

## REVIEW

# *Rhizophora* zonation, salinity, and nutrients in the western atlantic

Ivania Cerón-Souza<sup>1</sup>  | María Beatriz Barreto<sup>2</sup>  | Eduardo Barreto-Pittol<sup>2</sup> |  
Angie Silva<sup>3</sup> | Gonzalo N. Feliner<sup>4</sup>  | Ernesto Medina<sup>5</sup> <sup>1</sup>Smithsonian Tropical Research Institute (STRI), Balboa, Panama<sup>2</sup>Facultad de Ciencias, Instituto de Zoología y Ecología Tropical (IZET), Universidad Central de Venezuela, Caracas, Venezuela<sup>3</sup>Escuela de Biología, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela<sup>4</sup>Real Jardín Botánico, CSIC, Madrid, Spain<sup>5</sup>Centro de Ecología, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela and International Institute of Tropical Forestry, USDA-Forest Service, San Juan, Puerto Rico**Correspondence**Ivania Cerón-Souza, Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, C.I. Tibaitatá, Km 14 vía Bogotá - Mosquera, Cundinamarca, Colombia.  
Email: iceron@agrosavia.co**Present address**

Ivania Cerón-Souza, Corporación Colombiana de Investigación Agropecuaria, AGROSAVIA, Centro de Investigación Tibaitatá, Cundinamarca, Colombia.

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**Associate Editor:** Ferry Slik**Handling Editor:** Rakan Zahawi**Abstract**

*Rhizophora* is the dominant genus of mangrove forests on the Atlantic coast of northern South America. What determines the zonation frequently observed in sympatric populations of the two neotropical species, *R. mangle* and *R. racemosa*, and their hybrids, *R. × harrisonii*, is an open question. The most widely held hypothesis is that differences in salinity tolerance among the taxonomic groups explain the observed zonation. To address this question, we analyzed the elemental composition of soils and canopy leaves from 60 *Rhizophora* spp. trees distributed in different intertidal zones of an estuarine site of the Paria Gulf, Venezuela. The low intertidal zone showed lower salinity, organic matter, C, N, S, and Na, and higher bulk density, Al, Fe, and Mn concentrations compared with the higher intertidal zones. Using morphological characters and microsatellite molecular markers, we identified 39 pure *R. mangle*, 19 hybrids, and only two pure *R. racemosa*. We found that both intertidal position and taxonomic groups explained most of the differences in leaf variables measured across trees. The ratio Mg/Ca, however, was higher in *R. mangle* than in *R. racemosa* and hybrids regardless of intertidal position. Moreover, at some specific intertidal position, *R. mangle* differed from *R. racemosa* and hybrids in the values of C, N, K, Mg, Fe, Mn, C/N, K/Ca, S/Ca, and  $\delta^{13}\text{C}$ . We conclude that despite the scarcity of *R. racemosa* and the absence of a clear species zonation, our results suggest that *R. mangle* copes with salinity differently than *R. racemosa* and *R. × harrisonii*.

**KEYWORDS**

hybrids, Mangrove soils, nutrients, Paria Gulf, salinity, stable isotopes, zonation

## 1 | INTRODUCTION

*Rhizophora* is one of the most representative mangrove genera worldwide. In the Neotropics, it includes two species, *R. mangle* and *R. racemosa*. *Rhizophora mangle* has a broad geographic distribution,

whereas *R. racemosa* is more restricted, and its range overlaps with that of *R. mangle*. Wherever both species are in sympatry, they hybridize, generating individuals with different introgression levels collectively named as *R. × harrisonii* (Breteler, 1969; Cornejo, 2013; Saenger & Bellan, 1995; Tomlinson, 1986).

The two *Rhizophora* species and their hybrids coexist in environments with either high local rainfall or freshwater discharge from large rivers in both the Atlantic and Pacific coast (Breteler, 1969, 1977; Cerón-Souza et al., 2010; Colonnello et al., 2009, 2012; Cumana et al., 2010; Jiménez & Sauter, 1991; Ukpong, 1997) or in narrow coastal areas within the eastern Caribbean in Venezuela (Barreto & Barreto-Pittol, 2012).

*Rhizophora racemosa* predominates upriver, reaching deep into the continent, for example, along the Essequibo River in Guyana (Breteler, 1969), and tidal river systems such as the San Juan in Venezuela (Echezuría et al., 2002; Leechman, 1918; Medina & Francisco, 1997; Medina et al., 2008). In contrast, the reports of *R. mangle* identify it as the dominant species near the river mouths, where salinity tends to be higher (Beentje et al., 2007; Breteler, 1969, 1977; Colonnello et al., 2012).

These patterns of dominance suggest that *R. racemosa* is less salt-tolerant compared with *R. mangle* and *R. × harrisonii* (Breteler, 1977; Echezuría et al., 2002; Leechman, 1918; Medina & Francisco, 1997; Medina et al., 2008; Ukpong, 1997). To date, our study located on the Pacific coast is the only one that correlates the zonation of *Rhizophora* spp. with their tolerance to salinity (Cerón-Souza et al., 2014). Specifically, combining information of elements from soils and leaves of trees identified genetically, we found that pure *R. mangle* seems to be more tolerant to salinity than pure *R. racemosa*. However, a similar analysis on the Atlantic coast that could corroborate the pattern found in the Pacific is lacking.

