Research Article

Leaf Gas Exchange and Nutrient Use Efficiency Help Explain the Distribution of Two Neotropical Mangroves under Contrasting Flooding and Salinity

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Rhizophora mangle and *Laguncularia racemosa* cooccur along many intertidal floodplains in the Neotropics. Their patterns of dominance shift along various gradients, coincident with salinity, soil fertility, and tidal flooding. We used leaf gas exchange metrics to investigate the strategies of these two species in mixed culture to simulate competition under different salinity concentrations and hydroperiods. Semidiurnal tidal and permanent flooding hydroperiods at two constant salinity regimes (10 g L⁻¹ and 40 g L⁻¹) were simulated over 10 months. Assimilation (*A*), stomatal conductance (*g_w*), intercellular CO₂ concentration (*C_i*), instantaneous photosynthetic water use efficiency (PWUE), and photosynthetic nitrogen use efficiency (PNUE) were determined at the leaf level for both species over two time periods. *Rhizophora mangle* had significantly higher PWUE than did *L. racemosa* seedlings at low salinities; however, *L. racemosa* had higher PNUE and *g_w* and, accordingly, had greater intercellular CO₂ (calculated) during measurements. Both species maintained similar capacities for *A* at 10 and 40 g L⁻¹ salinity and during both permanent and tidal hydroperiod treatments. Hydroperiod alone had no detectable effect on leaf gas exchange. However, PWUE increased and PNUE decreased for both species at 40 g L⁻¹ salinity compared to 10 g L⁻¹. At 40 g L⁻¹ salinity, PNUE was higher for *L. racemosa* than *R. mangle* with tidal flooding. These treatments indicated that salinity influences gas exchange efficiency, might affect how gases are apportioned intercellularly, and accentuates different strategies for distributing leaf nitrogen to photosynthesis for these two species while growing competitively.

1. Introduction

Mangroves are forested wetland habitats characterized by individual tree species that can segregate along rather diffuse environmental gradients. Salinity and hydroperiod (i.e., frequency, depth, and duration of flooding) are considered two of the more important factors that control the local distribution of mangrove species [1–5], and both factors have the potential to interact strongly along spatial and temporal scales in many floodplains [6–9]. Indeed, interactions between salinity and hydroperiod have been implicated in the regulation of survival, growth, physiological proficiency, and zonation of individual seedlings, saplings, and trees

that colonize mangrove wetlands [10, 11]. Through repetitive experimentation, we are progressively gaining a better understanding of the interactive nature of these environmental drivers on seedling establishment and competitive outcomes within floodplain settings globally [12].

Rhizophora mangle L. and *Laguncularia racemosa* (L.) C. F. Gaertn. frequently coexist and are common mangrove species along coastal reaches of riverine floodplains in the Neotropics [13–15]. *Rhizophora mangle* can occupy a broad range of tidal flooding, from highly abundant in lower intertidal zones with high annual flood durations (up 50–60%) to less abundant in upper intertidal zones with lower annual flood durations [4, 16, 17]. *L. racemosa* is often found

coexisting with *R. mangle*, but the occurrence of *L. racemosa* can favor landward extremes where salinity might at times be higher than in lower intertidal areas [14, 18]. In addition to salinity [19, 20] and hydroperiod [15, 21–23], the distribution of *L. racemosa* and *R. mangle* is also correlated with soil resources such as nitrogen (N) and phosphorus (P) [24–26]. All site factors interact considerably along such riverine flood and tidal gradients, confusing our understanding of physiological preference in Neotropical coastal floodplains.

