

Differences in plant function in phosphorus- and nitrogen-limited mangrove ecosystems

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Summary

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- Mangrove ecosystems can be either nitrogen (N) or phosphorus (P) limited and are therefore vulnerable to nutrient pollution. Nutrient enrichment with either N or P may have differing effects on ecosystems because of underlying differences in plant physiological responses to these nutrients in either N- or P-limited settings.
- Using a common mangrove species, *Avicennia germinans*, in sites where growth was either N or P limited, we investigated differing physiological responses to N and P limitation and fertilization. We tested the hypothesis that water uptake and transport, and hydraulic architecture, were the main processes limiting productivity at the P-limited site, but that this was not the case at the N-limited site.
- We found that plants at the P-deficient site had lower leaf water potential, stomatal conductance and photosynthetic carbon-assimilation rates, and less conductive xylem, than those at the N-limited site. These differences were greatly reduced with P fertilization at the P-limited site. By contrast, fertilization with N at the N-limited site had little effect on either photosynthetic or hydraulic traits.
- We conclude that growth in N- and P-limited sites differentially affect the hydraulic pathways of mangroves. Plants experiencing P limitation appear to be water deficient and undergo more pronounced changes in structure and function with relief of nutrient deficiency than those in N-limited ecosystems.

Key words: *Avicennia germinans*, Belize, fertilization, Florida, hydraulic conductivity, leaf water potential, photosynthesis.

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Introduction

Mangroves are intertidal forests that occur in the tropics and subtropics. They inhabit saline sediments, and their growth and stature generally declines with increasing sediment salinity (Clough *et al.*, 1982). Recent studies have shown that nutrient limitations to growth are also common in mangrove environments (Feller, 1995; Koch, 1997; Feller *et al.*, 2002, 2003; Lovelock *et al.*, 2004). Although mangroves occur in environments that often have an ample supply of water, it is

costly for plants to extract water of low ionic content from highly saline soils (Ball *et al.*, 1988; Sobrado, 2000). Consequently, mangroves must limit water loss (i.e. transpiration), which occurs during photosynthetic CO₂ uptake. Thus, mangroves have evolved in a setting where the rates of photosynthesis and growth, which are stimulated by high nutrient availability, are restricted by the capacity to maintain a favourable water balance with minimum salt uptake, resulting in trade-offs between nutrient resource utilization and salt tolerance (Ball *et al.*, 1988; Ball, 1996; Lovelock &

Feller, 2003). However, different nutrient limitations could have divergent effects on this trade-off.

In tropical intertidal mangrove forests, growth of trees can be limited by nitrogen (N) or phosphorous (P) (Boto & Wellington, 1983; Feller, 1995; Feller *et al.*, 2002; Lovelock *et al.*, 2004). Experimental studies in flooded dwarf *Rhizophora mangle* L. (red mangrove) forests in Belize (Cheeseman & Lovelock, 2004; Lovelock *et al.*, 2006) and Panamá (Lovelock *et al.*, 2004) suggested that enhancements in growth with P fertilization are associated with an increased capacity of plants to transport water. In other plant species, deficiencies in P are associated with reductions in water uptake (Carvajal *et al.*, 1996), possibly because of the crucial role of phosphorylation in the function of water channels (aquaporins) in plant tissues (Johansson *et al.*, 1998).

In contrast to our studies in Belize and Panamá, growth of dwarf *Avicennia germinans* (L.) Stearn (black mangrove) mangroves in Florida was N limited (Feller *et al.*, 2003). When these N-limited trees were fertilized with N, growth enhancements were associated with higher photosynthetic capacity (Lovelock & Feller, 2003) and greater leaf area development (Feller *et al.*, 2003). Other studies of the effects of N fertilization on the hydraulic function of trees in other ecosystems found that increases in leaf area were associated with proportional increases in hydraulic conductivity, indicating a high degree of co-ordination between photosynthesis and hydraulic conductivity with N enrichment (Hubbard *et al.*, 2004). Because of the important role that P may have in water uptake (Carvajal *et al.*, 1996; Johansson *et al.*, 1998), and our previous observations of increases in hydraulic conductivity with relief of P limitation (Lovelock *et al.*, 2004, 2006), we predict that where P limits growth, plants should exhibit symptoms of water deficiency [lower leaf water potential (ψ_{leaf}) lower stomatal conductance and higher water-use efficiency (WUE)] that will be alleviated with the addition of P. By contrast, we predict that plant water relations will not be changed by the addition of N to N-limited plants. Rather, we expect N fertilization to stimulate sink strength or the number and activity of meristems, which instead limits photosynthesis and the growth of N-limited plants (Van der Werf & Nagel, 1996; Wilson, 2000). To test these hypotheses, we compared photosynthetic physiology, water relations and hydraulic architecture of fertilized dwarf trees of *A. germinans* in a P-limited setting in Belize and in an N-limited setting in Florida.

