductivity and vulnerability to cavitation of woody species are known to vary strongly with environmental conditions (e.g. Tyree, Velez & Dalling 1998; Ewers *et al.* 2000; Van der Willigen *et al.* 2000; Cavender-Bares & Holbrook 2001). Not all studies of variation in hydraulic conductivity with nutrient availability support the hypothesis that reduced relative allocation to roots under high nutrient conditions results in increased susceptibility to cavitation. Pine grown with low nutrient availability had both reduced specific hydraulic conductivity and increased vulnerability to xylem cavitation with declining water availability compared to plants grown in high nutrient availability (Ewers *et al.* 2000). In hybrid poplar, enhanced nutrient availability did not influence hydraulic conductivity of stems or xylem anatomy. However, N and P affected vulnerability to cavitation differently, with N increasing susceptibility, and P decreasing susceptibility (Harvey & Van der Driessche 1997). In the study of Harvey & Van der Driessche (1997), enhancement in vulnerability to cavitation in N fertilized treatment was associated with greater leaf area relative to root mass, while improvements with P were thought to be due to changes in xylem pit membranes. The dwarf mangrove trees in the current study are subject to high levels of solar radiation, low sediment redox potentials and moderate salinity, conditions that may make them susceptible to midday xylem cavitation and associated reductions in stomatal conductance and photosynthetic carbon gain (Tyree & Sperry 1989; Hubbard, Yoder & Ryan 1999; Brodribb *et al.* 2003). We expected that fertilization of dwarf trees with P would enhance hydraulic conductivity, but that fertilized trees would also be more susceptible to losses in hydraulic conductivity than unfertilized trees during periods of high evaporative demand.

METHODS

Site description

The study was conducted at Twin Cays, a 92 ha archipelago of mangrove islands located approximately 1.6 km inside the Belizean Barrier Reef Complex (see Feller 1995 and McKee 1993 for a full site description). The islands are composed of peat that rests on a Pleistocene coral reef platform. They are situated approximately 12 km from the mainland and do not receive terrigenous inputs of freshwater or sediments. Twin Cays is dominated by *Rhizophora mangle* L. (red mangrove) and *Avicennia germinans* (L.) Stearn. (black mangrove), with scattered *Laguncularia racemosa* (L.) Gaertn. f. (white mangrove).

There is a distinctive tree height gradient from a narrow fringe zone of tall trees (4–7 m in height) that occur low in the intertidal zone around the margins of the islands, decreasing to a dwarf zone composed of trees ≤ 1.5 m in height in the island interior. Soil surface elevation also varies along this height gradient, rising to a maximum in the transition zone (i.e. the zone intermediate between fringe and dwarf) and decreasing to a minimum in the dwarf zone (Feller *et al.* 2002; McKee *et al.* 2002). Flooding intensity follows the variation in elevation. The fringe zone is flooded and drained > 700 times per year while the dwarf zone is perennially flooded, except during unusually low tides. Sediment characteristics are described in detail in Feller *et al.* (2002). Salinity is relatively uniform over the sites and ranges from 35 to 37 g L⁻¹ in the fringe and 37–40 g L⁻¹ in the dwarf. Sediment redox potentials are higher in the fringe (Eh +50 to +150 mV) than the dwarf (–100 to +100 mV). Sulfide concentrations are relatively low and range from 0.1 mM in the fringe to 0.3 mM in the dwarf zone. Both fringe and dwarf zones are dominated by *R. mangle*.

Experimental design

Three transects 10 m apart were established at three different locations (total = 9 transects) at Twin Cays in January 1995 (see McKee *et al.* 2002 and Feller *et al.* 2002 for a full description). The transects were orientated perpendicular to the shoreline and traversed the tree-height gradient from shoreline to island interior. The transects were designated as either control, N-fertilized or P-fertilized in each site. They were then subdivided into three zones based on tree height, and three experimental trees per treatment were selected within each zone for a total of nine trees across each transect and 81 trees overall. *R. mangle* trees were each fertilized first in January 1995 and then at 6-month intervals with 300 g of N fertilizer as urea (45:0:0) or P fertilizer as P₂O₅ (0:45:0) as described in Feller (1995).

Forest structure

Variation in forest structure was measured in January 2003 by assessing the leaf area index (LAI) using a gap fraction method and by measuring tree heights. A hemispherical

photo was taken with a Nikon Coolpix digital camera (model 995, Nikon, Tokyo, Japan) fitted with fisheye lens under the canopy of each tree in the experiment. Images were processed using the computer program Hemiview Canopy Analysis Software (version 2.1, Delta-T Devices Ltd, Cambridge, United Kingdom). Tree heights were measured with a telescoping pole.