The analysis of elements and isotopes in leaves of mangrove species has been a useful approach to infer the distribution of mangrove species across the intertidal zone based on the soil nutrients and salinity tolerance (Ball, 1988; Sarangi et al., 2002). Thus, leaf Ca and Mg concentrations are a proxy for the salinity effects in the cell's structural functions. Moreover, the level of Na, K, and S in leaves reveal plant osmotic balance. Finally, leaf P and N concentrations inform about the soil nutritional conditions in the intertidal zones where mangroves grow (Lacerda et al., 1985; Medina et al., 1995). On the other hand, the  $\delta^{15}\text{N}$  reflects the supply of this nutrient in the intertidal zone, whereas  $\delta^{13}\text{C}$  is an indirect measure of water-use efficiency, which could inform about the salinity of the soil environment and the adaptation of mangroves to saline environments (Medina & Francisco, 1997; Medina et al., 2001).

To analyze the correlation among species zonation, salt tolerance, and soil composition in the Atlantic coast, we selected an undisturbed estuarine site with riverine mangroves within the Paria gulf in Venezuela. Previous studies in this area suggested that *R. mangle*, *R. racemosa*, and their hybrids coexist in sympatry (Colonnello et al., 2009; González, 2011; Medina et al., 2008).

In this site, following a similar methodological approach of our study on the Pacific coast of Panama, we tested the following hypotheses:

1. *R. mangle* trees occur in more saline soils than *R. racemosa*. Because this was the main result of our previous study on the Pacific coast of Panama, we hypothesized that this pattern

would be retained independently of the ocean basin where *Rhizophora* species and their hybrid coexist (Cerón-Souza et al., 2014).

2. As *R. mangle* is more salt-tolerant than *R. racemosa*, it should show higher concentrations in leaves of abundant seawater elements (Na, Mg, and S) and/or higher ratios of these elements to Ca. Because Ca is immobile in the phloem and remains in the leaves once it arrives, it is a reference element that allows for a comparison of seawater element uptake between *Rhizophora* spp. under different soil salinities within and among intertidal zones.
3. *Rhizophora* spp. trees occupying younger sediments located closest to the shoreline should have higher leaf  $\delta^{15}\text{N}$  (open N cycle) and  $\delta^{13}\text{C}$  values (less relative water stress caused by salinity). Several studies in mangrove forests have shown that the tidal regime determines the N cycle openness because it affects sediment transport and deposition near the shoreline (McKee et al., 2002; Medina et al., 2008, 2010). Na concentration generally increases landwards in mangroves sites, thus generating water stress leading to reduced stomatal conductance, higher water use efficiency, and less negative  $\delta^{13}\text{C}$  values (Medina & Francisco, 1997).

In this study area, one-third of the trees sampled turned out to be hybrids (*R. × harrisonii*), but we did not find enough individuals of *R. racemosa* to compare the physiological responses of *Rhizophora* spp. evenly. However, we found additional evidence suggesting that also in the Atlantic Ocean basin, *R. mangle* individuals cope with salinity differently than *R. racemosa* and *R. × harrisonii*.

## 2 | METHODS

### 2.1 | Study site

The study site is located in an estuarine–riverine mangrove forest dominated by red (*Rhizophora* spp.) and black (*Avicennia germinans*) mangroves with few individuals of white mangrove (*Laguncularia racemosa*). The location of this mangrove forest is within the Paria gulf in a site known as Punta de Piedra (10.421767 N; 62.796708 W), bordering Turuépano National Park (Colonnello et al., 2009). Tidal channels such as Turuépano, La Palma, Guariqué, Guarapichito, and Ajíes surround this area. In addition, the San Juan River discharge is 25 km southeast of the site (Lasso et al., 2004), so freshwater and sediment discharge strongly influence the mangrove forest of the area.

The study site was selected based on its accessibility, density, and homogeneity of the vegetation, and relative protection from the Paria Gulf heavy winds. Based on information from the closest meteorological stations (i.e., Caripito and Güiría), the area has a short dry season between February and March (Walter & Medina, 1971). However, data from a climate model covering a more extensive period (1982–2012) indicate a longer dry season from January to April, with averages of 1444 mm of precipitation and 26.9°C for

temperature (Climate-Data.org, 2016). The river freshwater discharge and the effect of large tidal amplitudes ( $\approx 1$  m, Herrera et al., 1981) compensate for the reduction in rainfall during the first quarter of the year. From June to December, rainfall exceeds 100 mm per month, contributing to significant leaching of salt from superficial soil layers. River discharge and rainfall maintains water salinity levels well below standard seawater (35‰) at the fringe of the mangrove-lined coast. For this study, we conducted fieldwork in December 2010 at the beginning of the dry season.

## 2.2 | Delimitation of the intertidal zones and sampling design

The tidal regime in the Paria Gulf is bi-diurnal, and the tidal range varies between 1.8 and 3.2 m. Therefore, the penetration of tidal waters into the forest varies accordingly. In this study area, the maximum penetration inland of the mangrove forest is about 200 m. Beyond 200 m, non-halophytic species such as *Symphonia globulifera* and *Cassipourea* spp. (Colonnello et al., 2009) begin to appear.