In this study, we tested whether leaf gas exchange responses in seedlings of *R. mangle* and *L. racemosa* differed under varying conditions of salinity and hydroperiod to understand the role of these drivers in mediating the competitive interactions among these two mangrove species at an early growth stage. Proficiency in assimilation (*A*) of carbon as CO₂ during photosynthesis can set the upper limit of individual tree growth and ecosystem productivity [27]. Species-specific differences in *A* can also be suggestive of among-species competitive relationships [28–30]. Lovelock and Feller [18], for example, attributed a switch in dominance between *Avicennia germinans* (L.) L. and *L. racemosa* along salinity and nutrient gradients to leaf-level gas exchange strategies and the trade-off between photosynthetic water use efficiency (PWUE) and photosynthetic nitrogen use efficiency (PNUE). We opted for a similar investigation here but include the competitive interactions between *L. racemosa* and a different species (*R. mangle*) for comparison.

2. Methods

2.1. Plant Material. Propagules of *R. mangle* and *L. racemosa* were collected from Terra Ceia Island near Bradenton, FL USA (27°N, 82°W). Propagules were transported to Lafayette, LA, USA (30°N, 92°W), sown directly into 0.4 L plastic cups with sand, irrigated with fresh water to the soil surface, and, after seedling emergence, were replanted into 27 L buckets. Soil in the buckets consisted of an equal mix of river sand and commercial potting mix (Sta-Green, Spectrum Brands, Atlanta, GA, USA). One-half of the seedlings were subjected to a 12-week acclimation period at 10 g L⁻¹ salinity with 10 cm of surface flooding. The remaining seedlings were acclimated identically until the last 20 d when salinity was gradually adjusted to a final concentration of 40 g L⁻¹. Water levels were adjusted from 10 to 20 cm by an increment of 1.5 cm d⁻¹. Thereafter, the entire volume of experimental water was replaced every 2.5 months. Initial mean heights of seedlings were 35.8 ± 0.4 (s.e.) cm for *R. mangle* and 40.1 ± 0.7 (s.e.) cm for *L. racemosa* at the point that treatments were imposed and competition began.

Average irradiance in the greenhouse was approximately 41% of ambient sunlight. Maximum instantaneous photosynthetically active radiation (PAR) in the greenhouse was approximately 800 μmol photons m⁻² s⁻¹ and generally did not appear limiting to leaf-level photosynthesis [31]. Greenhouse temperatures were maintained above 8°C during winter months and below 43°C during summer months (mean of 22°C during experiment). Relative humidity ranged from 45 to 90%.

2.2. Experimental Design. The experiment was maintained for approximately 10 months from August to May. Plants used for gas exchange measurements were selected from a larger experimental setup in which seedlings of both species were competing in paired seedling arrays (1 *L. racemosa*; 1 *R. mangle*) within each 27 L bucket [32]. Buckets were located on tables, which produced flooded hydroperiods (permanently) and tidal hydroperiods by circulating water through reservoirs below. Each table contained six buckets: three were subjected to flooded hydroperiods and three were subjected to tidal hydroperiods. Tables were assigned in pairs to salinity of either 10 g L⁻¹ or 40 g L⁻¹ for a total of six tables per salinity treatment. Salinity concentrations were selected as approximate average (10 g L⁻¹) and approximate maximum (40 g L⁻¹) concentrations recorded from floodplain mangroves along the lower Shark River, FL, USA [22]. The entire experimental design was replicated six times on 12 independently maintained tables for a total of 72 individual seedlings per species. Maximum water level for each hydroperiod treatment was 20 cm. Tidal hydroperiods were simulated as two floods per day (0900; 1600), with each tide flooding seedlings for approximately 6 h to simulate lower intertidal conditions along the Shark River ([23] site SRS-6). Soil oxidation-reduction (redox) potentials ranged from -48 to 149 mv for tidal hydroperiods and from -112 to -17 mv for flooded hydroperiods (mixed culture experiment [32]).

