
Mangroves in Contrasting Osmotic Environments: Photosynthetic Costs of High Salinity Tolerance

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Abstract

Mangrove trees of the salt secreting *Avicennia germinans* and the non-secreting *Rhizophora mangle* were investigated at the northern coast of Venezuela at a low salinity site (127 mmol kg⁻¹) and two hypersaline sites (1600–1800 mmol kg⁻¹). Leaf sap osmolality and mass/area ratio of both species were positively correlated, while size was negatively correlated with soil salinity. Leaf sap osmolality was always higher in *Avicennia* and exceeded soil solution osmolality. Salinity increased the concentration of 1D-1-O-methyl-muco-inositol (OMMI) in *Rhizophora* and glycinebetaine in *Avicennia*. The latter could make up to 21% of total leaf nitrogen (N). Nitrogen concentration was higher in *Avicennia*, but subtracting the N bound in glycinebetaine eliminated interspecific differences. Photosynthetic rates were higher in *Avicennia*, and they decreased with salinity in both species. Leaf conductance (g_s) and light saturated photosynthesis (A_{sat}) were highly correlated, but reduction of g_s at the hypersaline sites was more pronounced than A_{sat} increasing water use efficiency in both species. Lower values of ¹³C discrimination at the hypersaline sites evidenced higher long-term water use efficiency. Apparent quantum yield and carboxylation efficiency decreased with salinity in both species. *Rhizophora* was more sensitive to high salinity than *Avicennia*, suggesting that glycinebetaine is a better osmoprotectant than OMMI.

Keywords: mangroves, *Rhizophora mangle*, *Avicennia germinans*, soil salinity, compatible solutes, photosynthesis

1. Introduction

Mangrove species in the neotropics are found along large latitudinal ranges including dry and wet coastal environments [1, 2]. Their distribution along steep salinity gradients provide

an opportunity to test *in situ* the impact of soil salinity on osmolyte accumulation in plant tissues, and analyze under similar light, temperature, and air humidity conditions, on their leaf development and photosynthetic performance.

The structural development and complexity of mangrove communities including height, leaf area index, leaf size, stem diameter, branching, litter production, and productivity are inversely related to interstitial soil water salinity. In neotropical mangroves, these properties have been shown to be strongly correlated [3–8].

Photosynthesis decreases significantly with salinity of interstitial water in several mangrove species [7–11]. Some species appear to be more sensitive to soil salinity than others, a characteristic that may be associated with specific metabolic and structural properties such as synthesis of compatible solutes, root permeability, salt excretion, and compartmentation of excess ions [12–17].

We studied the differentiation in leaf morphology, accumulation of osmotically active solutes and the photosynthetic response of two mangrove species, *Rhizophora mangle* L. and *Avicennia germinans* (L.) Stearn, reportedly differing in their salt tolerance [18–20] (**Figure 1**). These species coexist in neotropical mangroves and differ in their mechanisms of salt tolerance. *Rhizophora mangle* is considered as salt excluder, dominant in fringe mangroves throughout the neotropics, whereas *A. germinans* possesses numerous salt secreting glands in their leaves and typically dominates basin mangrove vegetation [20, 21]. Measurements were conducted under field conditions, in contrasting environments regarding fresh water availability and salt concentration of the soil interstitial water. Our objective was to assess quantitatively the impact of high salinity environments on photosynthetic performance and leaf expansion in association with inorganic and organic osmolyte accumulation.

In this chapter, we present results relating leaf sap osmolality and concentration of compatible solutes (cyclitols and glycinebetaine) to leaf morphology and patterns of gas exchange. The compatible solutes are presumably accumulated in the cytoplasm and counteract the osmotic effect of inorganic ions predominantly accumulated in the vacuole [14]. Accumulation of these compounds requires energy and carbohydrates from photosynthesis, and in the case of glycinebetaine, it needs additional amounts of N. The latter probably affects photosynthesis through the reduction of N availability for synthesis of photosynthetic enzymes.

Our hypotheses for this study were: (1) the reduction of photosynthesis resulting from salt accumulation in leaf cell sap is stronger in the species assumed to have lower salt tolerance, *R. mangle*; (2) salinity affects photosynthesis through diminished nutrient uptake, such as N, affecting protein synthesis, and phosphorus (P), possibly affecting N use efficiency; and (3) water use efficiency is higher in the high salinity (drier) environment, as a result of the combined effect of increased cell sap and interstitial soil water osmolalities on leaf conductance, leading to a proportionally larger reduction of transpiration compared to photosynthesis.

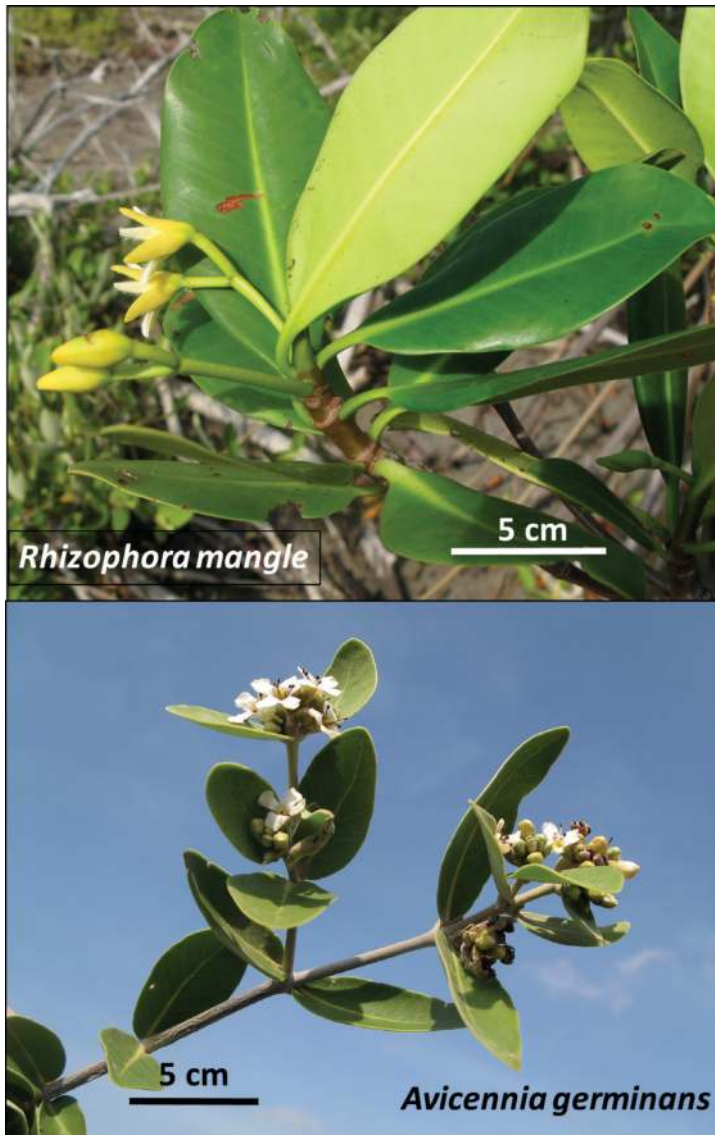


Figure 1. Flowering twig of the species studied. Notice the differences in leaf size.