Based on this characterization of the study site, we established two transects perpendicular to the coast border toward the upper tidal zone and into the mangrove forest, separated 200 m from each other. On each transect, we delimited the two extremes of the intertidal range. The low intertidal zone (LI) was delimited as a 20-m-wide stripe located at 2–20 m from the shoreline. Similarly, the upper intertidal zone (UI) was delimited as a 20 m-wide strip at 150–170 m from the shoreline. Finally, a 20 m-wide strip at 90–110 m from the shoreline was considered to represent the middle intertidal zone (MI). This intermediate area helped us test for gradients of different parameters across the intertidal range. Thus, our sampling design included two plots in each of the three intertidal zones, one in each transect, totaling six plots.

At the low intertidal zone (LI), each one of the two plots had an area of 900 m<sup>2</sup> (30 × 30 m) totaling 1800 m<sup>2</sup>. Because the density of *Rhizophora* spp. trees with DBH >5 cm increased from the shoreline (LI) toward the middle (MI) and upper (UI) intertidal zone, we reduced the size of the plots progressively. At the MI zone, each one of the two plots covered 400 m<sup>2</sup> (20 × 20 m), totaling 800 m<sup>2</sup>. At the UI zone, each one of the two plots covered 225 m<sup>2</sup> (15 × 15 m), totaling 450 m<sup>2</sup>.

## 2.3 | Measurements of interstitial water salinity

We measured interstitial water salinity *in situ* at eight distances, along the two transects, from the coastal border toward the upper tidal zone into the mangrove forest, spanning the three intertidal zones. The distances were 2 and 20 m (at LI); 90, 100, and 110 m (at MI); and 150, 160, and 170 m (at UI). At each distance point, we collected porewater samples at 20, 40, and 80 cm depth with a 1-m-long aluminum tube with perforations at the end, connected to a 60-mL syringe. Salinity was measured with a digital refractometer

(PAL-06S, Atago) and expressed in ‰. We used one or two replicates for each depth for a total of 72 measurements across the study site.

## 2.4 | Sampling of soils and canopy leaves of *Rhizophora* spp. individuals

We randomly collected four soil samples at 0–20 cm depth of each plot for 24 samples (i.e., four random samples × three intertidal zones × two plots per intertidal zone). We discarded one sample during processing. The remaining 23 samples were dried in the laboratory using a ventilated oven at 45°C to constant weight, ground, and then passed through a 20-mesh sieve. We separated each sample into two subsamples to measure the elemental composition (see section 2.5) and pH. For pH, the subsamples were hydrated with deionized water (1:5), homogenized manually for 5 min, and allowed to rest for 14 h before measurements with a HANNA instrument (model 991301). Following the same sampling design, we measured bulk density in soil samples collected between 0 and 10 cm depth using PVC tubing (5 cm diameter, 10 cm length) for 24 samples. Samples were dried in the laboratory using a ventilated oven (45°C) until constant weight. This measurement was the quotient of soil dry mass/original volume of the PVC cylinder (200 cm<sup>3</sup>).

Within each one of the six plots, we randomly selected ten trees of *Rhizophora* spp. with inflorescences totaling 60 individuals. Eight fully expanded green canopy leaves were collected from each of these trees for laboratory analysis of elements and isotopes (see Sections 2.5 and 2.6).

## 2.5 | Elemental composition of canopy leaves and soils

Sixty samples of canopy leaves were oven-dried at 65°C, grounded, and subsequently passed through an 18-mesh sieve. The powdered samples were ashed at 490°C and then dissolved in HCl 6 N. Using the thermogravimeter (LECO TGA701), we measured dry mass (105°C) and ash content (490°). In parallel, the 23 soil samples were digested sequentially in concentrated hot HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, and HCl (Huang & Schulte, 1985). Then, using digested subsamples of both leaves and soil samples, Na, K, Mg, Ca, Fe, Mn, S, and P concentrations were measured using the SPECTRO Analytical Instruments GmbH (Boschstr. 10, 47533 Kleve, Germany). Additionally, total carbon and nitrogen were also measured using a macro dry combustion method with the LECO CNS-2000 Analyzer (Leco Corp. 3000 Lakeview Ave. St. Joseph, Michigan 49085, USA).

## 2.6 | Leaf isotopic C and N ratios

Isotopic C and N ratios of the leaves sampled were measured for each one of the 60 *Rhizophora* spp. trees at the L7 isotope

laboratory of the University of Miami, Coral Gables (<http://pengu.in.bio.miami.edu>). Small tin spheres containing samples were placed in an automated elemental analyzer (Eurovector, Milan, Italy) and pyrolyzed. Gases from the pyrolysis were led into a mass spectrometer (Isoprime, Elementar) and analyzed for  $^{13}\text{C}$  and  $^{15}\text{N}$  abundance. Carbon and nitrogen isotope ratios are expressed as  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \cdot 10^3$ .  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the molar ratio of the heavier ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) to lighter isotope ( $^{12}\text{C}$  and  $^{14}\text{N}$ ) in the sample and standard, respectively. The standard for carbon isotope ratio was belemnite from the PeeDee Formation in South Carolina, with an analytical precision of 0.1‰. The standard for the nitrogen isotope ratio was atmospheric nitrogen, with an analytical precision of 0.3‰.