2.3. Photosynthetic Measurements. Photosynthetic gas exchange characteristics were determined for 48 seedlings of *L. racemosa* and *R. mangle* during two sampling months (September, November). Subsamples of two experimental buckets per hydroperiod treatment (permanently flooded versus tidally flooded) were chosen randomly, as was the daily measurement order, which yielded 24 sample replicates (2 salinities × 2 hydroperiods × 6 experimental replicates) per species per month. From each selected seedling, one leaf was systematically selected from either the highest node in a lag phase of growth as determined by a fully developed apical sheath atop the node or, if damaged or obscured, from the node below. Of the two leaves per nodal pair, we selected the best looking leaf.

To document bias associated with measuring seedlings under either flooded or drained states during respective hydroperiod treatments (sensu [31]), gas exchange measurements in tidal hydroperiods were made under both states. Parallel measurements were conducted on seedlings grown in flooded hydroperiods for comparison (under both salinities). There were no differences in the leaf gas exchange responses evaluated in this study, while tidal treatments were flooded versus drained ($\alpha = 0.05$), so the distinction was collapsed statistically for remaining analyses (see below). All measurements were made in random but variable order by treatment between 0900 and 1330 to account for response variation over the 4.5 h period.

Each leaf was placed inside the cuvette of a LI-6400 Portable Photosynthesis System (Li-COR, Lincoln, NE, USA). Conditions inside the cuvette were selected as follows:

TABLE 1: Mixed model ANOVA results describing leaf gas exchange variables for seedlings of *Rhizophora mangle* and *Laguncularia racemosa* grown in mixed culture under different salinity and hydroperiod treatments.

Source of variation	DF, DF _{error}	A	C _i	g _w	PWUE	DF, DF _{error}	PNUE
Species	1, 5	ns	***	***	***	1, 20.1	*
Salinity	1, 5	*	***	**	**	1, 20.8	***
Hydroperiod	1, 5	ns	ns	ns	ns	1, 20.8	ns
Species × salinity	1, 5	ns	**	ns	**	1, 20.1	ns
Species × hydroperiod	1, 5	ns	ns	ns	ns	1, 20.1	ns
Salinity × hydroperiod	1, 5	ns	**	*	ns	1, 20.8	ns
Species × salinity × hydroperiod	1, 5	*	ns	**	ns	1, 20.1	ns
Month	1, 5	***	***	***	***	—	—
Salinity × month	1, 5	ns	ns	**	**	—	—
Species × month	1, 5	ns	ns	*	ns	—	—
Hydroperiod × month	1, 5	ns	ns	*	ns	—	—
Salinity × species × month	1, 5	*	ns	ns	ns	—	—

NS: not significant at the 0.05 level; * significant at 0.01–0.05 level; ** significant at 0.01–0.001 level; *** significant at <0.001 level.

A: assimilation; C_i: leaf intercellular CO₂ concentration; g_w: stomatal conductance; PWUE: instantaneous photosynthetic water use efficiency; PNUE: photosynthetic nitrogen use efficiency.

ambient temperature, ambient humidity, PAR of 800 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (red/blue light source), and reference CO₂ of 375 $\mu\text{mol mol}^{-1}$ air. Midday leaf temperatures in September averaged 32.8°C (range: 29.5–37.2°C), while leaf temperatures in November averaged 29.2°C (ranging from 29.5–31.5°C). For comparison, sapling leaf temperatures for *R. mangle* along a south Florida estuary in October and December of a previous year ranged from 26.6 to 33.5°C (unpublished data from [31]). Depending on individual leaf response, each sample was allowed 2 to 8 min to equilibrate to a steady state for A (assimilation rate) and g_w (stomatal conductance). We included the most relevant variables in this study: A, g_w, T_r (transpiration), and intercellular CO₂ concentration (C_i). PWUE (instantaneous) was calculated as the quotient of A/T_r. We also measured PNUE by determining leaf nitrogen concentration with an elemental analyzer (Model NC2500, Carlo-Erba Elemental Analyzer, Lakewood, NJ, USA). Leaves were harvested at the end of the experiment, dried to a constant weight at 60°C, and ground prior to nitrogen analysis. PNUE was calculated as the quotient of A and leaf nitrogen concentration, was destructive, and, hence, determined only for November for 6 leaves per treatment combination (i.e., overall sample size of 24 leaves per species).