Hydraulic conductivity and anatomy

Hydraulic conductivity was measured on stem segments from each of the dwarf and fringe control and P-fertilized experimental trees in December 2002. N-fertilized trees were also sampled from the fringe zone but not from the dwarf zone because of the limited plant material available. The transition zone was omitted because of time restriction. Branches of each experimental tree, cut at least 0.5 m from the target stem segment, were harvested early on the morning of measurement. Stems were re-cut to 10 cm length under water prior to measurements and were trimmed with a razor blade. The length of 10 cm was chosen because it was the maximum length of unbranched stem segment available across all treatments that directly supported the terminal rosette of leaves. Longer segments were available in the fringing trees but not in the dwarfs. Measures of hydraulic conductivity can be influenced by the presence of open vessels, that is, those exceeding the stem segment length. The maximum vessel length reported for *R. mangle* is 35 cm, but 92% of the vessels were shorter than 10 cm (Sperry, Tyree & Donnelly 1988). Thus, the 10 cm stem lengths used in our study may lead to an overestimation of hydraulic conductivity, but were a compromise in order to measure hydraulic conductivity in stems of similar branching order with similar leaf areas distal to the measured stem segment.

Native specific K_s ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was measured using a flow meter that measures the pressure drop across the stem segment relative to that across a capillary tube of known resistance (Brodribb & Feild 2000). We used calibrated tubes of differing resistance so as to match the resistance of the tube to that of the branch to minimize errors. Stems were inserted in-line and allowed to equilibrate to steady state (approximately 5 to 10 min) before measurement. The pressure applied to the stem varied between 2.2 and 2.7 kPa, and the pressure drop across the stem segment ranged between 0 and 0.16 kPa (mean 0.065 kPa). After the conductivity of the stem segment was measured, xylem vessels were dyed using 0.1% basic fuchsin dye drawn gently into the stem segment by suction, which aided in identification of functional sapwood for calculation of K_s . Variation in hydraulic anatomy of stem segments was assessed by measuring diameters of approximately 50–100 xylem vessels in a randomly chosen patch of xylem tissue at $\times 100$ magnification from each stem section.

To test for midday xylem cavitation in dwarf trees and the effects of P fertilization on daily variation in native hydraulic conductivity, measurements were made on an additional set of branches from control and P-fertilized dwarf trees at all sites. These branches were cut under water

in the field at 1300 h over two sunny days, and transported in darkness to the laboratory (approximately 20 min).

Photosynthetic gas exchange and leaf water potentials

Rates of photosynthetic gas exchange were measured with a Li-Cor 6400 photosynthesis measuring system (Li-Cor Corp., Lincoln, NE, USA) in February 2001. Photosynthesis was measured on sunny days with little or no cloud cover under natural light. Light levels were generally saturating for photosynthesis (greater than $800 \mu\text{mol m}^{-2} \text{s}^{-1}$), thus photosynthetic rates were assumed to be close to maximal. Measurements were made on the youngest, fully expanded leaves that were exposed to full sunlight at the time of measurement. After each measurement was completed (usually in approximately 1 min), the leaf was harvested. Leaf area was measured using a Li-Cor leaf area meter (Li-Cor Corp.). The harvested leaves were then dried in an oven at 60°C and later weighed. Dried leaf material was ground to a fine powder in a small mill. Nitrogen concentration within the leaves was analysed in a CHN analyser (Perkin Elmer, Norwalk, CT, USA) using a small subsample of the ground leaf tissue. Leaf P concentrations were determined using Inductively Coupled Plasma Mass Spectrometer analysis at the Agricultural Testing Laboratory of Pennsylvania State University.

Leaf water potential of leaf discs were made in January 2004 using leaves of similar age and exposure as those used for photosynthesis measurements. Water potentials were measured psychrometrically on leaf discs (5.6 mm diameter) obtained with Peltier-type leaf-cutter psychrometers (Merrill Instruments, Logan, UT, USA). The psychrometers were temperature equilibrated in an insulated water bath for 3–4 h prior to measurement with an automated multichannel microvoltmeter (Model CR7, Campbell Scientific, Logan, UT, USA). The psychrometers were calibrated with NaCl solution, and were capable of measurements down to -7.5 MPa . Leaf discs were collected in the morning when photosynthetic rates were high (between 0830 and 1030 h local time), and in the afternoon (1300–1400 h local time) when photosynthetic rates were declining.