2. Study sites

Field work was carried out at two locations in the Caribbean coast of northern Venezuela, both in the State of Falcón. The site, further on called Tacuato, is a low stature mangrove

stand (<5 m tall) of the species *Rhizophora mangle* and *Avicennia germinans* growing in a hypersaline lagoon (salinity >1000 mmol kg⁻¹ ≈ 35 ppt) located south of the village Tacuato on the Paraguaná peninsula (11°41'40"N, 69°49'52"W). The climate is dry (<400 mm rainfall) with one rainy season from September to December. The lagoon has access to the gulf of Tacuato with an average salinity of 45 ppt (1600 mmol kg⁻¹). The water depth of the lagoon in the sampling area varied between 0 and 20 cm, depending on rainfall events and tides. Diurnal air humidity was about 70–80%, whereas day air temperatures ranged from 27 to 37°C. The tallest trees of both species in the middle part of the lagoon reached a height of 5 m. Trees used for measurements were smaller, but mature, as they were flowering and fruiting. The site was divided into two sub-sites: the fringe-region and an inner site nearly 20 m apart from the fringe, differing in their average osmolality of interstitial soil water (1600 and 1800 mmol kg⁻¹, respectively).

The second study site, further on called Ricoa, is located at the fringes of the estuary of the Ricoa River west to the village of Tocópero (11°30'21"N, 69°12'19"W). Annual precipitation is about twice that of Tacuato (970 mm in average) with peaks in May–July and November–December. The soil water salinity averaged 127 mol kg⁻¹ (2–3 ppt), the diurnal air humidity was about 70–80%, like that at Tacuato; day air temperatures were in general lower with highest values around 33°C. Reduced soil salinity was a consequence of higher rainfall and the contribution of the river water run-off. At this site, the trees used for measurements were located at the estuary flood plain, and had approximately the same height as the plants used in Tacuato. Measurements and sample collection of *R. mangle* and *A. germinans* (from now on designated by their genus names) were carried out during seven field trips distributed over 9 months (from October to June), thus including dry and rainy seasons at both sites.

3. Materials and methods

3.1. Interstitial water

Interstitial water was sampled by digging 10–20 cm into the mud with a perforated plastic tube. Water salinity was determined *in situ* with a refractometer (ATAGO) calibrated with distilled water just before the measurement. Osmolality of the sampled water was calculated from salinity (in ppt) as in [22].

3.2. Gas exchange measurements

Gas exchange measurements were carried out with an open IRGA system of the type LCA 3 (ADC3, Analytical Development Co.) combined with a Parkinson leaf chamber of 6.25 cm². A photometer and a thermocouple attached to the chamber allowed the measurement of incoming light intensity and leaf temperature. Photosynthetic rates used for correlations with leaf conductance (g_l), and the concentrations of N and chlorophyll, were measured under natural conditions at saturating intensities of photosynthetic active radiation (PAR) $\geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (A_{sat}). Leaves were oriented at 90° to the incoming radiation during measurements. To obtain a range of quantum fluxes, leaves were shaded in the field by a set of fine wire nets. The wire

nets covered the photosynthesis chamber until g_i stabilized (2–3 min). A light response curve was a composite of measurements conducted on four leaves. Curves were fitted to the data using Sigmaplot 2.01 (Jandel Corporation 1994) and the following equation [23, 24]:

$$A = ((\Phi Q + A_{\text{sat}}) - \text{sqrt}((\Phi Q + A_{\text{sat}})^2 - 4 \theta \Phi Q A_{\text{sat}})) / 2\theta - R_d \quad (1)$$

where Q is the measured quantum flux and A is the rate of photosynthesis. By this procedure, we obtained the maximum photosynthetic capacity at saturating light intensity (A_{sat}) and the apparent quantum yield (ϕ).

To obtain different leaf internal CO_2 concentrations (c_i), the concentration of CO_2 in the air entering the leaf chamber was reduced stepwise below ambient by passing a part of the air flow over soda lime. Photosynthetic rates were found to be higher in the second and third leaves below the branch apex, and these leaves were used for all measurements. Photosynthesis was measured during late morning and early afternoon (10–15 hours).

3.3. Chemical analyses of the samples

After gas exchange measurements, leaves were detached and gently cleaned with a wet tissue to remove salt from their surfaces. About 7–10 leaves were used to obtain one sample. Petioles and midribs were removed. Every leaf was cut into halves of which one was put into a plastic syringe (for leaf sap extraction) and the other was put into a plastic bag (for the determination of chlorophyll, N, and P). The samples prepared in that way were immediately frozen on dry ice. Upon returning to the laboratory, they were stored in a freezer at -5°C .

Fresh mass was determined in the field by a battery powered balance (precision ± 0.01 g). Samples were dried at approximately 70°C in a ventilated oven until constant weight. Total chlorophyll (a + b) ($\text{Chlor}_{\text{tot}}$) concentration of leaf disks was measured by spectrophotometry of acetone extracts [25]. Syringes containing the samples were thawed, and leaf sap was squeezed out with a pressure device [20]. Osmolality of the leaf sap was determined with a dew point osmometer (WESCOR 5500). Total P concentration was measured in acid digested dry leaf material following the procedure of Murphy and Riley [26]. Nitrogen concentration was measured using a standard microKjeldahl procedure [27]. These measurements were contrasted with the parallel analysis of calibrated leaf material (peach leaf or citrus leaf, National Institute of Standards and Technology, USA). Sample preparation for organic compounds analyses and the chromatographic determinations of cyclitols in *Rhizophora mangle* and glycinebetain in *Avicennia germinans* have been described in detail elsewhere [14, 28, 29]. Measurement of carbon isotope ratios ($\delta^{13}\text{C}$ values) of leaf material was performed at the Institute of Botany and Microbiology, University of Munich following standard procedures described elsewhere [30].