Materials and Methods

Site descriptions and experimental design

The study was conducted at Twin Cays, a 92-ha archipelago of mangrove islands located approx. 1.6 km inside the Belizean Barrier Reef Complex (16°50'N, 88°06'W, see Feller, 1995 and McKee, 1993 and references therein for a full

site description) and in the Indian River Lagoon, North Hutchinson Island, Florida (27°33'N, 80°20'W, see Feller *et al.*, 2003 for a full description). Briefly, both sites are microtidal, with tidal range of approx. 0.5 m. Mean minimum and maximum air temperatures are, respectively, 25.4 and 28.8°C at the site in Belize and 18.0 and 27.9°C at the site in Florida. Annual rainfall is approx. 2.5 m at Twin Cays, distributed relatively uniformly over the year, while in Florida annual rainfall is 1.37 m, falling mainly in the summer months. Twin Cays is 12 km from the mainland and does not receive terrigenous inputs of freshwater or sediments. The soil is composed of highly organic peat, comprising mainly red mangrove roots, and rests on the Pleistocene coral reef platform. The dwarf forests, where trees are less than 1.5 m tall, occur in the interior of the islands where tidal inundation is frequent and sediments are reduced (−30 mV, Feller *et al.*, 2003). Within the Indian River Lagoon, our site in Florida is an abandoned mosquito impoundment (number 23) in which natural tidal flow and the mangrove forest was re-established in 1976. The mangrove soils are sandy with abundant shell fragments and are highly oxidized (+95 mV, Feller *et al.*, 2003). Pore water salinity was hypersaline in both sites – mean 50 practical salinity units (PSU) – but these conditions are not likely to inhibit the growth of *A. germinans* to any great extent (see Cintrón *et al.*, 1978). At Twin Cays, the dwarf forests are dominated by *R. mangle*, but dwarf *A. germinans* are also present. In Florida, dwarf forests are dominated by *A. germinans* with scattered *Laguncularia racemosa* (Feller *et al.*, 2003).

At each site we fertilized eight to nine individual dwarf trees of *A. germinans* with N or P fertilizer, or designated them as controls (no fertilizer). In Belize, eight replicate trees were selected per treatment (24 total) and the treatments were 10 m apart. For the Florida site, we used nine replicate dwarf trees (27 total), previously described in Feller *et al.* (2003). The trees were each fertilized first in January 1997, and then at 6-month intervals until April 2001, with 300 g of N fertilizer as urea (45 : 0 : 0; PCS Sales, North Brook, IL, USA), or P fertilizer, as P₂O₅ (0 : 45 : 0, triple superphosphate; Diamond R Fertilizer Company, Winter Garden, FL, USA), or cored but not fertilized (controls) as described in Feller (1995). Briefly, fertilizer was encased in dialysis tubing (Spectropore; Spectrum, Gardena, CA, USA) and inserted in 30 cm deep × 7 cm wide holes, which were cored in the soil on either side of the tree at the dripline of the canopy. After inserting the fertilizer, the holes were then plugged with the extracted soil core. The same holes were used each time the fertilizer was applied.

Hydraulic conductivity and architecture

Hydraulic conductivity was measured on stem segments of each of the trees in December 2002 in Belize and in March 2004 in Florida. A branch from each experimental tree, cut at

least 0.3 m from the target stem segment, was harvested in the morning (before 09:00 h) of measurement. All branches used were produced after the experimental fertilization had been imposed. Stems were transported to the laboratory and re-cut to approx. 7–10 cm length under water, before the measurement, and trimmed with a razor blade. Stems were inserted inline with a perfusion solution of 95% distilled water augmented with 5% seawater (collected locally) and allowed to equilibrate to steady state (approx. 5 min) before measurement. Native stem hydraulic conductivity (K_H , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) 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 (PEEKTM tubing; Upchurch Scientific, Oak Harbor, WA, USA) in order to match the resistance of the tube to that of the branch to minimize errors. The capillary tubing and stem segment were placed in series. The pressure applied to the stem was provided by an elevated reservoir, which varied between 2.2 and 2.7 kPa. After measurement, leaves were detached from the stem and their area measured using a Li-Cor leaf area meter (Li-Cor Corp., Lincoln, NE, USA). The K_H was divided by the leaf area supported by the stem to give leaf area specific hydraulic conductivity (K_L). The mean leaf area of stems sampled for measurements of hydraulic conductivity was 108 cm² (standard error = 9 cm²).