2.4. Statistical Analysis. Data were analyzed with an analysis of variance (ANOVA) as a split-split-plot design with salinity designated as the whole plot, and hydroperiod and species designated as the sub- and sub-subplots, respectively. Month was also treated as a subplot; repeated measures could not be used because the sample unit (i.e., individual plant) changed between months, even though the experimental unit (i.e., table) remained constant. Two separate analyses were conducted on the overall dataset, both included species and hydroperiod as fixed effects and salinity as a random effect. A, C_i, g_w, and PWUE were analyzed as a general linear model using ANOVA with month (September versus November)

specified as a block. PNUE was determined only in November and was analyzed similarly to the other variables but with month collapsed using mixed model procedures.

Prior to all analyses, data were inspected for normality in the distribution of their residual errors and transformed when appropriate. All graphs report untransformed means. Analyses were performed by using either Proc GLM or Proc Mixed of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Assimilation and Intercellular CO₂ Concentration. At the leaf level, A did not differ between *R. mangle* and *L. racemosa* (Table 1). Salinity and month, but not hydroperiod, affected assimilation rate, even though there was a significant interaction among species, salinity, and hydroperiod (Table 1). Higher values of A were generally obtained at lower salinity, but these effects were not attributable to specific treatment combinations after Bonferroni adjustment (Figure 1). A was higher in September (Table 1) and decreased by 34.2% for *L. racemosa* and 33.7% for *R. mangle* from September to November, perhaps reflecting photoperiod, temperature, and/or relative humidity sensitivity.

Species did show significant differences in C_i, but did not display the significant interactions among species, salinity, and hydroperiod as for A (Table 1). Higher C_i was registered by *L. racemosa* at 10 g L⁻¹ salinity (Figure 2). C_i was not significantly affected by hydroperiod, but C_i was slightly depressed with experimental duration which ultimately affected A. Seasonal responses by the two species were also similar, that is, consistently lower C_i in November as ambient temperature during sampling dropped by 3.6°C and PAR increased by 146 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

There was a significant correlation between A and C_i for *L. racemosa* ($P < 0.001$; Figure 3(a)) and *R. mangle* ($P < 0.001$; Figure 3(b)). The stronger correlation and steeper slope

FIGURE 1: Instantaneous carbon assimilation (A) and stomatal conductance (g_w) for *Laguncularia racemosa* (L) and *Rhizophora mangle* (R) leaves in response to tidal flooding (TF) or permanent flooding (PF) with 10 g/L (10S) or 40 g/L (40S) salinity. Data are displayed according to the highest order significant interaction (Table 1). Values are means $\pm$ 1SE. Effects annotated with the same letter indicate lack of significance ($P > 0.05$) after Bonferroni adjustment ($\alpha = 0.05/12$) for a particular graph.

for *R. mangle* suggest that *R. mangle* tends to have higher A at a given C_i under all treatment combinations tested.

3.2. Stomatal Conductance, PWUE, and PNUE. Mean g_w was significantly greater in *L. racemosa* than in *R. mangle* across all combinations (Table 1). As was the case for A , these effects were not clear among specific treatment combinations after Bonferroni adjustment (Figure 1). Again, salinity and month significantly affected stomatal response, whereas hydroperiod did not (Figure 1), even though the two hydroperiods simulated had different effects on g_w at contrasting salinities for both species as indicated by significant salinity $\times$ hydroperiod interactions (Table 1). It is also important to stress the strong monthly response on g_w . Higher overall values were recorded in September ($0.115\text{--}0.279 \text{ mol m}^{-2} \text{ s}^{-1}$) than in November ($0.063\text{--}0.138 \text{ mol m}^{-2} \text{ s}^{-1}$) for both species.