3.4. Statistical analysis of data

Significant differences between means of species and sites were tested with a one-way analysis of variance (ANOVA) and a multiple range test after Scheffé. Differences were considered

significant when $P \leq 0.05$. Differences between means of measured parameters in the two species at the same site were tested with students t-test at the level of $P = 0.05$. All statistical analyses were done using Statgraphics 5.0.

4. Results

4.1. Leaf morphology

Leaves of *Rhizophora* showed large differences between sites. They were thin and green at Ricoa, but showed a leathery texture and a yellowish color at the two hypersaline sites. At the Tacuato sites, leaves were smaller, had their edges bent downward, and showed an angle well above 45° from the horizontal. In *Avicennia*, differences in leaf morphology were not that obvious, but leaf inclination was also more pronounced in the hypersaline sites. In *Avicennia*, crystals of secreted salt could be observed on the leaf surface at both Tacuato sites.

Leaf length (L) in both species was reduced in the hypersaline sites, while leaf width (W) was reduced only in *Rhizophora* (Table 1). As a result, *Avicennia* leaves from hypersaline sites tended to be rounder than those of the low salinity site (smaller L/W ratio).

For both species, average area of a single fully expanded leaf was greater at the low salinity site (Table 2). Leaves from the two hypersaline sites differed in size only for *Rhizophora*. Reduction in leaf area from low to high salinity site was more pronounced for *Rhizophora* (37–59%) than for *Avicennia* (26–34%). Leaf dry mass decreased significantly in hypersaline sites only in the case of *Rhizophora*, but fresh mass decreased in both species. The fresh mass/dry mass ratio was higher for *Rhizophora* at all sites. Leaf dry mass/area ratios were significantly lower for both species at the Ricoa site, and the differences between species within sites were only significant in the case of Tacuato-lagoon (Table 2).

Site	n	Length (cm)	Width (cm)	L/W
<i>Rhizophora mangle</i>				
Ricoa	21	12.1 ^{a,0}	5.6 ^{a,0}	2.2 ^{a,0}
Tacuato-fringe	78	9.8 ^{b,0}	4.3 ^{b,0}	2.3 ^{a,0}
Tacuato-lagoon	41	8.6 ^{c,0}	3.3 ^{c,0}	2.6 ^{a,0}
<i>Avicennia germinans</i>				
Ricoa	27	7.9 ^{A,1}	2.6 ^{A,1}	3.1 ^{A,1}
Tacuato-fringe	85	5.4 ^{B,1}	2.6 ^{A,1}	2.1 ^{B,1}
Tacuato-lagoon	87	5.1 ^{B,1}	2.5 ^{A,1}	2.1 ^{B,1}

In columns, different superscript letters denote significant differences ($P < 0.05$) between sites and leaves of one species in different sites; different superscript numbers denote significant differences ($P < 0.05$) between species at the same site

Table 1. Average values of leaf dimensions from adult leaves collected at the different sites.

Species and Sites	n	Area	Dry mass	Fresh mass	Dry mass/Area	Fresh/Dry mass
		cm ²	g	g	g m ⁻²	g g ⁻¹
<i>Rhizophora mangle</i>						
Ricoa	11	50.9 ^{a,0}	0.92 ^{1,0}	2.89 ^{a,0}	180 ^{a,0}	3.16 ^{a,0}
Tacuato-fringe	21	32.1 ^{b,0}	0.77 ^{b,0}	2.11 ^{b,0}	239 ^{b,0}	2.73 ^{b,0}
Tacuato-lagoon	15	21.1 ^{c,0}	0.50 ^{c,0}	1.28 ^{c,0}	237 ^{b,0}	2.58 ^{c,0}
<i>Avicennia germinans</i>						
Ricoa	9	14.8 ^{A,1}	0.28 ^{A,1}	0.75 ^{A,1}	187 ^{A,0}	2.70 ^{A,1}
Tacuato-fringe	20	10.9 ^{B,1}	0.27 ^{A,1}	0.68 ^{AB,1}	250 ^{B,0}	2.41 ^{B,1}
Tacuato-lagoon	14	9.7 ^{B,1}	0.26 ^{A,1}	0.63 ^{B,1}	267 ^{B,1}	2.38 ^{B,1}

Statistical notations as in **Table 1**.

Table 2. Area/mass relationships in adult leaves collected at the different sites.

4.2. Osmotic adaptation to salinity of the soil solution

Salinity of interstitial water differed by more than one order of magnitude between the low and high salinity sites (**Table 3**). Within the high salinity site, the Tacuato-lagoon showed always larger osmolalities than the Tacuato-fringe, because the former was not always in contact with the bay water, so that concentration through evaporation could not be compensated by tides.

Leaf sap osmolality was also higher for both species in the high salinity sites, particularly at Tacuato-lagoon, but absolute values were only 1.5–1.8 times higher than at Ricoa. Average data of **Table 3** show that osmolality was well above 100 mmol kg⁻¹ higher in *Avicennia* than in *Rhizophora* for all values of soil salinity. The leaf sap-soil osmolality difference in *Rhizophora* decreased from nearly 900 mmol kg⁻¹ in Ricoa to negative values approaching 100 mmol kg⁻¹ in Tacuato. In *Avicennia*, the reduction of Δ leaf sap-soil was also very strong, but average values were always positive. The sap-soil differences were larger in *Avicennia*, and the differences between sites were all significant.

Site	n	Osmolality soil solution	<i>Rhizophora mangle</i>			<i>Avicennia germinans</i>		
			n	Osmolality of leaf sap	Δ sap-soil	n	Osmolality of leaf sap	Δ sap-soil
Ricoa	3	127 ^a	11	1037 ^{a,0}	914 ^{a,0}	9	1226 ^{A,1}	1103 ^{A,1}
Tacuato-fringe	6	1666 ^b	21	1631 ^{b,0}	-63 ^{b,0}	20	1859 ^{B,1}	180 ^{B,1}
Tacuato-lagoon	5	1862 ^c	15	1893 ^{c,0}	-92 ^{b,0}	14	2027 ^{B,1}	54 ^{C,0}

Units: mmol kg⁻¹

Statistical notations as in **Table 1**

Table 3. Average values of osmolality of soil solution and leaf sap of *R. mangle* and *A. germinans* at the different sites.

4.3. Concentration of compatible solutes

In *Rhizophora*, the main compatible solute is the cyclitol 1D-1-O-methyl-muco-inositol (OMMI) [29]. Its concentration in the leaf sap increased with osmolality from nearly 80 mmol L⁻¹ in Ricoa to about 160 mmol L⁻¹ at Tacuato (**Table 4**). The other cyclitols present in *Rhizophora* (L-quebrachitol, L-chiro-inositol, and D-pinitol) were present only as minor components.