After measuring the hydraulic conductivity of the stem segment, xylem vessels were stained using basic fuchsin red dye drawn into the stem segment by attaching the segment to a 50-ml syringe. Hand-cut sections were mounted in glycerol, then photographed at $\times 100$ magnification (Nikon Coolpix 995; Nikon Corp., Tokyo, Japan). Variation in xylem structure of stem segments was assessed by measuring diameters of 50–100 xylem vessels at $\times 100$ magnification on a randomly selected section from each stem segment using the program SCION IMAGE (Scion Corp., Frederick, MD, USA). Xylem diameters were weighted to reflect the enhanced flow rates possible with larger-diameter xylem vessels. Hydraulically weighted xylem diameter was calculated as $(\sum r^5)/(\sum r^4)$, where r is the xylem vessel radius (Sperry *et al.*, 1994). We estimated the magnitude of hydraulic conductivity of the whole hydraulic system, K_{S-L} , using the Soil–Plant continuum model and Cohesion–Tension theory (Tyree & Ewers, 1996; Sperry, 2000), as follows:

$$E \text{ (transpiration or flux per unit leaf area)} = K_{S-L} \times (\Psi_{\text{soil}} - \Psi_{\text{leaf}}).$$

Water potential of leaves and porewater

The Ψ_{leaf} values were measured in January 2004 in Belize, and in March 2004 in Florida. We measured mid-morning Ψ_{leaf} values on bright sunny days when photosynthetic rates are at a maximum. One fully expanded leaf, exposed to full sunlight, was measured for each experimental plant.

The leaves of *A. germinans* have salt-excreting glands. To remove salt from the leaf surface, the leaves were washed with distilled water, carefully blotted dry with paper tissues, allowed to air-dry for approx. 15 min and then visually checked for any remaining moisture before sampling. The Ψ_{leaf} values were measured on leaf punches (5.6 mm diameter) using Peltier-type leaf-cutter psychrometers (Merrill Instruments, Logan, UT, USA) and an automated multichannel microvoltmeter (Model CR7; Campbell Scientific, Logan, UT, USA). Psychrometers were calibrated with NaCl solutions and were capable of measurements down to at least -7.5 MPa. The psychrometers were temperature equilibrated in an insulated water bath for 3–4 h before measurements. Measurements were taken after applying a 2.5 mV excitation voltage for 45 s.

Pore water was extracted from the base of each experimental tree at each site at approx. 30 cm depth using a 50-ml syringe attached to rigid tubing (McKee, 1993). Porewater salinity was measured with a handheld refractometer. The Ψ of porewater was measured using leaf-cutter psychrometers (Merrill Instruments; see above) by placing filter papers, thoroughly soaked with porewater, into the chambers.

Photosynthetic gas exchange

Rates of photosynthetic gas exchange were measured with a Li-Cor 6400 photosynthesis measuring system (Li-Cor Corp.) in February 2001 in Belize, and in March 2004 in Florida. Photosynthesis was measured on sunny days with little or no cloud cover, using sunlight under ambient conditions of CO₂, air temperature and humidity. The quantum flux density of photosynthetically active radiation was generally saturating for photosynthesis ($> 800 \mu\text{mol m}^{-2} \text{s}^{-1}$), and thus photosynthetic rates were assumed to be close to maximal. Measurements were made on three young, fully expanded leaves per tree, which were then averaged to obtain one value for each experimental tree. Instantaneous WUE was calculated as CO₂ assimilation/transpiration.

After each measurement was completed (usually in approx. 1 min), the leaf was harvested. Leaf area was measured using a Li-Cor leaf area meter (Li-Cor Corp.). Leaves were then dried in an oven at 60°C and then weighed. Dried leaf material was ground to a fine powder in a small mill. The nitrogen concentration within the leaves was analyzed in a CHN analyzer (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 (PA, USA). Integrated measures of WUE of leaves were obtained by measuring the leaf carbon isotope discrimination ratio ($\delta^{13}\text{C}$), using an Isotope Ratio Mass Spectrometer at the UC Davis Stable Isotope Facility of the Department of Plant Sciences, University of California, Davis (CA, USA).

Data analysis

We used a generalized linear model with site and nutrient treatment as fixed effects in the model. Where a significant main effect or interaction between site and nutrient treatment occurred, we used Fisher's Least Significant Difference post hoc 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

Photosynthesis and stomatal conductance

In control plants, photosynthetic rates and stomatal conductance were lower in the P-limited Belize plants than in the N-limited Florida plants (Fig. 1, main effect of site $F_{1,43} = 42.03$, $P < 0.0001$). In Belize, P-fertilized plants had photosynthetic rates that were similar to those of the controls in Florida (Fig. 1, site \times treatment interaction, $F_{2,43} = 12.27$, $P < 0.0001$). In both sites, photosynthetic carbon (C) gain was linearly correlated with stomatal conductance, with both sites showing a similar slope of the relationship between stomatal conductance and photosynthetic C gain (Fig. 2a, $R^2 = 0.78$). Rates of photosynthetic C gain were not correlated with leaf N concentration at either site (leaf N ranged from 0.97 to 2.88%, data not shown), but were correlated with leaf P concentrations at the P-limited site (Fig. 2b, $R^2 = 0.40$). On a dry weight basis, leaf P ranged from 0.028 to 0.21%. The leaf to air vapour pressure difference was variable (range 1.9–2.8 kPa) but did not vary significantly over the sites (data not shown).