## 2.7 | Mangrove species identification by morphological characteristics

In each of the six plots at three different intertidal zones, we identified mangrove trees of *Rhizophora* spp., *Avicennia germinans*, and *Laguncularia racemosa*. Identification of *A. germinans* and *L. racemosa* was straightforward because there are no other species of these genera in the area; however, identification of *Rhizophora* trees was complex. The only morphological character distinguishing the two species and the hybrids is the inflorescence type (i.e., the number of bifurcations in the inflorescence). Based on the inflorescence type, the 60 sampled *Rhizophora* trees were identified as follows: those with 1–2 bifurcations as *R. mangle*, those with 5–6–7 bifurcations as *R. racemosa*, and those with 3–4–5 bifurcations as hybrids, *R. × harriisonii* (Cerón-Souza et al., 2010; Jiménez, 1987). Due to the partly overlapping ranges of variation of inflorescence bifurcation in the three taxonomic units, which is associated with ongoing hybridization and introgression, field identification should ideally be corroborated genetically with molecular markers (Cerón-Souza et al., 2010, 2014).

## 2.8 | Genetic identification of sampled *Rhizophora* individuals

Of the 60 *Rhizophora* trees sampled, we genotyped 46 individuals to validate the taxonomic identification based on inflorescence type using DNA extracted from leaves. We used ten microsatellite loci (i.e., RM07, RM11, RM19, RM21, RM36, RM46, RM05, RM50, RM59, and RS67) following the protocols in Cerón-Souza et al. (2010). The genetic composition of individuals and the hybrid class were determined using the Bayesian genetic clustering analyses with STRUCTURE 2.3.3 (Falush et al., 2003, 2007; Pritchard et al., 2000) and NEWHYBRIDS 1.1 beta (Anderson & Thompson, 2002). In neither of the two analyses was the taxonomic identification based on inflorescence types used as prior information. For STRUCTURE, an admixture model was assumed, with correlated allele frequencies and a uniform prior probability of  $K$ . We ran ten iterations for

each number of groups assayed, from  $K = 1$  through  $K = 10$ , using 500,000 replicates of MCMC after a burn-in of length 50,000 replicates. We applied the criterion of Evanno et al. (2005) to select the best estimate of the number of genetic groups based on DK calculation. For NEWHYBRIDS, we performed the analysis five times, each starting with a different random number of seeds and including 500,000 iterations and 50,000 burn-in steps of the MCMC chain using a uniform allele and genetic frequency prior. Based on this approach, the 46 individuals were assigned to one of six possible genotype classes: 1) pure *R. mangle* parent, 2) pure *R. racemosa* parent, 3)  $F_1$  hybrid (50% of the genome originated from *R. mangle* and 50% from *R. racemosa*), 4)  $F_2$  hybrid (50% from  $F_1$  hybrid and 25% from each of the parents *R. mangle* and *R. racemosa*), 5) backcrosses with *R. mangle* (50% from  $F_1$  hybrid and 50% from parent *R. mangle*), and 6) backcrosses with *R. racemosa* (50% from  $F_1$  hybrid and 50% from parent *R. racemosa*).

## 2.9 | Statistical analysis

To test differences in soil characteristics, we used a one-way ANOVA comparing LI, MI, and HI intertidal zones. We also performed a principal component analysis of element concentration and element ratios to determine their correlation and characterize their spatial distribution across the intertidal zone. For the interstitial soil salinity, we used a two-way ANOVA to compare the effect of intertidal zone and depth.

To test element concentrations, ratios of elements, and isotope differences across *Rhizophora* individuals, we performed a two-way ANOVA comparing the effect of the position in the intertidal zone and the taxonomic identification combining morphology and molecular markers.

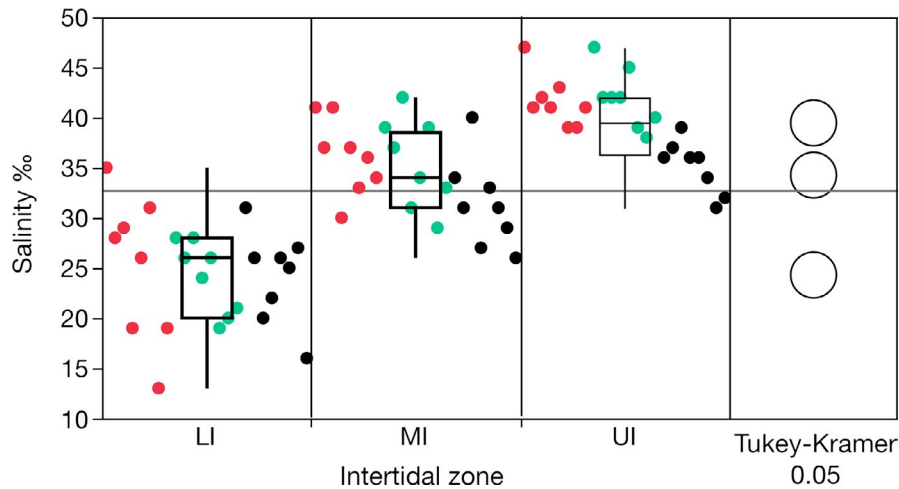
In all analyses for both soil and leaf variables, we confirmed the homogeneity of variances (Bartlett test). For the few variables in which unequal variances were detected, we used Welch's test for means allowing for unequal standard deviations. We compared the means using the Tukey–Kramer HSD test at  $p < 0.05$ . For all statistical tests, we used JMP 13 (SAS Institute Inc. 2016).