The only compatible solute in *Avicennia* is the quaternary ammonium compound glycinebetaine [14]. It reached concentrations of about 120 mmol L⁻¹ at Ricoa to 180 mmol L⁻¹ at Tacuato. Glycinebetaine contained between 15 and 21% of total leaf N with higher values found at the hypersaline sites (calculated with values from **Tables 4** and **5**). At all sites, concentrations of glycinebetaine in the leaf sap of *Avicennia* were higher than those of cyclitols in *Rhizophora*.

4.4. Total phosphorus, nitrogen, and chlorophyll concentrations

Both total P and N concentrations per unit leaf mass were higher in *Avicennia* than in *Rhizophora* (**Table 5**). In both species, no differences in total P concentration between sites were detected. However, concentrations of P and N per unit leaf area increased with salinity in both species because of the higher leaf mass/area ratios. The N to P molar ratios varied between 23 and 29 in both species, suggesting that P was not a limiting nutrient in these soils.

Concentration of Chl_{tot} per leaf area at a given site was always higher in *Avicennia* than in *Rhizophora*, but there was not a clear pattern relating chlorophyll concentration with salinity (**Table 5**). The ratio Chlor_{tot}/N was higher in *Rhizophora*, but the differences disappear if the amount of N invested in glycinebetaine is subtracted, suggesting similar N allocation to photosynthetic structures. In both species, this ratio decreases significantly in hypersaline sites.

Site	n	mmol L ⁻¹	
		OMMI	∑cyclitols
<i>Rhizophora mangle</i>			
Ricoa	11	77.2 ^a	88.1 ^a
Tacuato-fringe	19	125.4 ^b	141.3 ^b
Tacuato-lagoon	15	159.4 ^c	172.6 ^c
<i>Avicennia germinans</i>			
		Glycinebetaine mmol L ⁻¹	GB N/Total N %
Ricoa	9	120.1 ^A	14.8 ^A
Tacuato-fringe	20	165.8 ^B	20.1 ^B
Tacuato-lagoon	14	178.1 ^B	21.1 ^B

OMMI = ortho-methyl-muco-inositol (other cyclitols include quebrachitol, chiroinositol and pinitol).

Statistical notations as in **Table 1**

Table 4. Concentration of compatible solutes in *Rhizophora mangle* (cyclitols) and *Avicennia germinans* (glycinebetaine) in adult leaves collected at the different sites.

SITE		P	N	N	N/P	Chlor _{tot}	Chlor/N	GB
		μmol g ⁻¹	μmol g ⁻¹	mmol m ⁻²	molar	μmol m ⁻²	mmol mol ⁻¹	mmol mol ⁻¹
<i>Rhizophora mangle</i>								
RICOA	(5) *	31 ^{a,0}	(11)** 910 ^{ab,0}	161 ^{a,0}	29	328 ^{a,0}	2.04 ^{a,0}	
TACUATO-fringe	(5)	33 ^{a,0}	(21) 817 ^{a,0}	195 ^{b,0}	25	325 ^{a,0}	1.67 ^{b,0}	
TACUATO-lagoon	(5)	31 ^{a,0}	(15) 1017 ^{b,0}	240 ^{b,0}	33	383 ^{b,0}	1.61 ^{b,0}	
<i>Avicennia germinans</i>								
RICOA	(4)	50 ^{A,1}	(9) 1365 ^{A,1}	258 ^{A,1}	27	467 ^{A,1}	1.82 ^{A,1}	2.1
TACUATO-fringe	(5)	52 ^{A,1}	(20) 1208 ^{B,1}	304 ^{B,1}	23	359 ^{B,1}	1.19 ^{B,1}	1.5
TACUATO-lagoon	(5)	50 ^{A,1}	(14) 1204 ^{B,1}	324 ^{B,1}	24	411 ^{c,0}	1.29 ^{B,1}	1.6

*Number of samples for P.

**Number of samples for the rest of the columns.

Statistical notation as in **Table 1**.

Table 5. Average nitrogen, phosphorus, and chlorophyll concentrations of leaf samples taken from the different sites.

4.5. Photosynthetic response to light intensities and internal CO₂ concentration

Light response curves for both species showed a clear reduction in light saturated photosynthesis in hypersaline sites, 36% in *Rhizophora* and 21% in *Avicennia* (**Figure 2**). *Avicennia* had higher rates of A_{sat} than *Rhizophora* at low and high salinity sites. The reductions in photosynthetic light use efficiency (Φ) reached 45% in *Rhizophora* and nearly 37% in *Avicennia*. Light compensation points were not so much affected by salinity, although the number of curves measured (4) does not allow a definitive conclusion.

Photosynthetic response to increasing intercellular CO₂ was measured at CO₂ concentrations near that of ambient air (≈350 ppm) and below (**Figure 3**). Hence, the transition from the linear part of the curve to the plateau of CO₂ saturation was not reached. The initial slope of the curve, representing carboxylation efficiency, was steeper in *Avicennia*, and in this species compensation values were lower than in *Rhizophora*. The carboxylation efficiency was reduced at the hypersaline site by 39% in *Rhizophora* and 26% in *Avicennia*, compared to that at the freshwater site, while compensation values were nearly unchanged.

4.6. Average values of gas exchange parameters

Average light intensities and temperature recorded during the measurement of A_{sat} under natural conditions were similar for both species at each site, but the temperature was higher at the hypersaline sites (**Figure 4**). At any given site, A_{sat} was higher in *Avicennia* than in *Rhizophora*. Differences between sites were significant for both species. At Tacuato-fringe, the values of A_{sat} in both species were only about 70% of those measured at Ricoa. At the Tacuato-lagoon site, A_{sat} was even lower, especially in *Rhizophora*.