Hydraulic conductivity and xylem architecture

The native hydraulic conductivity of stems was similar in the P- and N-limited sites (K_H , Fig. 3a, $P > 0.05$), although in the P-limited site, P-fertilized trees had a significantly higher K_H (site \times treatment interaction $F_{2,43} = 4.00$, $P = 0.025$). When conductivity was adjusted for leaf area, no significant differences were found among sites or treatments (K_L , Fig. 3b, $P > 0.05$). Data were variable, particularly in control stems in the N-limited site. Calculation of hydraulic conductivity of the whole soil to leaf pathway, K_{S-L} , found that total pathway hydraulic conductivity was lower in the P-limited compared with the N-limited site (Table 1, main effect of site $F_{1,39} = 17.41$, $P = 0.002$). Although the main effect of treatment was not significant ($P > 0.05$), fertilization treatments tended to have differing effects at different sites (site \times treatment interaction $F_{2,39} = 3.16$, $P = 0.052$). In the P-limited site, fertilization with P tended to increase K_{S-L} , while N fertilization had little effect on K_{S-L} at either

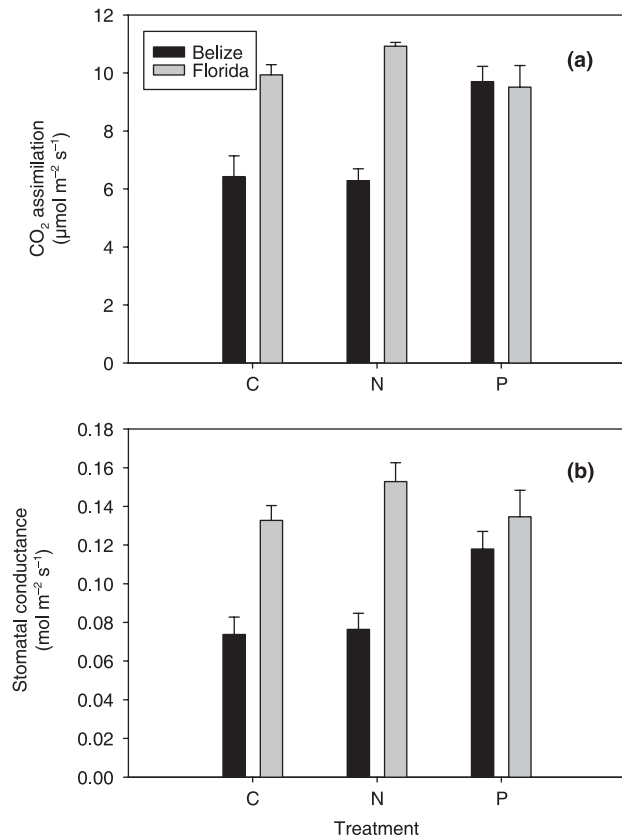


Fig. 1 Photosynthetic CO₂ fixation (a) and stomatal conductance (b) in *Avicennia germinans* trees fertilized with nitrogen (N) and phosphorus (P), or unfertilized controls (C), in Belize where growth is limited by P (dark bars) and in Florida where growth of trees is limited by N (light bars). Values are the mean and standard errors of eight to nine trees per treatment.

site. Fertilization with P at the N-limited site tended to reduce K_{S-L} .

Mean xylem diameters, and hydraulically weighted vessel diameters, were smaller at the N-limited than at the P-limited site (main effect of site $F_{1,43} = 17.35$, $P < 0.0001$). Xylem diameter was not significantly greater in N-fertilized trees at the N-limited site, but was much larger in P- and N-fertilized trees than in control trees at the P-limited site (Table 2, treatment $F_{2,43} = 3.93$, $P = 0.027$). The cross-sectional area of xylem that is vessel lumen was higher in the N-limited site than in the P-limited site (main effect of site $F_{1,43} = 5.48$, $P = 0.024$). Fertilization enhanced the proportion of xylem lumen in P-fertilized plants in the P-limited Belize site and in N- and P-fertilized trees in the N-limited Florida site (treatment $F_{2,43} = 3.17$, $P = 0.052$). The cross-sectional area of the stem that was xylem tissue was higher in P-fertilized plants than in control and N-fertilized trees in the P-limited Belize site (Table 2). For this parameter, comparative data were not available for the Florida site.