Data analysis

We used a 3×3 factorial (nutrient treatment \times zone) analysis of variance (ANOVA), blocked at three sites to test for differences in plant response. Where trees in the transition zone were not examined, we used a 3×2 factorial ANOVA. For stem hydraulic conductivity data, N-treated plants were not available in the dwarf zone. Where this occurred, we conducted separate ANOVAs using a 2×2 factorial for a comparison of P fertilization across zones, and one-way ANOVA to test for fertilization effects (control, N and P) within the fringe. When an ANOVA found a significant main effect or interaction between nutrient treatment and zone, we used Fisher's Least Significant Difference post hoc

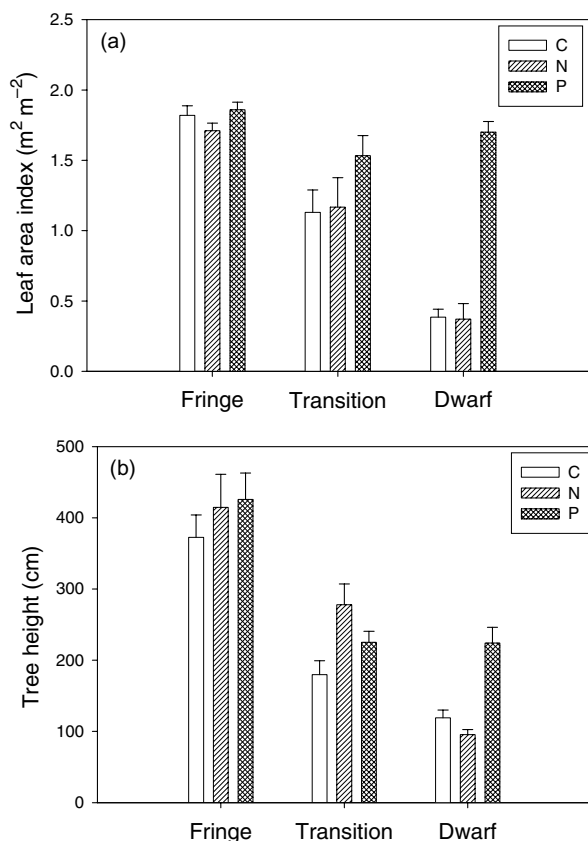


Figure 1. Leaf area index (a) and tree height (b) of mangrove trees across the forest tree height gradient at Twin Cays, Belize. The tree height gradient extends from forest fringing the ocean (Fringe), through the transitional forest (Transition), and into the central dwarf stand (Dwarf). Trees were either fertilized with nitrogen (N) or phosphorus (P), or were unfertilized controls (C). Data are means \pm standard errors.

hypothesis test to examine pairwise differences within and among the treatment levels. To analyse for heteroscedasticity, probability plots of all variables and residual plots were examined. For heterogeneous variances, we transformed continuous data using logarithms.

RESULTS

Canopy structure

LAI of control and N-fertilized treatments was higher in the fringe than in the transition and dwarf zones (Fig. 1). After 8 years of fertilization, P-fertilized transition and dwarf trees had equivalent LAI to fringe trees (zone \times treatment $F_{4,53} = 3.93$, $P = 0.0182$). Tree height of dwarf trees was also increased with P fertilization, doubling in 8 years (zone \times treatment $F_{4,54} = 11.01$, $P = 0.0024$).

Hydraulic conductivity and anatomy

Native Hydraulic conductivity from control plants, expressed either on a xylem area or leaf area basis (Fig. 2a &

b, respectively), was similar in fringe and dwarf trees. Fertilization with P significantly increased K_s in the dwarf trees ($F_{1,24} = 10.775$, $P = 0.0111$). Hydraulic conductivity normalized for leaf area (K_{leaf}) showed similar trends with fertilization in the dwarf trees, but data were more variable and differences between treatments were not significant. Fertilizer had no significant effect on hydraulic conductivity in fringe trees.

In control dwarf trees, K_s of stems harvested at midday was similar to those harvested in the morning (Fig. 3a). In contrast, K_s of P-fertilized trees was significantly lower at midday than in the morning (time \times treatment, $F_{1,24} = 14.93$, $P = 0.0048$). Leaf water potential of control dwarf trees tended to be lower in the morning compared to P-fertilized trees, but this difference was not significant (Fig. 3b, time \times treatment, $F_{1,22} = 3.59$, $P = 0.198$). In the afternoon, control and P-fertilized trees had similar leaf water potential.

Mean diameters of xylem vessels were enhanced with P fertilization in both the fringe and the dwarf trees (Table 1, treatment effect, $F_{2,4} = 26.44$, $P = 0.004$ and $F_{2,4} = 9.17$, $P = 0.032$, respectively), but the density of xylem vessels were not significantly affected. Frequency distributions of xylem vessel diameters were shifted to