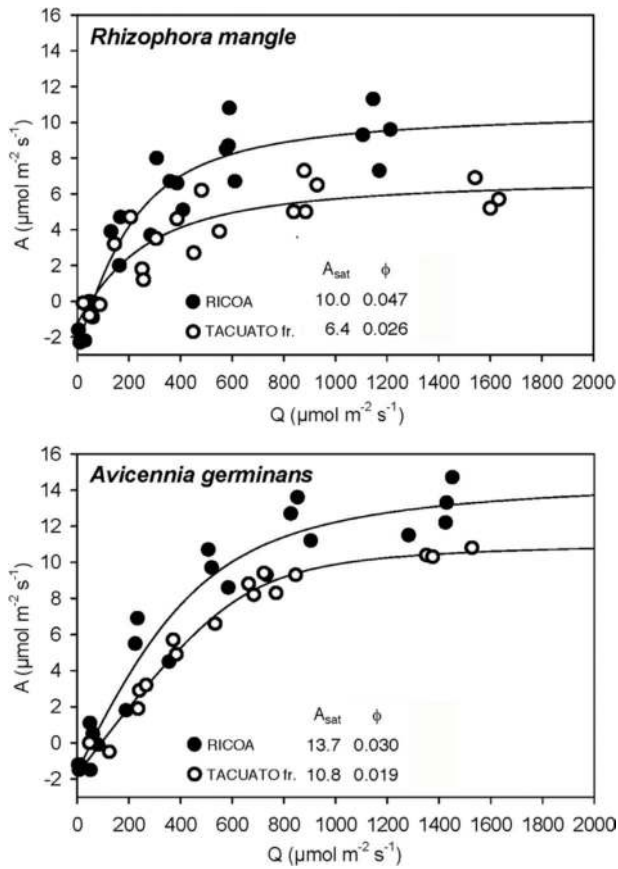


Figure 2. Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) versus light intensity ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) measured in four leaves per species at each site. ϕ : Apparent quantum yield; A_{sat} : light saturated photosynthetic rate.

Leaf conductance to water vapor (g_l) showed a similar pattern to that of A_{sat} ; however, relative differences between the low salinity site and the hypersaline sites were more pronounced in the former. Differences between the two hypersaline sites were significant in *Rhizophora* only (**Figure 4**). Intrinsic water use efficiency (A/g_l) was higher in both species at the hypersaline sites, maximum values corresponding to *Rhizophora* at Tacuato.

4.7. Carbon isotope discrimination

As expected, values for $\delta^{13}\text{C}$ followed a pattern opposite to A/g_l (**Figure 4**). ^{13}C discrimination (Δ) was calculated from leaf $\delta^{13}\text{C}$ values using formulation of Farquhar *et al.* [51] ($\delta^{13}\text{C}$ air = -8‰). Discrimination values were lower at the hypersaline sites for both species, whereas they showed no significant differences at the hypersaline site, between Tacuato-fringe and Tacuato-lagoon. Differences between species were only significant at the Ricoa site, with *Rhizophora* having higher Δ values. This pattern in Ricoa was expected as *Rhizophora* showed distinctly higher leaf conductance.

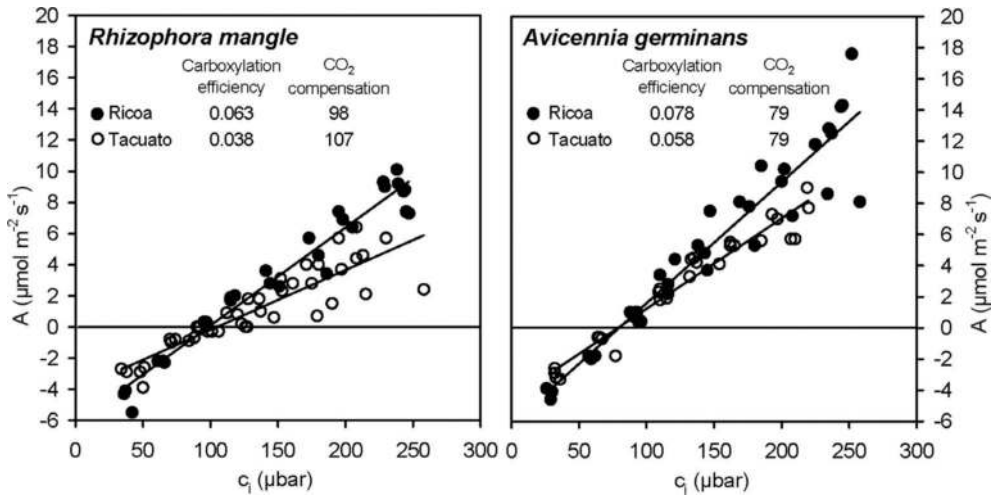


Figure 3. Dependence of photosynthetic rate on intercellular CO₂ concentration measured in four leaves from each species at each site.

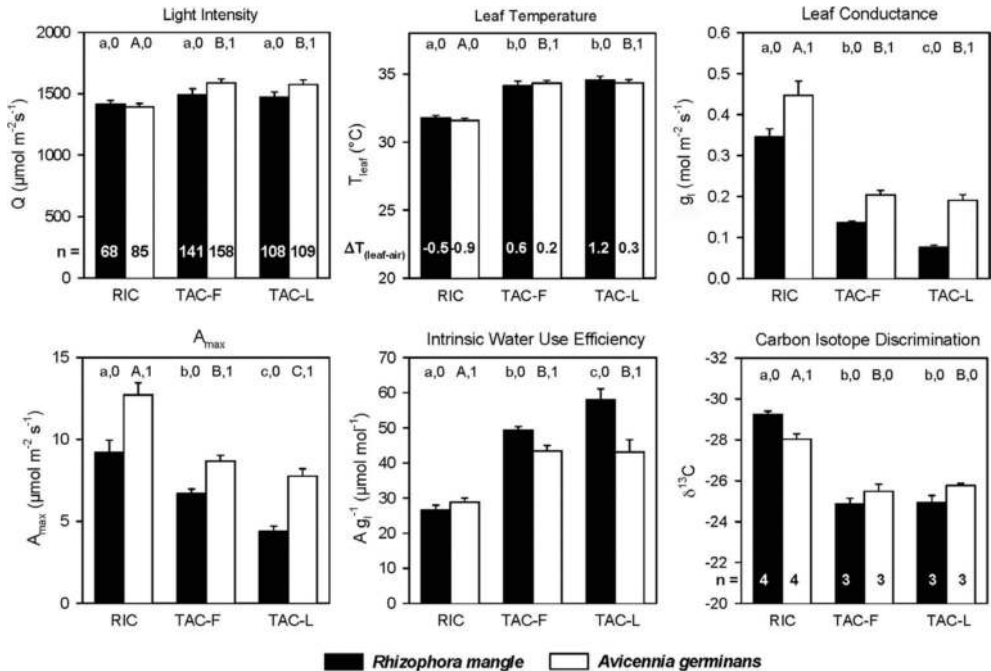


Figure 4. Average light intensity, leaf temperature, and gas exchange characteristics of the investigated species. In the upper left panel, the numbers within the columns indicate the number of leaves measured, while in the right-hand panel, they indicate the difference in temperature between the leaf and the surrounding air. On top of the columns, letters indicate significance of differences between sites for a given species. Numbers indicate differences between species at the same site.