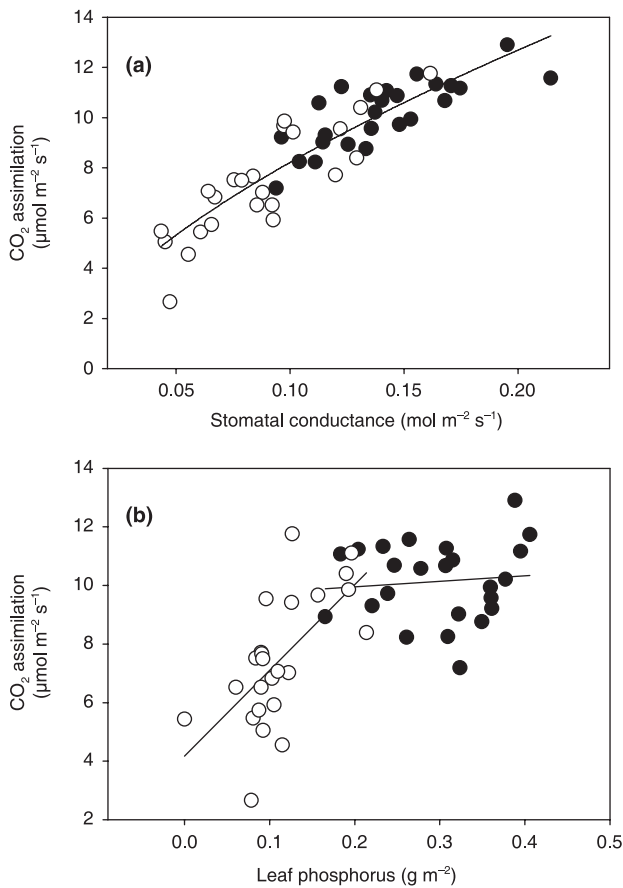


Fig. 2 Variation in photosynthetic CO₂ fixation with stomatal conductance (a), and leaf phosphorus concentrations (b) in *Avicennia germinans* trees fertilized with either nitrogen (N) or phosphorus (P), and in unfertilized controls (C), in Belize where growth is limited by P (open symbols) and in Florida where growth of trees is limited by N (closed symbols). Lines in plots are lines of best fit of the form $y = 3.06 + 50.2x$, $R^2 = 0.78$ (a, for both sites), $y = 3.72 + 3.27x$, $R^2 = 0.40$ (b, open symbols, P-limited Belize) and $y = 9.58 + 0.18x$, $R^2 = 0.01$ (b, closed symbols, N-limited Florida).

Water potential and water use efficiency

The ψ values were approx. 0.5 MPa lower in the P-limited than in the N-limited site (Fig. 4a, main effect of site $F_{1,43} = 11.39$, $P = 0.0015$), despite no significant differences in substrate salinity between sites or among treatments (Fig. 4b). Instantaneous WUE was higher in the P-limited site than the N-limited site (Site $F_{1,43} = 9.32$, $P = 0.0039$). However, $\delta^{13}\text{C}$, which gives a time-integrated indication of WUE, was lower (indicating lower WUE) in the P-limited site than in the N-limited site (Fig. 5, Site $F_{1,43} = 21.27$, $P < 0.0001$). Fertilization had no significant effects on ψ_{leaf} or WUE at either site, but $\delta^{13}\text{C}$ was higher in P-fertilized compared with control plants at the P-limited site ($P = 0.0037$).

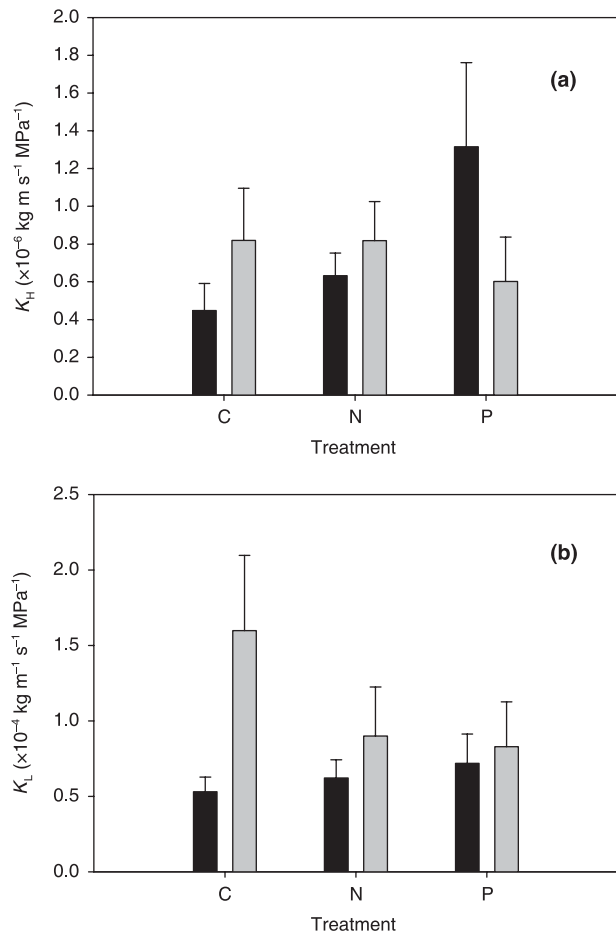


Fig. 3 Native hydraulic conductivity of stems (K_{st}) (a), and hydraulic conductivity per unit leaf area (K_{L}) (b) for *Avicennia germinans* trees fertilized with either nitrogen (N) or phosphorus (P), and for unfertilized controls (C), in Belize where growth of trees is limited by P (dark bars) and in Florida where growth of trees is limited by N (light bars). Values are the mean and standard errors of eight to nine trees per treatment.