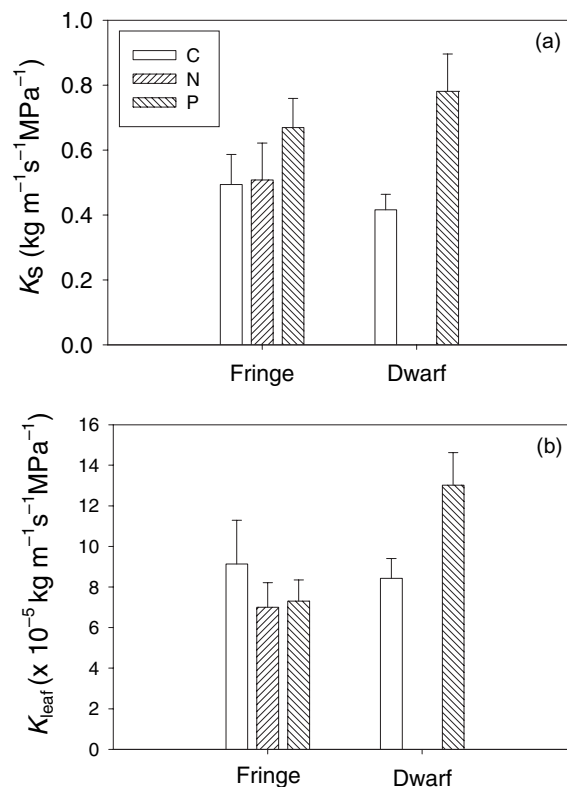


Figure 2. Hydraulic conductivity (K_s , kg m⁻¹ s⁻¹ MPa⁻¹, a) and hydraulic conductivity adjusted for leaf area (K_{leaf} , b) of stem segments of *Rhizophora mangle* in trees fringing the ocean (Fringe) and in dwarf trees (Dwarf). Trees were either fertilized with nitrogen (N) or phosphorus (P), or were unfertilized controls (C). Data are means \pm standard errors.

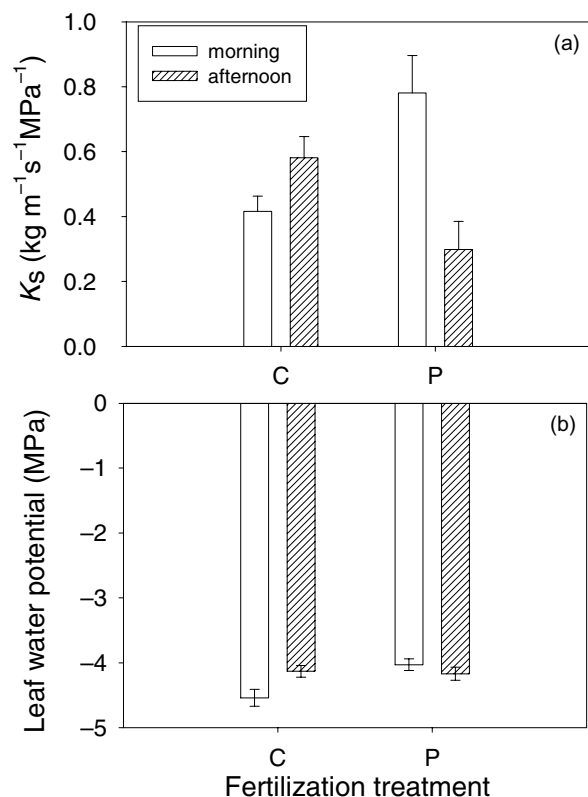


Figure 3. Hydraulic conductivity of stems (K_s , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$, a) and leaf water potential (b) of dwarf trees of *Rhizophora mangle*. Stems and leaf discs for water potential measurements were from control trees (C) and phosphorus-fertilized trees (P) harvested in the morning and in the afternoon. Data are means \pm standard errors.

higher size classes in P-fertilized trees (Fig. 4a & b, $\chi^2 = 175.1$, $P < 0.0001$). Additionally, in the fringe trees, fertilization with N significantly increased the fraction of vessels with larger diameters (Fig. 4a, $\chi^2 = 18.82$, $P = 0.0426$). When xylem diameters were weighted to reflect the enhanced flow rates possible with larger diameter xylem vessels (Lewis & Boose 1995), P fertilization resulted in a 27% enhancement in hydraulically weighted xylem vessel diameter ($F_{2,4} = 69.26$, $P = 0.008$, Table 1).