4.8. Efficiency of photosynthetic resource use

Mass-based assimilation rate can be used as a measure of the biomass use efficiency in photosynthesis. As previously shown for A_{sat} per unit area, at any given site, *Avicennia* also showed higher assimilation rates per unit leaf dry mass than *Rhizophora* (Table 6). Comparing sites, values were higher at the low salinity site and decreased with salinity.

Water use efficiency was estimated as the ratio of A to transpiration E (calculated using leaf conductance, ambient relative humidity, and temperature). The ratio A/E showed the same pattern of intrinsic water use efficiency for short-term water use, that is, similar for both species at Ricoa, but higher for *Rhizophora* in the hypersaline sites.

Nitrogen use efficiency (A_{sat}/N) was lower in *Avicennia* at all sites, when using total N concentration as basis for calculation. When the amount of N bound in glycinebetaine from total N in *Avicennia* is subtracted, differences between the species disappeared at Ricoa and Tacuato-fringe, but at Tacuato-lagoon, *Rhizophora* still showed a lower A/N index.

4.9. Relationships between leaf gas exchange and specific leaf area, and osmotic properties

We calculated correlations for a complete subset of data including A_{sat} on a dry mass basis, leaf conductance, specific leaf area, osmolality, and N. For these calculations, average values of photosynthesis of the leaves pooled for chemical analyses were used. In all cases, higher correlations were found when using dry mass as a reference basis.

In both species, leaf conductance, specific leaf area, and N concentration were positively, whereas osmolality was negatively, and significantly correlated with photosynthetic rate (Table 7). The N-photosynthesis correlation in *Avicennia* increased from 0.67 to 0.79 when the glycinebetaine-N was subtracted from total N.

	n	A_{sat} $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$	A/E mmol mol^{-1}	A/N	A/N-GIBet.N $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ s}^{-1}$	A/Chlor
<i>Rhizophora mangle</i>						
Ricoa	11	51.6 ^{a,0}	1.5 ^{a,0}	56.7 ^{a,0}	—	27.7 ^{a,0}
Tacuato-fringe	21	28.3 ^{b,0}	2.5 ^{b,0}	34.6 ^{b,0}	—	20.9 ^{b,0}
Tacuato-lagoon	15	18.6 ^{c,0}	2.8 ^{c,0}	18.4 ^{c,0}	—	11.7 ^{c,0}
<i>Avicennia germinans</i>						
Ricoa	9	68.5 ^{A,1}	1.7 ^{A,0}	49.7 ^{A,0}	58.8 ^{A,0}	27.2 ^{A,0}
Tacuato-fringe	20	35.0 ^{B,1}	2.3 ^{B,1}	28.7 ^{B,0}	34.6 ^{B,0}	24.1 ^{A,1}
Tacuato-lagoon	14	29.4 ^{B,1}	2.2 ^{B,1}	24.3 ^{B,1}	28.1 ^{B,1}	18.8 ^{B,1}

A: Maximum photosynthetic rate; E: Transpiration rate, N: Nitrogen concentration; N-GIBet. N: Nitrogen concentration minus nitrogen bound in glycine betaine; Chlor: Total chlorophyll concentration.

Statistical notations as in Table 1.

Table 6. Efficiency of resource use in photosynthesis.

Photosynthesis	<i>Rhizophora mangle</i>	<i>Avicennia germinans</i>
$\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ dry mass. s}^{-1}$ vs	(n = 47)	(n = 43)
Leaf conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)	0.91***	0.90***
Specific leaf area ($\text{m}^2 \text{ g}^{-1}$ dry mass)	0.79***	0.88***
Osmolality (mmol kg^{-1})	-0.79**	-0.81***
Nitrogen ($\mu\text{mol g}^{-1}$ dry mass)	0.78** (n = 11)	0.67***
N-glycinebetaine ($\mu\text{mol g}^{-1}$ dry mass)	—	0.79***

Statistical significance:** (P = 0.05);
*** (P = 0.01).

Table 7. Correlation coefficients between saturated photosynthesis under field conditions and specific leaf area, and leaf conductance to water vapor and nitrogen concentration.

5. Discussion

5.1. Leaf morphology, leaf size, and leaf water content

A conspicuous visual feature observed in the field was that leaves had a high degree of inclination at the hypersaline sites. Ball *et al.* [31] showed that the high degree of leaf inclination found in mangrove species in nature effectively reduces the intensity of radiant heat loading. Furthermore, in both species, a marked reduction in leaf area in hypersaline sites was observed. This may also improve the energy balance in saline and dry sites, as in smaller leaves convective cooling is more effective.

Lin and Sternberg [32] found a reduction in leaf size in dwarf scrub mangroves in contrast to tall growing fringe mangroves in Florida. Salinity, as well as nutrient level, may cause reductions in leaf area while neither sulfide nor original growth form had an influence [33].

In the present study, reductions in leaf area and dry mass associated with salinity were, respectively, 59 and 46% in *Rhizophora* compared to the much lower reductions of 34 and 7% in *Avicennia*, suggesting a higher salt sensitivity in the former species. In *Avicennia*, not only leaf size but also leaf shape was affected by high salinity, leaves of high salinity sites being rounder than those of low salinity sites.

Larger leaf dry mass/area ratios at the hypersaline sites have been reported before and attributed to increased succulence [34, 35, 36], although there was also evidence for scleromorphy [34]. We observed in both species a significant decrease in the fresh mass/dry mass ratio that together with the increase in leaf mass/area ratio are rather symptoms of scleromorphy than of succulence. Generally, higher leaf mass/area ratios increase heat capacity and may be of importance in controlling leaf temperature [31].

5.2. Osmotic adaptation

Increases of leaf sap osmolality with soil salinity in both species counteracted the lower soil water potential at the hypersaline sites. In *Rhizophora*, differences between leaf sap and soil

solution osmolalities were small or negative at the hypersaline sites, whereas in *Avicennia* differences were always positive. Scholander *et al.* [21], and Walter and Steiner [37] found that the osmotic potential in mangrove leaves exceeded that of the seawater surrounding them. However, in a field study in Venezuela, Rada *et al.* [38] found that turgor loss occurred at mid-day in leaves of *Conocarpus erectus* and *Rhizophora mangle* during drought periods. We did not measure water potential in the investigated plants, but both conductance and photosynthetic rates measured did not indicate turgor loss even at the most stressful sites.

At the low salinity site for both species, osmolalities of the leaf sap were found to be about 10 times higher than that of the soil solution, indicating their halophytic (salt accumulating behavior) character [15, 17]. The generally larger values of leaf sap osmolality in *Avicennia* are consistent with the higher salinity tolerance of species of this genus [13, 14, 19, 20, 37].