# 3 | RESULTS

## 3.1 | Physical and chemical soil properties

The two-way ANOVA indicated that, when measures from all soil depths were pooled, interstitial water salinity increased with distance from the shore ( $24 \pm 5\%$  in the LI zone,  $34 \pm 5\%$  in the MI, and  $40 \pm 4\%$  in the UI zone,  $p < 0.0001$ ). Moreover, only at UI, the salinity at depths 40 and 80 cm was higher than at 20 cm ( $p < 0.0001$ ). There was no interaction between the intertidal zone and depth (Figure 1).

All soil variables showed significant differences in LI compared with MI and UI, except for K, Mg, and C/N (Table 1). Bulk density and



**FIGURE 1** Salinity of interstitial soil water obtained from three intertidal zones at 2–20 m (lower—LI), at 90–110 m (intermediate—MI), and at 150–170 m (upper—UI), at three depths: 20 cm (black dots), 40 cm (green dots), and 80 cm (red dots). A two-way ANOVA using intertidal zone and soil as factors showed that intertidal zones had the strongest influence on salinity (LI 24, MI 34, UI 40; Fratio 77.7,  $p < 0.0001$ ) compared with soil depth (20 cm 30, 40 cm 34, 80 cm 34; Fratio 6.4,  $p = 0.0029$ ). Only at UI was the salinity higher at 40 and 80 cm compared with 20 cm. There was no significant interaction between the factors (Fratio 1.5,  $p = 0.217$ ). Differences in means for the whole sample set were significant according to the Tukey–Kramer test

pH were lower in MI and UI zones corresponding to the increases in LOI% and total C, which suggests an enrichment in the organic matter beyond the LI zone (Table 1). The alkaline metals Na and Ca showed significantly higher concentrations at MI and UI in a pattern opposite to metals Al, Fe, and Mn. Soil C and N were strongly correlated ( $R^2 = 0.97$ ,  $p < 0.001$ ), suggesting their common origin from plant organic matter. Moreover, the ratios K/Na, S/Ca, and Mg/Ca indicated that salinity conditions are less favorable at MI and UI compared with LI zones.

We found two element groups based on interelement correlations that explain at least 50% of the variance ( $R \geq 0.71$ ;  $p < 0.0001$ ). Ca, Mg, Na, C, N, and S were positively correlated and increased toward the upper intertidal zone, whereas Al, K, and P were also positively correlated but decreased. The PCA confirmed the variance analysis and revealed a clear separation between LI plots on one side and MI and UI plots, which overlapped extensively (Figure 2). Based on this result, for the two-way ANOVA of leaf element composition, we considered the categories LI vs. HI (i.e., pooling MI and HI) for the intertidal zone variable (see Section 3.3).

### 3.2 | Zonation of *Rhizophora* species along the transects

Among the 60 *Rhizophora* sampled trees, we identified 37 *R. mangle*, two *R. racemosa*, and 21 hybrids based on both inflorescence type (i.e., the number of bifurcations—NB) and genetic information. The STRUCTURE analysis for those 46 of the 60 individuals identified two clusters ( $K = 2$ ) as the best genetic partition of the data corresponding to two genetic groups: *R. mangle* and *R. racemosa* + *R. × harrisonii*. Results from STRUCTURE analysis are consistent

with morphological identification. All individuals with a “mangle” inflorescence type (i.e., NB between 1 and 2) were in the pure parental *R. mangle* genetic group, and those with a “racemosa” inflorescence type (i.e.,  $>6$  NB) were in the pure parental *R. racemosa* group. All individuals with an intermediate inflorescence type (i.e., NB of 2–6) were hybrids. These hybrids represent different classes (i.e.,  $F_1$ ,  $F_2$ , or backcrosses toward both paternal species), suggesting more than two advanced generations of hybridization or backcrossing (Figure 3). Zonation across the intertidal zone was weak. The 37 *R. mangle* individuals occurred in the three intertidal zones. However, 30 of them occurred in the high intertidal zones—HI (UI and MI), and seven in the LI. The hybrids also occurred throughout the intertidal zones: 11 individuals were in the LI plots and ten in the HI (UI+MI). *Rhizophora racemosa* was remarkably scarce in our study site. We identified only two individuals, both located in the LI zone (Figure 3). Based on this result, we considered two categories for the taxonomic group variable (*R. mangle* vs. *R. racemosa* + *R. × harrisonii*) instead of three for the two-way ANOVA of leaf element composition (see Section 3.3).

### 3.3 | Leaf elemental composition across intertidal zones and taxonomic group

The two-way ANOVA focusing on the effect of intertidal location (i.e., LI vs. HI) and taxonomic group (*R. mangle* vs. *R. racemosa* + *R. × harrisonii*) indicated that the results varied depending on the leaf variable. We found no variation in Na. By contrast, the intertidal position alone explained the variation of  $\delta^{15}N$ , P, and S. In comparison, both factors explained the differences in N, K, Mg, Ca, Fe, Mn, and the molar ratios of C/N, K/Na, K/Ca, and S/Ca. However, the taxonomic group effect predominated in N, K, Fe,