There was a decrease in g_w in both species between months, with differences for *L. racemosa* and *R. mangle* remaining comparable (0.13 versus $0.10 \text{ mol m}^{-2} \text{ s}^{-1}$, resp., or a 58% versus 56% decrease).

PWUE was significantly higher in *R. mangle* than in *L. racemosa* at low salinity (Figure 2). Not only was there a difference between species and sensitivity to salinity and month, but also there was no effect of hydroperiod on PWUE (Table 1). *L. racemosa* had significantly higher PWUE at higher salinity (Figure 2), where PWUE increased from 2.64 ± 0.64 (s.e.) $\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$ at salinities of 10 g L^{-1} to 3.27 ± 0.77 (s.e.) $\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$ at salinities of 40 g L^{-1} .

Finally, *L. racemosa* exhibited significantly higher overall PNUE than *R. mangle* (Table 1), with differences becoming most disparate under tidal treatments at 40 g L^{-1} (Figure 4).

FIGURE 2: Leaf intercellular CO_2 concentration (C_i) and photosynthetic water use efficiency (PWUE) for *Laguncularia racemosa* (L) and *Rhizophora mangle* (R) in response to 10 g/L (10S) or 40 g/L (40S) salinity. Data are displayed according to the highest order significant interaction (Table 1). Values are means $\pm$ 1SE. Effects annotated with the same letter indicate lack of significance ($P > 0.05$) after Bonferroni adjustment ($\alpha = 0.05/12$) for a particular graph.

FIGURE 3: Relationship between intercellular CO_2 concentration (C_i) and carbon assimilation rate (A) for (a) *Laguncularia racemosa* and (b) *Rhizophora mangle* seedlings grown at different hydroperiods and salinity concentrations.

FIGURE 4: Photosynthetic nitrogen use efficiency (PNUE) and leaf nitrogen (N) concentration of *Laguncularia racemosa* (L) and *Rhizophora mangle* (R) in response to tidal flooding (TF) or permanent flooding (PF) with 10 g/L (10S) or 40 g/L (40S) salinity. Values are means $\pm$ 1SE. Effects annotated with the same letter above the bar (for PNUE) indicate lack of significance ($P > 0.05$) after Bonferroni adjustment.

Both species generally had higher PNUE at low salinity (Table 1) by tending to concentrate more foliar N at the higher salinity concentration; foliar N concentrations displayed inconsistent responses between tidally and permanently flooded hydroperiods (Figure 4).

4. Discussion

4.1. Interspecific and Seasonal Differences in Leaf Gas Exchange. Average A for *R. mangle* in this study was comparable to the 7.5 to 14.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ reported by others under similar light levels and salinity concentrations [30, 31, 33–35]. Accordingly, A for *L. racemosa*, which averaged 9.6 ± 0.29 (s.e.) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, was also comparable to field results of Lovelock and Feller [18], where A averaged $7.7 \pm 0.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for trees fertilized with P and $9.5 \pm 0.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for trees fertilized with N. On the other hand, g_w for *R. mangle* in our study (0.06–0.24 $\text{mol m}^{-2} \text{ s}^{-1}$) can only be compared to lower ranges reported by others [30, 36–38] and highlights the importance of intrinsic water conservation strategies in *R. mangle* as salinity is increased under different hydroperiods in experimental culture and presumably field settings.