Concentrations of compatible solutes were clearly correlated to soil salinity and were within the range reported in other field studies [14]. Glycinebetaine concentrations were higher than those of cyclitols at each site, and in both cases their concentration increased with leaf sap osmolality. This is in accordance with their postulated role in keeping osmotic equilibrium between cytoplasm and vacuole. However, the cyclitol/osmolality ratio was 1.25 in *Rhizophora*, while the glycinebetaine/osmolality ratio in *Avicennia* was only 0.7, suggesting a higher osmo-protective efficiency of this compound.

5.3. Phosphorus, nitrogen, and chlorophyll

Studies of nutrient availability in soils under several mangrove stands showed that P availability may be limiting growth, especially under oxidized conditions of well drained soils [39]. Growth limitation by P was confirmed by fertilization studies on dwarf red mangrove in Belize [40], where an increase in growth was brought about only by application of NPK or P. In the present study, leaf P concentration did not differ between sites but was always higher in *Avicennia* compared to *Rhizophora*. Besides, N to P molar ratios were below 35 suggesting that P supply was not limiting mangrove growth in the study sites.

Leaf N concentrations of *Avicennia* were significantly higher than those of *Rhizophora* as has been found earlier [20, 41] and reported for Australian species of these genera by Popp *et al.* [14]. Part of the difference can be explained by the amount of glycinebetaine in *Avicennia*, representing 15–21% of total leaf N. Differences in N concentration between species disappear when this fraction is subtracted from total N.

Differences in N concentrations between sites were mainly related to differences in leaf mass/area ratio in both species. While N concentration decreased with salinity when calculated on a dry mass basis, they increased based on leaf area. *Rhizophora* leaves at Tacuato-lagoon, however, showed a higher concentration of N per leaf area as well as per g dry mass, indicating a strong reduction in nitrogen use efficiency.

Chlorophyll concentrations per leaf area were similar or lower than those reported earlier for the same species in dry and wet habitats [20], or for mangrove species in Australia and India growing on a range soil salinities [42, 43]. The average Chl_{tot}/N ratios decrease markedly in hypersaline sites, pointing to a reduction in N investment in photosynthetic structures due to salt stress. Besides, these ratios were low compared with earlier reports on these species [20], a fact perhaps related to the much lower leaf N/P ratios found in the

present study. Values of $\text{Chl}_{\text{tot}}/\text{N}$ at a given site did not differ significantly between species, when the amount of N bound in glycinebetaine in *Avicennia* was subtracted from total N. It seems that under similar salinity stress, both species invest a similar N fraction into the construction of photosynthetic structures.

The fractional investment of leaf N into chloroplast protein-pigment complexes can be calculated using the N to chlorophyll ratio in thylakoids estimated by Evans [44] (50 mol thylakoid N/mol chlorophyll). Both species had values ranging from 9% of leaf N in low salinity sites to 5–6% in high salinity sites. *Rhizophora* had always slightly higher values than *Avicennia*. Those values are about half of the average reported for lowland trees in humid tropical forest (107 species, $23.7 \pm 0.8\%$ of leaf N) [45]. The large difference underscores the photosynthetic cost of high salinity tolerance.

5.4. Photosynthetic capacity

Avicennia showed consistently higher assimilation rates than *Rhizophora*, in accordance with previous reports on other species of the same genera [11, 46, 47]. Our results showed that both species had lower A_{sat} at the hypersaline sites. However, the depression of A_{sat} related to high salinity was more pronounced in *Rhizophora*.

Light saturated photosynthetic capacity reflects the maximum possible benefits from a given investment in photosynthetic machinery [48]. Zotz and Winter [49] showed a linear relationship between diurnal carbon gain and maximum rate of CO_2 uptake in a range of rainforest canopy species. This would explain the low growth rates and the shrubby stature of the plants at the hypersaline sites. In addition, as constructing and maintaining photosynthetic machinery is energetically expensive, photosynthetic capacity should be tuned to the constraints of the environment [48]. The most prominent factor in mangrove habitats is salinity. In *Aegiceras corniculatum* and *Avicennia marina*, photosynthetic capacity was found to decrease with increasing salinity [50, 51], and A_{sat} was negatively related to salinity in a range of mangrove species under field conditions [11]. Other environmental factors such as low nutrient availability [33] and temporal variation in salinity [52] are also known to depress maximum assimilation rate of mangroves. Extreme low values of A_{sat} in *Rhizophora* leaves at Tacuato-lagoon may be related to a combination of salinity with one or more of the latter mentioned factors.

Values of light saturated A calculated from light response curves confirmed that the photosynthetic capacity was generally higher in *Avicennia* compared to *Rhizophora* and was reduced in both species at the hypersaline sites.

Quantum yield (ϕ) on an incident light basis was depressed at the hypersaline sites in both species. Björkman *et al.* [46] found that quantum yield in mangrove leaves decreased due to the combination of low leaf water potentials with high irradiance; whereas salinity, and the resulting leaf water deficit, had no negative effect on the quantum yield of mangrove leaves protected from direct sunlight.

The C_i - A_{sat} relationships obtained for CO_2 concentrations equal or below ambient were linear, with a slope proportional to the *in vivo* activity of Rubisco (carboxylation efficiency, CE [53]). The lower CE of both species at the hypersaline site indicates that decreases of A_{sat} with salinity were not only due to stomatal limitation, but also due to the result of changes in the biochemical properties of photosynthesis.

CO₂ compensation points were higher in *Rhizophora* than in *Avicennia*, suggesting higher photorespiration rates in the former species. However, species characteristic compensation values were similar at Tacuato and Ricoa. Similar results were obtained by Ball and Farquhar [12] with mangrove species grown at different salinities.

5.5. Water use and N use efficiency in photosynthesis

At the low salinity site, g_i was significantly higher for both species. The generally higher values of g_i of *Avicennia* compared to those in *Rhizophora* are correlated with their assimilation rates. At the hypersaline sites, g_i was reduced to a greater proportion than A_{sat} in both species, but in *Rhizophora*, the reduction of these parameters was more pronounced indicating the lower salinity tolerance of this species.

Intrinsic water use efficiency (A/g_i) evaluates the role of biological components in determining water-carbon exchange relationships [50, 51]. In both species, A/g_i was higher at the hypersaline sites, because of the relatively larger reduction of g_i compared to A_{sat} . Values of A/g_i were similar to those calculated from data from Smith *et al.* [54] for *Avicennia germinans* and *Conocarpus erectus* at a coastal site in northern Venezuela and to those calculated from data from Lin and Sternberg [32] for *Rhizophora mangle* at a site in coastal Florida. In an extensive field study, Clough and Sim [11] found higher water use efficiency in mangroves with increasing salinity and decreasing air humidity. As air humidity in our study did not differ much between sites, changes in A/g_i with salinity were less drastic than in the study mentioned above.