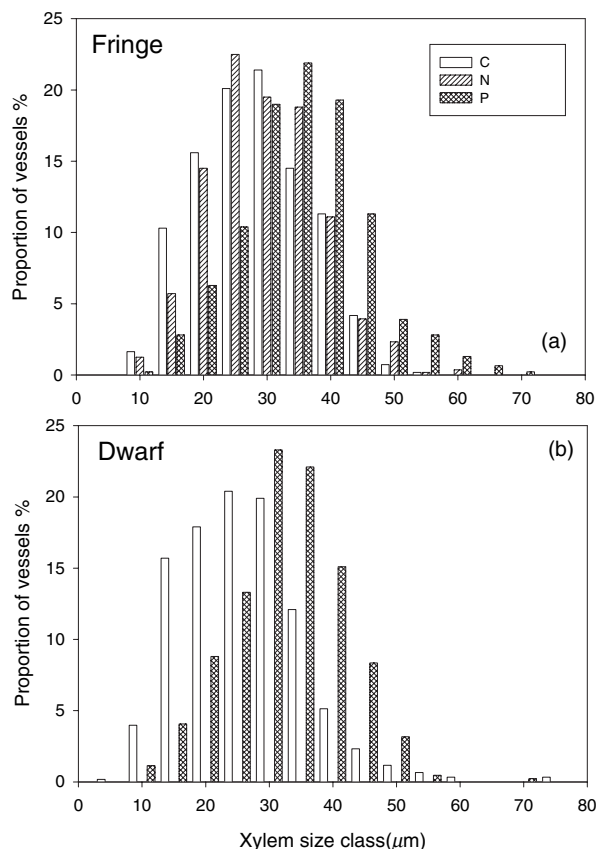


Figure 4. Frequency distribution of xylem vessel diameter in Fringe (a) and Dwarf (b) trees of *Rhizophora mangle* that were fertilized with nitrogen (N) or phosphorus (P), or were unfertilized controls (C).

The leaf area of rosettes supported by the conductive tissue of the sampled stem segments was significantly greater in P-fertilized trees compared to control trees in both the dwarf and the fringe ($F_{2,4} = 22.37$, $P = 0.042$, Table 1). The Huber Value, which is the ratio of the cross-sectional area of xylem to the leaf area supplied by the xylem (Tyree & Ewers 1996), was not significantly altered by fertilization or between the fringe and dwarf trees (Table 1).

Table 1. Summary of hydraulic architectural characteristics of dwarf and taller fringe trees of *Rhizophora mangle*

| | Dwarf | | | Fringe | | |
|-----------------------------------------------------------|------------------------------|---|------------------------------|------------------------------|------------------------------|------------------------------|
| | Control | N | P | Control | N | P |
| Mean xylem diameter (μm) | 23.9 \pm 2.0 ^a | – | 31.1 \pm 1.4 ^{bc} | 26.3 \pm 1.3 ^{ac} | 27.1 \pm 1.0 ^{ac} | 32.7 \pm 0.9 ^{bc} |
| Hydraulically weighted average diameter (μm) | 30.0 \pm 1.4 ^a | – | 38.0 \pm 1.3 ^{bc} | 34.3 \pm 1.6 ^b | 35.0 \pm 1.4 ^b | 41.9 \pm 1.2 ^c |
| Density of xylem vessels (mm^{-2}) | 257 \pm 49 ^a | – | 213 \pm 24 ^a | 236 \pm 25 ^a | 197 \pm 11 ^a | 178 \pm 16 ^a |
| Huber Value ($\times 10^{-5}$) | 1.03 \pm 0.15 ^a | – | 0.79 \pm 0.10 ^a | 0.87 \pm 0.11 ^a | 1.04 \pm 0.14 ^a | 0.98 \pm 0.15 ^a |
| Leaf area per rosette (cm^2) | 290 \pm 31 ^a | – | 336 \pm 35 ^b | 303 \pm 26 ^a | 358 \pm 32 ^b | 388 \pm 31 ^c |
| No. nodes (m^{-1}) | 243 \pm 18 ^a | – | 71 \pm 17 ^b | 113 \pm 30 ^b | 124 \pm 27 ^b | 94 \pm 24 ^b |

Hydraulically weighted xylem diameter is calculated as $(\sum r^5) / (\sum r^4)$, where r is the xylem vessel radius. Data are means \pm standard errors.

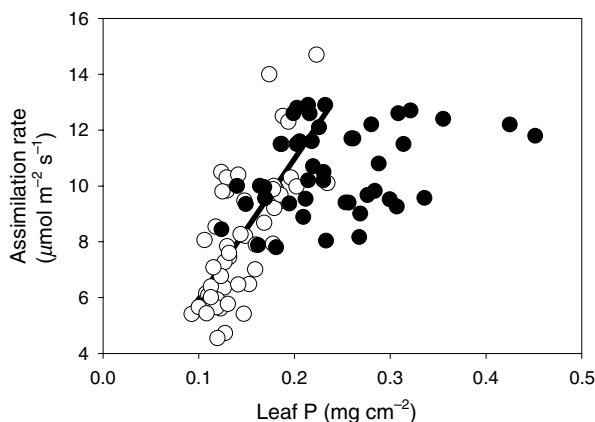


Figure 5. The relationship between photosynthetic carbon assimilation and leaf phosphorus (P) concentration in dwarf (open circles) and fringe (closed circles) trees of *Rhizophora mangle*. The line is the line of best fit for dwarf trees ($y = 50.3x + 0.92$, $r^2 = 0.51$).

Photosynthetic gas exchange

In the control trees, rates of photosynthetic CO_2 assimilation were greater in the fringe than in the dwarf (main effect of zone $F_{1,24} = 23.94$, $P = 0.0018$). In the fringe trees, assimilation rates were not significantly affected by fertilization. In dwarf trees, assimilation rates were significantly enhanced by P fertilization (zone \times treatment effect $F_{2,24} = 8.71$, $P = 0.0126$). Within the dwarf zone, assimilation rates were significantly correlated with leaf P concentration (Fig. 5, dwarf $r^2 = 0.51$, $P < 0.0001$) and stomatal conductance (see Cheeseman & Lovelock 2004) while in the fringe, assimilation was only weakly correlated with leaf P (Fig. 5, Fringe $r^2 = 0.10$, $P < 0.038$).