Discussion

Because of the important role that P can have for water uptake and transport in plants (Caravajal *et al.*, 1996; Clarkson *et al.*, 2000), we hypothesized that P-deficient mangroves would be effectively water deficient, while this would not be the case at our N-limited site. At the P-limited site we found that the plants had lower ψ_{leaf} values, lower stomatal conductance and lower CO₂-assimilation rates, but higher instantaneous WUE, compared with those at the N-limited site, characteristics that are consistent with water deficiency. Contrary to enhanced instantaneous WUE, $\delta^{13}\text{C}$ was lower at the P-limited site than at the N-limited site, suggesting that long-term WUE was lower at the P-limited site (Farquhar *et al.*, 1989). It is possible that this contrary pattern, of higher instantaneous WUE and lower $\delta^{13}\text{C}$ observed at the P-limited site

Table 1 Total hydraulic conductivity of the soil–leaf pathway, K_{S-L} , in a phosphorus (P)-limited (Belize) and a nitrogen (N)-limited (Florida) site with fertilization with N and P, or left as controls (C)

Site	Treatment	Transpiration (mmol m ⁻² s ⁻¹)	Ψ_{soil} (MPa)	Ψ_{leaf} (MPa)	K_{S-L} (mmol m ⁻² s ⁻¹ MPa ⁻¹)
Belize	C	1.63 ± 0.15	-4.00 ± 0.19	-4.97 ± 0.14	1.91 ± 0.42
	N	1.67 ± 0.16	-4.06 ± 0.17	-4.88 ± 0.22	2.05 ± 1.18
	P	2.28 ± 0.16	-4.23 ± 0.24	-4.80 ± 0.19	3.19 ± 1.31
Florida	C	2.78 ± 0.15	-3.82 ± 0.06	-4.36 ± 0.14	9.77 ± 4.27
	N	3.36 ± 0.16	-4.31 ± 0.07	-4.47 ± 0.12	8.28 ± 2.50
	P	2.93 ± 0.25	-3.80 ± 0.05	-4.46 ± 0.16	4.08 ± 0.97

Calculated from: Flux per unit leaf area (i.e. transpiration) = $K_{S-L} \times (\Psi_{soil} - \Psi_{leaf})$. Ψ_{leaf} , leaf water potential; Ψ_{soil} , soil water potential. The Ψ_{soil} is estimated from the relationship between porewater soil salinity, measured with a handheld refractometer, and Ψ_{soil} measured by psychrometry [$\Psi_{soil} = -0.073 - 0.08 \times \text{salinity in practical salinity units (PSU)}$, $R^2 = 0.52$].

Table 2 Xylem characteristics of *Avicennia germinans* in a phosphorus (P)-limited (Belize) and a nitrogen (N)-limited (Florida) site with fertilization with N and P, or left as controls (C)

Site	Treatment	Mean vessel diameter (µm)	Hydraulically weighted vessel diameter (µm)	Percentage of the xylem in vessels	Proportion of the stem that is xylem
Belize	C	17.8 ± 1.4a	24.8 ± 1.6a	7.5 ± 0.3a	27.3 ± 3.1
	N	21.8 ± 0.9b	31.6 ± 0.9b	7.5 ± 0.9a	37.5 ± 4.6
	P	23.0 ± 2.4b	33.1 ± 3.5b	9.7 ± 1.3ab	48.5 ± 7.1
Florida	C	15.6 ± 0.7a	21.2 ± 0.8a	8.5 ± 0.9a	–
	N	17.3 ± 0.2a	22.7 ± 0.8a	10.5 ± 0.6b	–
	P	16.6 ± 1.0a	20.1 ± 1.3a	11.0 ± 1.1b	–

Values are means ± standard error of seven to nine plants. Different letters after the mean values in each column indicate that the means are different at $P < 0.05$.

compared with the N-limited site, indicates that instantaneous values obtained when photosynthetic rates are at a maximum do not reflect longer-term patterns (Martin & Thorstenson, 1988). Rates of photosynthetic C fixation show marked daily depressions at the P-limited site (Cheeseman & Lovelock, 2004) and in other mangrove systems (Cheeseman, 1994), and therefore WUE may be lower than we recorded for much of the day. Lower $\delta^{13}\text{C}$ values have also been observed in other nutrient-deficient trees compared with nutrient-replete treatments, indicating there may be a loss of stomatal efficiency with severe nutrient deficiency (Guehl *et al.*, 1995).