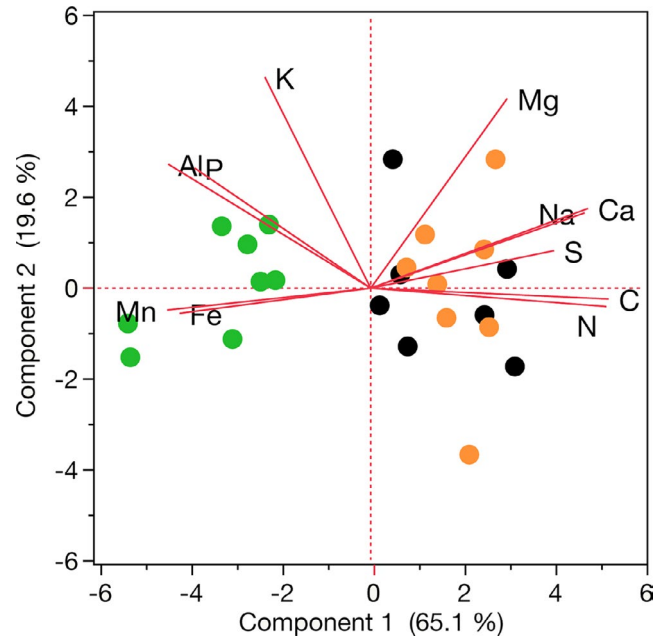
**TABLE 1** Means and analysis of variance (ANOVA) of soil bulk density, pH, element concentrations, and their ratios from the low (LI), intermediate (MI), and upper (UI) intertidal zones

Variable	LI	MI	UI	ANOVA p-Value
No. of samples	8	8	7	
Bulk density g/cm <sup>3</sup>	0.48 <sup>a</sup>	0.34 <sup>b</sup>	0.33 <sup>b</sup>	<0.0001
pH	6.2 <sup>a</sup>	5.5 <sup>b</sup>	5.6 <sup>b</sup>	0.003
LOI %	16.2 <sup>a</sup>	29.4 <sup>b</sup>	28.7 <sup>b</sup>	<0.0001
Metals (mmol/kg)				
Al	817 <sup>a</sup>	612 <sup>b</sup>	626 <sup>b</sup>	<0.0001
Fe	559 <sup>a</sup>	361 <sup>b</sup>	317 <sup>b</sup>	<0.0001
Mn*	5.8 <sup>a</sup>	2.1 <sup>b</sup>	2.2 <sup>b</sup>	0.0312
Na	504 <sup>a</sup>	787 <sup>b</sup>	756 <sup>b</sup>	0.0002
K	98	89	89	ns
Mg	272	304	293	ns
Ca	63 <sup>a</sup>	96 <sup>b</sup>	86 <sup>b</sup>	<0.0001
Non-metals (mmol/kg)				
C	5598 <sup>a</sup>	11798 <sup>b</sup>	11514 <sup>b</sup>	<0.0001
N	259 <sup>a</sup>	506 <sup>b</sup>	543 <sup>b</sup>	<0.0001
S	123 <sup>a</sup>	333 <sup>b</sup>	369 <sup>b</sup>	<0.0001
P	26 <sup>a</sup>	17 <sup>b</sup>	18 <sup>b</sup>	<0.0001
Ratios				
N/P	10.0 <sup>a</sup>	29.8 <sup>b</sup>	30.0 <sup>b</sup>	<0.0001
C/N	21.3	23.3	22	ns
K/Na	0.2 <sup>a</sup>	0.1 <sup>b</sup>	0.1 <sup>b</sup>	<0.0001
S/Ca	1.9 <sup>a</sup>	3.5 <sup>b</sup>	4.3 <sup>b</sup>	0.002
Mg/Ca	4.4 <sup>a</sup>	3.2 <sup>b</sup>	3.3 <sup>b</sup>	<0.0001

Each intertidal zone had eight samples. However, one sample from UI was eliminated because of low quality. Different letters after means for each variable indicate significant differences after the Tukey–Kramer HSD test at  $p < 0.05$ . An asterisk indicates that the variable data set had unequal variances (Bartlett test), and the probability corresponds to Welch's test. Significant  $p$ -values are in bold, and ns are the non-significant  $p$ -values.

and K/Ca because of its lower  $p$ -value compared with the intertidal position. Finally, the taxonomic group alone explained the variation of C,  $\delta^{13}\text{C}$ , N/P, Na/Ca, and Mg/Ca. No significant interaction between factors was detected for element composition except for C, Al, and Fe (Table 2).

Comparing the means, we corroborated the differences in leaf variables that are relevant to explain the salinity tolerance of the taxonomic groups. In *R. mangle*, the Mg and S concentrations increased more rapidly with increasing Ca concentrations than in *R. racemosa* + *R. × harrisonii* group across the two intertidal zones (Table 3 and Figure 4). Moreover, *R. mangle* had lower C and higher Fe than the *R. racemosa* + *R. × harrisonii* group at LI. Furthermore, the  $\delta^{13}\text{C}$  in *R. mangle* at HI was less negative than *R. racemosa* + *R. × harrisonii* at LI. Likewise, *R. mangle* at HI had higher N and Mg concentrations and lower Mn and C/N concentrations than