At one of the sites, the Lair Channel, we assessed diurnal variation in CO_2 assimilation rates in the dwarf trees (Fig. 6). Assimilation rates were highest in the morning, and declined over time, such that by the end of the day, there was no significant difference between control and P fertilized trees.

DISCUSSION

Spatial gradients in tree height in barrier islands of Belize, associated with nutrient deficiency in the absence of a strong salinity gradient (Feller *et al.* 2002; McKee *et al.* 2002), are consistent with the findings of Boto & Wellington (1983) that certain nutrients limited the growth of a mangrove forest in Queensland, Australia, and those of Lovelock *et al.* (2004), who observed the same for mangrove forests in Panamá. While P deficiency is likely to affect many parameters (Grossman & Takahashi 2001), here we show that addition of P to dwarf mangroves stimulated increases in diameters of xylem vessels, leaf area of terminal branches and tree height, and through increased branching (i.e. the number of new shoots initiated per shoot per time interval was > fourfold in P fertilized plants com-

pared with controls, Feller *et al.* 2002) resulted in fourfold increase in the LAI. These changes in structure were consistent with related changes in function as addition of P fertilizer also increased hydraulic conductivity of terminal stems, stomatal conductance and photosynthetic assimilation rates to the same levels as measured in fringe trees. Although we did not measure sapflow in the main stem of the trees, we anticipated that this would scale with enhancements of leaf area, as has been observed in other tree species (Hubbard *et al.* 2004). Overall, the evidence presented suggests that alleviation of P deficiency reduces structural and functional distinctions between dwarf and fringe tree forms of *R. mangle*.

A similar increase in hydraulic conductivity of terminal shoots was also observed in P-fertilized dwarf *R. mangle* trees in Panamá (Lovelock *et al.* 2004). However, absolute increases in hydraulic conductance in Panamá were greater than those observed in Belize. For example, K_{leaf} in Panamá increased from 16 to $47 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$, while in the present study, K_{leaf} increased from 8.4 to $13.0 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ when trees were fertilized with P. Additionally, although K_{leaf} was enhanced in P-fertilized plants in Panamá, there was no observable correlated enhancement in rates of photosynthetic carbon gain, and new shoot and leaf initiation was lower compared to the current study of trees in Belize. While we cannot be certain which factors differing among the two sites could contribute to the observed discrepancies in hydraulic conductivity and photosynthetic processes and their response to nutrient addition, it is known that sediment sulfide concentrations can strongly influence stomatal conductance, photosynthesis and growth of mangroves (Youseff & Saenger 1998), possibly through the inhibition of root metabolic processes (Koch, Mendelssohn & McKee 1990). Sediment sulfide concentrations in Belize are low compared to those at the Panamá site (i.e. 0.3 mM in Belize compared to 0.9 mM in Panamá, Feller *et al.* 2002; Lovelock *et al.* 2004). Thus, it is possible that differences in sulfide concentrations between the sites are

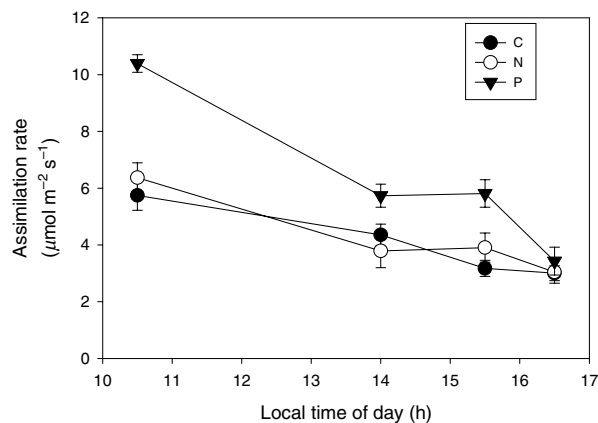


Figure 6. Variation in photosynthetic carbon assimilation over a day in dwarf trees of *Rhizophora mangle* that were either fertilized with nitrogen (N, open circles) or phosphorus (P, closed triangles), or were unfertilized controls (C, closed circles).

responsible for the observed differences in hydraulic conductivity, photosynthetic processes and growth.

Comparison of dwarf and fringe trees

Large differences in LAI between dwarf and fringe forests (approximately sixfold) and differences in growth rates (see Feller *et al.* 2002) were associated with a range of differences in physiology and hydraulic properties of the trees. For example, rates of photosynthetic carbon gain, stomatal conductance (Fig. 5, Cheeseman & Lovelock 2004), lengths of nodes (Table 1) and frequency of larger sized xylem vessels (Fig. 4) are significantly different between control fringe and dwarf trees.