Additional support for our hypothesis, that P-deficient mangroves are water deficient, is that fertilization with P at the P-limited site significantly increased stomatal conductance, $\delta^{13}\text{C}$, mean xylem diameter, abundance of conductive tissue and K_H , and tended to enhance K_{S-L} . Enhancements in K_H were tightly coupled with increased leaf area, such that K_L was not significantly affected by fertilization treatments. Similar co-ordination of hydraulic conductivity, CO_2 assimilation and allocation to leaf area has previously been observed in other species (see review Hubbard *et al.*, 1999, 2001, 2004; Sperry, 2000; Brodribb *et al.*, 2002, 2003; Meinzer *et al.*, 2004).

In our N-limited site, fertilization with N slightly increased xylem diameter and the proportion of the xylem that was

vessel lumen, but did not significantly enhance stomatal conductance, K_H , K_L or K_{S-L} . Apparently, relief from N deficiency in these plants is not tightly linked to improving water-transport processes, but is caused by other processes, such as enhanced meristem activity and associated increase in the strength of C sinks, as the addition of new tissues demands more C for their function (Wilson, 2000, I. C. Feller, unpublished). Fertilization with N in the P-limited site, and with P in the N-limited site, had significant effects on xylem anatomy in some cases, with N significantly increasing vessel size in P-limited plants in Belize, and fertilization with P enhancing the proportion of the vessel lumen in N-limited plants in Florida. The addition of nonlimiting macronutrients therefore does exert some influence on tissue structure and, possibly, function. Changes in vulnerability to mid-day embolism have been observed to occur with fertilization in other species (Harvey & van der Driessche, 1997; Ewers *et al.*, 2000). However, plant hydraulic function was only influenced by the addition of limiting nutrients in the P-limited site in this study.

Unlike stomatal conductance and K_H , the lower values of Ψ_{leaf} at the P-limited site were not ameliorated by fertilization with P. These results suggest that some factor, in addition to P limitation, contributes to the lower Ψ_{leaf} at the P-limited Belize site. This other factor is unlikely to be salinity, as soil

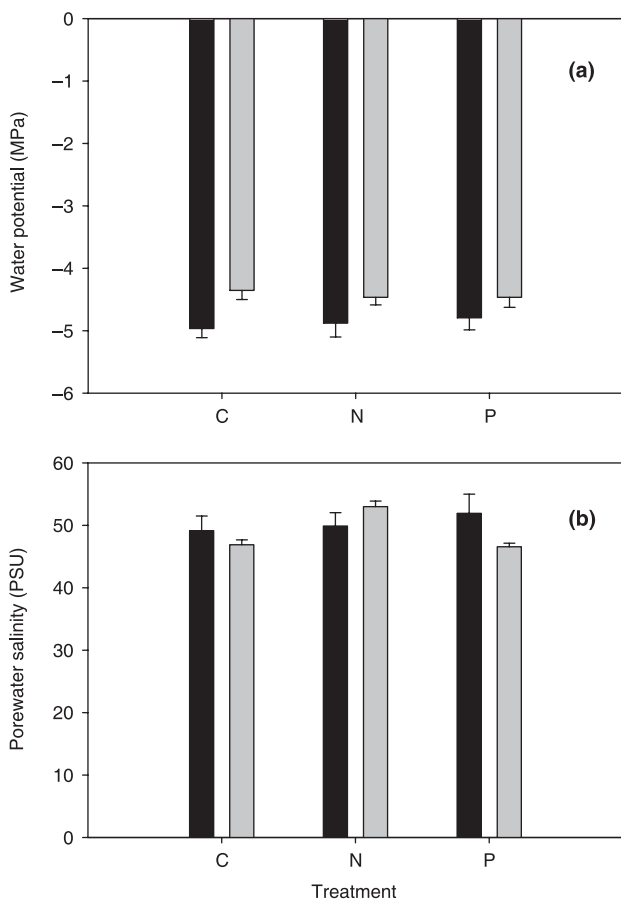


Fig. 4 Leaf water potential (ψ_{leaf}) (a), and soil porewater salinity in practical salinity units (PSU) (b) in *Avicennia germinans* trees fertilized with either nitrogen (N) or phosphorus (P), and in unfertilized controls (C), in Belize where growth is limited by P (dark bars) and in Florida where growth of trees is limited by N (light bars). Values are the mean and standard errors of eight to nine trees per treatment.