A appeared to be only loosely controlled by g_w in *L. racemosa* during gas exchange measurements, thus reflecting a short-term buildup of intercellular CO_2 as sustained high light levels during measurements opened stomata acutely to CO_2 diffusion. C_i ranged from 138 to 285 $\mu\text{mol CO}_2 (\text{mol air})^{-1}$ in *L. racemosa* and from 107 to 310 in *R. mangle* across both months; C_i for healthy mangrove leaves are typically around 170 $\mu\text{mol CO}_2 (\text{mol air})^{-1}$ [39]. Yet, it is often noted that stomatal conductance has a strong effect on photosynthesis [40, 41], and T_r and g_w appeared to be synchronic in our study; dedicated studies of specific A/C_i

FIGURE 5: Stomatal conductance (g_w) as a function of the relative humidity within the sample cuvette of the Li-6400 for *Laguncularia racemosa* and *Rhizophora mangle* seedlings during two time periods. Data were pooled across species.

responses would be more conclusive (Figure 3). Apparently, however, the occurrence of higher C_i does not guarantee higher carbon assimilation if the photosynthetic capacity of the species or imposed environment is low [42]. *L. racemosa* certainly grew faster and to a larger size in this mixed-culture experiment than *R. mangle* [32]. Interspecific differences in the sensitivity of g_w with relative humidity can also be an important determinant of stomatal behavior. g_w increased exponentially with an increase in the relative humidity inside of the leaf cuvette across both species in our study (Figure 5).

Ambient temperature at the leaf surface of seedlings in September averaged 3.6°C higher than in November and likely affected the seasonal drop in leaf gas exchange. Mangrove leaf temperatures can exert an important influence on photosynthesis independent of stomatal activity [39, 42]. At physiologically acceptable low temperatures, an increase in temperature leads to spikes in A , but any increase above temperature thresholds causes a strong decrease in A [43]. This threshold for mangroves is about 35°C [10, 39]; leaf temperatures in this study were measured to a maximum level of 37.2°C (mean, 32.8°C) during September but only to a maximum level of 31.5°C (mean, 29.2°C) during November.

4.2. Leaf Gas Exchange and Salinity. Salinity has been identified repeatedly as constraining mangrove plant community growth and development at different concentrations depending on species [44, 45]. Mangroves have evolved conservative water use strategies at both the leaf level [10] and individual tree level [46, 47], which are especially evident at higher salinity concentrations (e.g., [48]). Low stomatal conductance is characteristic of this strategy as freshwater loss through stomata is balanced against limited CO_2 gain [49]. Species more tolerant to higher salinity are typically more efficient in water use [50]. When growing in high salinity habitats, for example, the mangrove *A. germinans* exhibits lower g_w and higher PWUE than does *L. racemosa*, which is typically regarded as less salt tolerant than *A. germinans* [18]. Certainly, growth

increment was more affected by salinity in *L. racemosa* than in *R. mangle* in mixed culture here [32]. Also, most species in the genus *Rhizophora* tend to increase PWUE with increasing salinity [29]; this increase has a metabolic cost to mangroves because of the unavoidable reduction in CO₂ assimilation [10, 49, 50]. In our study, g_w was affected by salinity differently under contrasting hydroperiods (Table 1), while PWUE was not.

PWUE increased at higher salinities in our study, but PNUE dropped appreciably as salinity increased in concentration by 30 g L⁻¹ (Figure 4). Recent studies have suggested a similar lowering of PNUE with salinity [18, 29, 51, 52] and have indicated that individual species possess different limits to absolute rates of either PWUE or PNUE depending upon growth condition [18, 31]. Actual foliar N concentrations, as a component of PNUE, reflected greater foliar loading of N at higher salinities in both species as a priority adjustment in lieu of enhanced *A* in our study (Figure 4).

Indeed, the relative proportion of soil N and P concentrations have been found to differ along natural gradients as well [22, 53], and the relative importance values for *R. mangle* versus *L. racemosa* dominance also shift along the same gradient. For example, at locations of 1.9 and 4.1 km upstream from the mouth of the Shark River along the floodplain (south Florida), *R. mangle* and *L. racemosa* share dominance. But farther up river, *R. mangle* dominates at 9.9 and 18.2 km as soil N/P ratios increase with a decrease in soil total P relative to soil total N [22]. Yet, N availability seems most important. Near the mouth of the river in higher salinity zones and also higher concentrations of total P, N is 2–4 times more available than in the lower salinity zones of the floodplain (18.2 km in land). This increase in inorganic nitrogen was attributable to higher ammonification rates measured near the mouth of the river and is correlated with phosphorus fertility of mangrove soils [22].