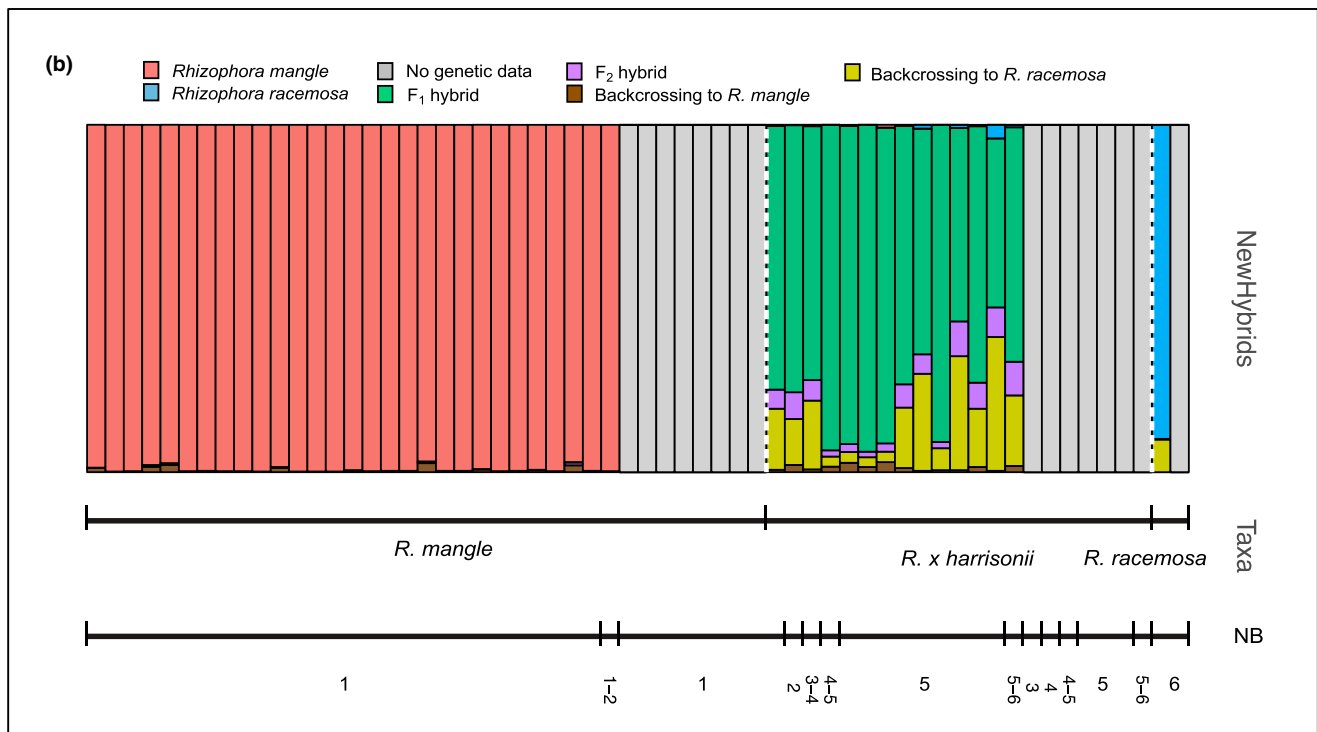
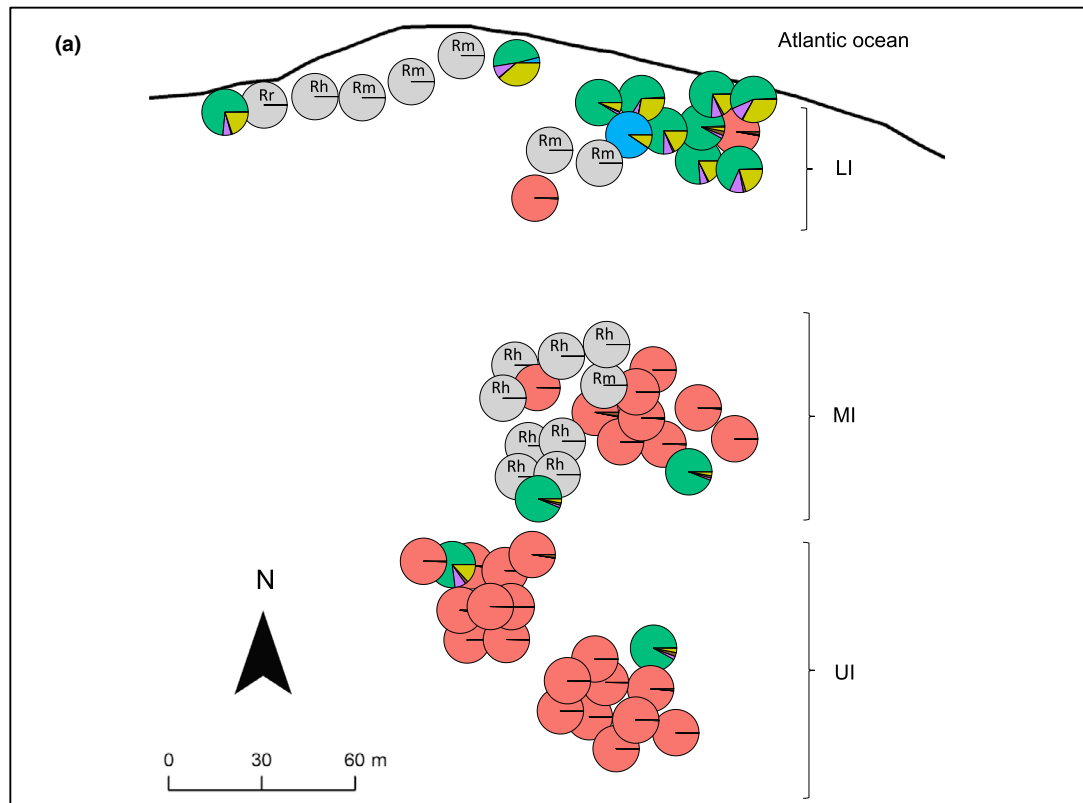
**FIGURE 2** Principal components analysis of soil elements (0–20 cm depth) sampled at low (LI, green dots,  $N = 8$ ), intermediate (MI, black dots,  $N = 8$ ), and upper (UI, brown dots,  $N = 7$ ) intertidal zones. One sampled of UI was discarded because of low quality