Mean xylem vessel diameter tended to be higher in the fringe compared to the dwarf control trees (Table 1), but this was not significant. Although hydraulically weighted xylem diameters of control fringe trees were significantly greater than control dwarf trees, we did not detect enhancements in hydraulic conductivity in control fringe trees compared to dwarfs (Fig. 2), as might be expected from differences in tree stature and growth rates. Explanations for this similarity may lie in the larger variation in the light conditions experienced by fringe trees compared to dwarfs, which is caused by the complex topography of the canopy of fringe trees and the much sparser canopy of the dwarfs. Differences in light environments experienced by fringe and dwarf trees are evident in the overall higher trait plasticity in fringe compared to dwarf trees (Table 2). The surprising similar-

ity among control dwarf and fringe trees in hydraulic conductivity and xylem anatomy of recent wood given the vast difference in stature may also be due to temporal changes in environmental conditions, including resource availability (Mencuccini 2003). For example, as in other woody species, fringe trees may have grown vigorously for some period, allowing them to attain their current stature, and then as time progresses they have reverted to relatively low growth rates and construction of branching modules with relatively low hydraulic conductivity (Ryan, Binckley & Fownes 1997). There is presently no detailed understanding of the growth trajectory of mangrove trees, but this may be important to understanding the maintenance of productivity and habitat stability of on mangrove peat islands.

Hydraulic conductivity and growth

Covariation of physiological and structural characteristics of mangroves over environmental gradients (e.g. Ball & Farquhar 1984; Ball, Cowan & Farquhar 1988; Clough & Sim 1989; Lin & Sternberg 1992; Cheeseman & Lovelock 2004) makes it difficult to determine which processes have the greater effect on growth rate and canopy development. Variation in trait plasticity can be used to indicate which processes are more variable, and therefore more likely to be important in determining overall fitness (e.g. enhanced growth rates and competitive ability) of plants (Agrawal 2001). Comparison of trait plasticity (Table 2), expressed as the mean coefficient of

| Plant traits | Dwarf | Fringe |
|------------------------------------------------------------|---------------|---------------|
| Hydraulic anatomy | | |
| Mean xylem diameter | 0.064 ± 0.019 | 0.038 ± 0.006 |
| Hydraulically weighted xylem diameter | 0.041 ± 0.005 | 0.038 ± 0.005 |
| Density of xylem vessels | 0.152 ± 0.039 | 0.084 ± 0.015 |
| Huber Value | 0.136 ± 0.009 | 0.138 ± 0.008 |
| Leaf area of terminal branches | 0.105 ± 0.001 | 0.085 ± 0.003 |
| Nodes per unit stem length | 0.157 ± 0.084 | 0.246 ± 0.017 |
| Hydraulic conductance | | |
| K_s | 0.131 ± 0.016 | 0.190 ± 0.033 |
| K_{leaf} | 0.120 ± 0.003 | 0.199 ± 0.029 |
| Theoretical flow rate (xylem vessel diameter) ⁴ | 0.488 ± 0.068 | 0.454 ± 0.021 |
| Leaf physiology | | |
| Photosynthetic CO ₂ assimilation rate | 0.050 ± 0.005 | 0.039 ± 0.005 |
| $\delta^{13}C$ | 0.006 ± 0.001 | 0.012 ± 0.002 |
| Specific leaf area | 0.018 ± 0.002 | 0.016 ± 0.005 |
| Leaf N concentration | 0.027 ± 0.002 | 0.057 ± 0.005 |
| Leaf P concentration | 0.029 ± 0.002 | 0.048 ± 0.006 |
| Plant growth | | |
| Stem extension | 0.279 ± 0.063 | 0.358 ± 0.100 |
| Shoot initiation | 0.279 ± 0.055 | 0.326 ± 0.065 |
| Whole stand | | |
| LAI | 0.486 ± 0.073 | 0.098 ± 0.002 |

Table 2. Trait plasticity over fertilization treatments, measured as the mean coefficient of variation (\pm standard error) of physiological and morphological characters of dwarf and fringe trees of *Rhizophora mangle*

Data for plant growth is from Feller *et al.* 2003, and $\delta^{13}C$ from McKee *et al.* (2002).

K_s , stem hydraulic conductivity; K_{leaf} , hydraulic conductivity for leaf area; N, nitrogen; P, phosphorus; LAI, leaf area index.

variation (i.e. standard error/mean over the fertilization treatment groups) shows that leaf level characteristics (light saturated rates of CO₂ assimilation, specific leaf area and leaf nutrient concentrations) were less plastic than hydraulic conductivity, anatomy and plant growth in response to nutrient additions. Results of other studies are consistent with this pattern. For example, stomatal size and density do not vary significantly in *R. mangle* across a similar tree height gradient in Panamá (C.E. Lovelock unpublished data). In addition, high levels of variability in xylem characteristics (Sun & Suzuki 2001; Yanez-Espinosa, Terrazas & Lopez-Mata 2001) and stem hydraulic conductivity (Sperry *et al.* 1988; Melcher *et al.* 2001) have been observed in other studies of the Rhizophoraceae. Thus, in *R. mangle*, the characteristics of leaves vary less across the tree height gradient than the hydraulic properties of the stems supplying them with water.