Our research suggests that the differences in PNUE for the two species may also contribute to patterns of community dominance along a coastal floodplain gradient as salinity changes. Where salinity is higher, greater leaf-level PNUE for *L. racemosa* might facilitate competitive dominance over *R. mangle* until exchangeable N increases. Enhanced competition between the two species might be especially important in zones of higher salinity and higher relative P, whereby ammonification is facilitated (cf., [22]), and more inorganic N becomes available for storage within leaf tissue. Lovelock and Feller [18] found similar results for *L. racemosa* in Florida (low PWUE; high PNUE) and discovered that *L. racemosa* was not able to increase foliar levels of N even after sites were fertilized with N and P because of low PWUE. Whereas our PNUE data indicate codominance of the *L. racemosa* and *R. mangle* regeneration pool at high salinity and higher N exchange and dominance of *L. racemosa* over *R. mangle* at high salinity and low N exchange, coexistence of *L. racemosa* with *Avicennia germinans* was predicted to occur with low N fertility at moderate salinities [18].

4.3. Leaf Gas Exchange and Tidal Flooding. For most plants, short-term flooding causes acute reductions in leaf gas

exchange [54]. Prolonged flooding can induce persistently depressed overall physiological activity and growth in flood sensitive species [55, 56]. Anaerobic soils, as a consequence of flooding, have been associated with decreased g_w and depressed water potential in flood tolerant mangrove leaves as well [57–59]. Except under extremely reduced soils (e.g., [59]), g_w and *A* appear unaffected by permanent flooding of *R. mangle* and *L. racemosa* seedlings or saplings [31, 59, 60], even though prolonged flooding and/or increases in flood depth can prompt changes in other types of physiological parameters (e.g., root oxidase activity, expression of chlorophyll pigments [61, 62]). Mangrove leaf gas exchange, thus, has a capacity to function under a range of hydroperiods, as long as flooding does not significantly lower soil redox conditions or, as we show here, create different salinity conditions.

Redox state ranged from –112 to 149 mv in this study, commensurate with a range of –212 to 292 mv for field measurements from the literature [21, 63–66]. While we were surprised that there were no consistent differences in leaf gas exchange for tidal versus flooded hydroperiods in our study, similar redox conditions (*sensu* [32]) suggest that perhaps our 12 h day⁻¹ simulated tidal hydroperiod did not differ enough from permanent flooding to stimulate a critical disparity in soil condition. Ultimately, interactions between salinity and hydroperiod will need to be directed at a wider range of hydroperiods in order to simulate early growing conditions of these Neotropical mangroves.

5. Conclusions

From the perspective of leaf-level gas exchange, it is difficult to understand why *R. mangle* and *L. racemosa* have different field preferences for salinity and hydroperiod unless we expand on our understanding of specific nutrient and water use relationships. It is especially important to consider whether PNUE also decreases with increases in salinity without regard to hydroperiod in the field as in the greenhouse (Figure 4), as evidence does suggest that interactions between soil condition and hydroperiod affect relationships among supply, plant demand, and root acquisition of nutrients [67]. The coexistence of *L. racemosa* and *A. germinans* is facilitated by shifts in PNUE and PWUE with edaphic fertility [18]. We suggest here that similar among-species partitioning at the leaf level with shifts in salinity can explain the success of *L. racemosa* versus *R. mangle* seedlings in some Neotropical floodplain locations through the differential ability to use nitrogen and water efficiently in photosynthesis on sites with contrasting N fertility. In contrast, differences in PNUE and PWUE imposed by hydroperiod alone explained very little. Including tidal flood frequencies and durations more indicative of upper intertidal locations (lower flood durations) will improve our overall understanding of flooding effects across the floodplain.

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