salinity and ψ of porewater in both sites were similar. In Belize – the P-limited site – sediments are reduced more than those in the N-limited Florida site because of consistent inundation (Feller *et al.*, 2002, 2003; McKee *et al.*, 2002). Low-sediment oxygen concentrations inhibit root function, including water uptake (Greenway & Gibbs, 2003), and can alter xylem anatomy (Yanez-Espinosa *et al.*, 2001). Therefore, differences in the ψ across sites could be partially associated with low oxygen concentrations in soils at the P-limited site. Flooded plants have also been observed to transport solutes from roots to shoots, decreasing the ψ_{leaf} by 0.08 MPa and providing important signals that give rise to altered physiology and allocation patterns (Jackson *et al.*, 1996). The functional consequences of lower ψ_{leaf} and K_H in the P-limited compared with the N-limited site may be an increased sensitivity of stomata, and thus transpiration and photosynthesis, to variation in environmental conditions (e.g. leaf temperature, humidity,

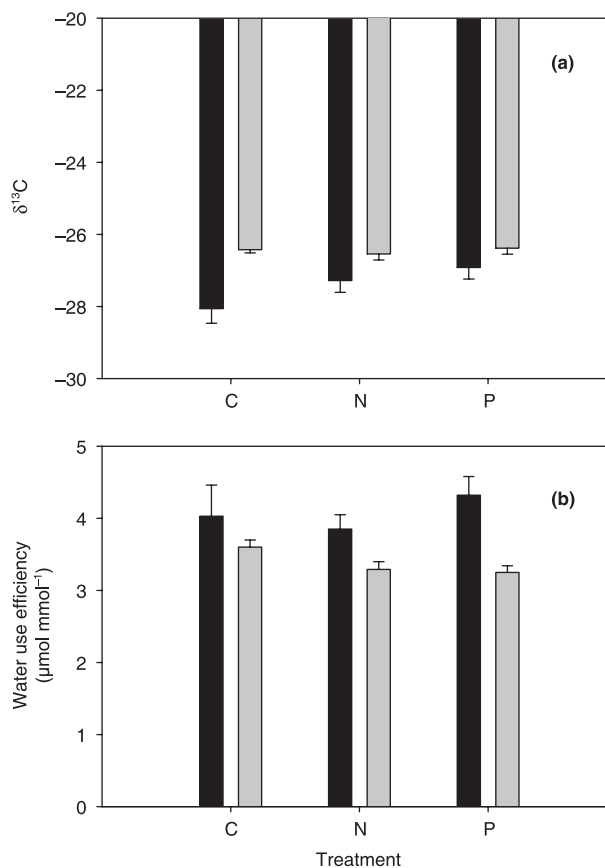


Fig. 5 The leaf carbon isotope discrimination ratio ($\delta^{13}C$) (a), and instantaneous water use efficiency (WUE) (b) in *Avicennia germinans* trees fertilized with either nitrogen (N) or phosphorus (P), or in unfertilized control trees (C), in Belize where tree growth is limited by P (dark bars) and in Florida where tree growth is limited by N (light bars). Values are the mean and standard errors of eight to nine trees per treatment.

salinity and redox potential), which would promote survival during unfavourable conditions (Sperry, 2000).

Although our hypothesis that P-limited plants would be water deficient because of the important role of P in water uptake in roots through aquaporins (Caravajal *et al.*, 1996; Clarkson *et al.*, 2000), hydraulic limitations at P-limited sites may also be relieved with P fertilization because P fertilization increases the root surface per unit leaf area of plants (i.e. thinner, longer roots). In addition to increasing root surface area, enhanced allocation to roots in P-fertilized tree of the mangrove, *R. mangle*, has been observed to contribute to vertical accretion (i.e. soil elevation), thereby reducing inundation and enhancing oxygenation of the root zone (McKee *et al.*, 2002; K. L. McKee, unpublished). Understanding the mechanisms underlying P limitation of hydraulic conductivity requires more detailed study of allocation to roots and root function at both N- and P-limited sites.

Conclusions

This study shows that in the mangrove species *A. germinans*, over N- and P-limited sites, physiological characteristics conform to similar relationships (e.g. Fig. 2), but physiological processes which result in enhancements in growth with relief of nutrient limitation differ between N- and P-limited mangrove ecosystems. In our P-limited site, hydraulic conductivity appeared to be a key process limiting photosynthetic C gain and thus growth. By contrast, in the N-limited site, hydraulic conductivity was not improved by fertilization with limiting N. Instead allocation to leaf area, by increasing the number of branching modules, is implicated in improving the performance of N-fertilized trees (Feller *et al.*, 2003, I. C. Feller, unpublished). At the P-limited site, plants had lower ψ , lower K_H and lower $\delta^{13}C$ than at the N-limited site. With P fertilization, the $\delta^{13}C$ approached that of the N-limited site, together with improved hydraulic conductivity, greater xylem vessel diameter and rates of photosynthetic C gain, suggesting that relief from P deficiency improved plant water uptake and transport.

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