There may be other physiological processes, in addition to changes in xylem anatomy, by which P fertilization leads to the observed dramatic enhancements in hydraulic conductivity in dwarf trees. In agricultural herbaceous plant species, impaired functioning of water channels (aquaporins) with nutrient deprivation was relieved when nutrient availability was enhanced (e.g. Carvajal *et al.* 1996; Clarkson *et al.* 2000). Similar enhancement of aquaporin function in mangrove roots with relief of P deficiency could also occur, but enhanced rates of root growth and enhanced numbers of fine roots could also be responsible.

Although other physiological mechanisms may also be important in improving water transport in dwarf *R. mangle*, the increase in hydraulic conductivity resulting from fertilization with P was clearly associated with two structural changes in the xylem tissue. First, the average diameter of vessels was enhanced. Because flow through cylinders increases with the radius raised to the fourth power, we calculated the hydraulically weighted average diameter (Lewis & Boose 1995; Comstock & Sperry 2000) to take into account the disproportionate contribution of large vessels to total flow. In the present study, the average hydraulically weighted vessel diameter increased 26.7%, from 30 to 38 μm , which could potentially increase flow by as much as 57%. Furthermore, fertilization with P increased internode length by a factor of 8 (Table 1). While vessel length was not measured, it would be expected to increase in association with internode length and increase in vessel diameter. This would additionally enhance hydraulic conductivity by decreasing the number of times that water must cross pit membranes between conduit lumen. The change in xylem anatomy was also coincident with enhancements in leaf area supported by the xylem tissue, resulting in consistency in Huber Values across the gradient in tree stature and fertilizer treatment (Table 1). Enhancements in rosette leaf area with addition of P were on the order of 16–28%. Based on our calculations of a potential 57% increase in water flow in P-fertilized trees, we would predict that stomatal conductance of leaf area could also be substantially increased in P-fertilized trees.

The increase in hydraulic conductivity was consistent with the observed enhancement of assimilation rates in P-fertilized plants. Unfortunately, measurements of stomatal conductance and transpiration were accidentally lost in the present study. However, Cheeseman & Lovelock (2004) found a linear relationship between assimilation rate and stomatal conductance in the same plants as the present study. According to their results, the increase in assimilation rate in the present study from 5.3 to 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ with P fertilization in dwarf mangroves (Fig. 6) would have been associated with a 62% increase in stomatal conductance from 68 to 110 $\text{mmol m}^{-2} \text{ s}^{-1}$, respectively. This difference accords well, with the measured hydraulic conductivity in the morning being greater and the potential increase in flow due to larger vessel diameters being 57% greater in the P-fertilized plants (Fig. 3). These results are consistent with previous reports linking hydraulic conductivity and photosynthetic capacity over a range of species (Brodribb & Feild 2000).

Consequences of enhanced hydraulic conductivity

Midday loss in K_s in P-fertilized dwarf plants compared to control trees (Fig. 3) suggests that the dwarf plants have an increased vulnerability to cavitation compared to control trees (Tyree & Sperry 1989; Comstock & Sperry 2000), which supports our initial hypothesis. Leaf water potential was relatively constant between the morning and afternoon measurements, indicating that stomatal closure is tightly coupled to hydraulic conductivity, as has been observed in other studies (Hubbard *et al.* 2001). Diurnal reduction in hydraulic conductivity has been observed in another study of *R. mangle* (Melcher *et al.* 2001) and other species (Hubbard *et al.* 1999; Brodribb & Feild 2000; Nadini & Salleo 2000; Sobrado 2000; Sperry 2000; Hubbard *et al.* 2001, 2002; Brodribb *et al.* 2003). Our current study suggests that higher rates of assimilation and water loss in the morning in P-fertilized plants may come at the expense of low carbon gain in the afternoon and any additional energetic cost of refilling embolized vessels. In contrast, in dwarf control trees, low levels of hydraulic conductivity appear to limit emboli and any necessity to repair this damage. However, low hydraulic conductivity of control dwarfs also reduces rates of photosynthetic carbon gain.

Explicit links between forest structure and physiological function

In mangroves, productivity is broadly correlated with forest height (Saenger & Snedaker 1993). In our study, the change of dwarf habit from low LAI and low stature to that which resembles fringe forest, that is, enhanced LAI and taller stature, was caused by addition of P and resulted in a greater capacity for stems to elongate and to conduct water. These are necessary requirements for high levels of transpiration and enhancement of photosynthesis and carbon gain.

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