Water use efficiency was higher in *Avicennia* at the low salinity site, but it was higher in *Rhizophora* in the high salinity sites. More conservative water use in the latter species at the hypersaline sites is probably related to its non-salt secreting character. Water loss is minimized as salt exclusion mechanisms at the roots impose a large resistance to water flow [55]. The higher water use efficiency in *Avicennia* at the low salinity site may be related to the association of this species with the more saline soils [15]. However, as in *Avicennia*, leaf-to-soil osmolality difference was at least 50 mmol kg⁻¹ at the hypersaline sites, and as accumulation of NaCl can be counteracted by salt secretion, and in this species restriction of water loss from leaves under hypersaline conditions was lower than in *Rhizophora*.

Nitrogen use efficiency in photosynthesis based on total leaf N was higher in *Rhizophora* at all sites. Similar results were reported by Alongi *et al.* [5] in Australian mangrove forests of *R. stylosa* and *A. marina*. However, those differences disappear if the amount of N invested in glycinebetain is subtracted from the total amount of N. In both species, the NUE decreases in hypersaline sites. In an experimental study, Cardona-Olarte *et al.* [16] did not find differences in WUE based on gas exchange of seedling grown in nutrient solutions with salinities between 10 and 40 ppt; however, PNUE decreased from about 85 μmol/mol N at 10 ppt to nearly 60 at 40 ppt.

5.6. Carbon isotope discrimination

The carbon isotope ratio $\delta^{13}C$ is related to a long-term average of c_i and can be taken as an indicator of water use efficiency [56, 57]. Values of $\delta^{13}C$ ranged between -24.3‰ and -29.4‰ ($\Delta = 16.7$ to 22.1‰), with a larger variation found in *Rhizophora*. They were well within the

range reported for mangroves in the literature [9, 20, 32, 47, 58]. These results confirmed for the long-term, the patterns found for short-term water use efficiency discussed above.

6. Conclusions

The relationships between the set of physiological properties associated with high salinity stress in both mangrove species studied here can be depicted along two sequences of events operating simultaneously (Figure 5). Increases in interstitial water salinity affect

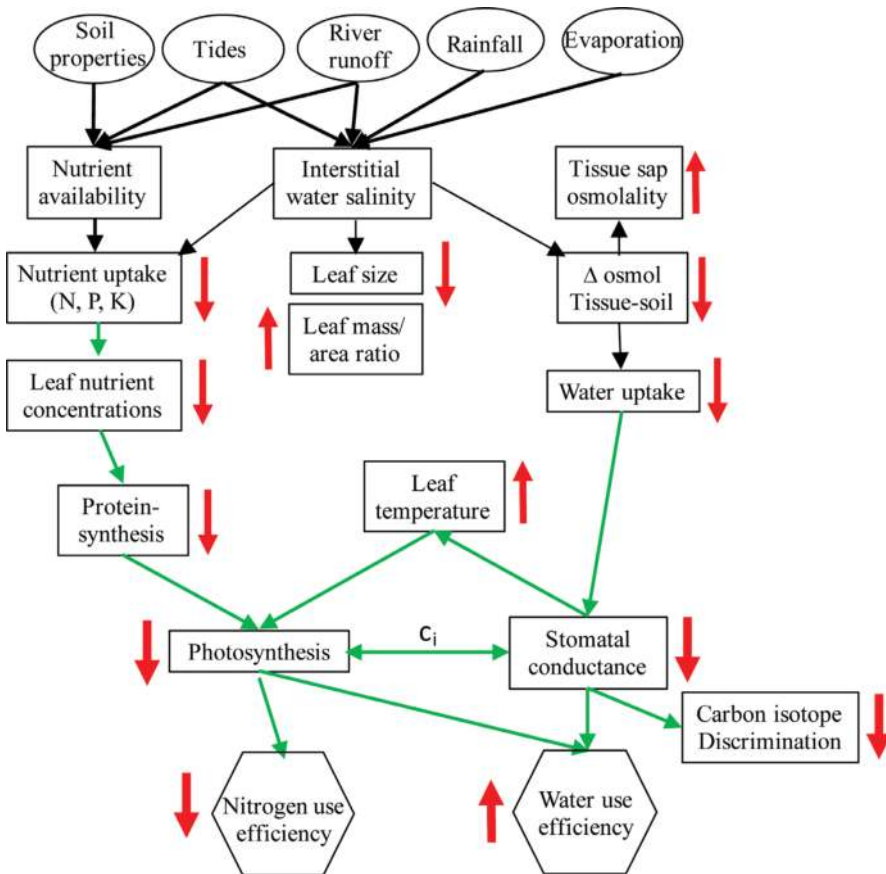


Figure 5. Scheme depicting the assumed sequence of events affecting photosynthesis and resource use efficiency caused by exposure to high salinity conditions. The driving forces for environmental salinity are encapsulated as tides (sea water supply), rainfall (dilution and washing-out effects), atmospheric evaporative demand (air water saturation deficit), and soil properties. Thick red arrows indicate the direction of change resulting from long-term exposure to high salinity. Thin black arrows indicate the plant-environment interface. Green arrows depict the hypothesized dependence of biological processes triggered by increases in cell sap osmolality and leaf nutrient status. The connections between the boxes are not necessarily linear, and processes affected may show differential sensitivity toward interstitial soil water salinity. Generally, mangrove-environment interactions under high salinity conditions lead to higher water use and lower N use efficiencies.

essential nutrients uptake and salt accumulation, determining increases of tissue sap osmolality. Both processes lead to a nutritional limitation of photosynthesis resulting in a strong reduction of nitrogen use efficiency for growth. In addition, the differential soil–plant osmotic potential decreases reducing the amount of water available for transpiration, and inducing an accumulation of compatible solutes that protect cytoplasmic organelles from dehydration and toxic ionic effects. As a result, leaf conductance is reduced to a larger proportion than photosynthesis, thereby increasing leaf temperature and water use efficiency. The connections between the boxes of **Figure 5** are not necessarily linear, and processes affected may show differential sensitivity toward interstitial soil salinity (as shown in the ratio of conductance to photosynthesis). In the processes documented in the present chapter, *Rhizophora* appears to be more sensitive than *Avicennia*, and we speculate that the ultimate cause of this difference may reside in the higher efficiency of glycinebetaine as an osmoprotectant compared to cyclitols.

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