*R. racemosa* + *R. × harrisonii* at LI. Finally, the concentration of K and the ratio K/Ca were higher in *R. mangle* than in the *R. mangle* + *R. × harrisonii* group at HI (Table 3).

## 4 | DISCUSSION

### 4.1 | Spatial differences in soil properties

Our study site showed clear differences in soil characteristics in the LI zone compared with combined HI zones. These differences most likely reflect the predominant organic matter exportation and sediment input by tides in the LI zone. Our study site is at the interface of hydrological processes (river discharge and tides) and organic matter production by vegetation established on consolidated sediments. Thus, predominant processes in the LI zone are sediment deposition from the Amazon and Orinoco rivers combined with constant flushing by the influence of an extensive drainage network located in the northeast of the Paria Gulf. In contrast, predominant processes in higher tidal zones are the accumulation of organic matter derived from litter production by the vegetation (both above- and belowground) and slow organic matter decomposition (Bout-Roumazailles et al., 2013; Milliman et al., 1982; Pujos et al., 1997; Warne et al., 2002). Thus, we could expect not only an accumulation of elements associated with biomolecules (C, N, S) at higher intertidal zones, but also a decrease in element concentration such as Al, Fe, and Mn compared with low intertidal zones (Alongi et al., 2003; Chen & Twilley, 1999; Deborde et al., 2015; Medina et al., 2008).



**FIGURE 3** Location of 60 *Rhizophora* spp. trees in the study site and genetic ancestry based on ten microsatellite molecular markers analyzed on 46 of these trees with the NEWHYBRIDS software. (a) The 60 individuals were located at different distances from the channel border within three delimited intertidal zones: low (LI), middle (MI), and upper (UI). (b) Each vertical bar represents one of the 60 individuals, color-coded to indicate each of six genetic groups: pure *R. mangle* (red), pure *R. racemosa* (blue),  $F_1$  hybrids (green),  $F_2$  hybrids (pink), backcrosses to *R. mangle* (brown), backcrosses to *R. racemosa* (yellow), and no genetic information (gray). Below the bars is the morphological identification of individuals as *R. mangle* (Rm), hybrids *R. × harrisonii* (Rh), or *R. racemosa* (Rr) by the number of bifurcations in the inflorescence (NB)

**TABLE 2** Two-way analysis of variance of leaf elemental composition and molar ratios considering the effects and the interaction of two factors

Elements	Intertidal zone <i>p</i> -value	taxonomic group <i>p</i> -value	Interaction <i>p</i> -value
C	0.1205	<b>0.0067</b>	<b>0.0104</b>
δ <sup>13</sup> C	0.2072	<b>0.0449</b>	0.4943
N	<b>0.0115</b>	<b>0.0323</b>	0.9772
δ <sup>15</sup> N	<b>&lt;0.001</b>	0.387	0.6160
P	<b>0.0074</b>	0.6134	0.7373
S	<b>&lt;0.0001</b>	0.4374	0.6038
Na	0.3379	0.1511	0.6727
K	<b>0.0117</b>	<b>&lt;0.0001</b>	0.4177
Mg	<b>&lt;0.0001</b>	<b>0.0083</b>	0.2531
Ca	<b>0.0119</b>	<b>0.0191</b>	0.9700
Al	0.2201	0.0749	<b>0.0328</b>
Fe	<b>0.0296</b>	<b>0.0071</b>	<b>0.0067</b>
Mn	<b>&lt;0.0001</b>	<b>0.0048</b>	0.4258
C/N	<b>0.002</b>	<b>0.0068</b>	0.4800
N/P	0.8607	<b>0.0242</b>	0.7666
K/Na	<b>0.0347</b>	<b>0.0404</b>	0.3027
Na/Ca	0.1554	<b>0.0107</b>	0.5251
K/Ca	<b>0.0011</b>	<b>0.0002</b>	0.9713
Mg/Ca	0.3149	<b>&lt;0.0001</b>	<b>0.0484</b>
S/Ca	<b>&lt;0.0001</b>	<b>0.0029</b>	0.1993
Effect summary			
Log worth	14.412	7.131	2.1740
P	<b>0.0000</b>	<b>0.0000</b>	<b>0.0067</b>

The spatial position in the intertidal zones where LI (*N* = 20) vs. HI (i.e., MI and UI, *N* = 40). The taxonomic categories based on both genetic diversity and morphological characters were *Rhizophora mangle* (*N* = 37) vs. *R. racemosa* + *R. × harrisonii* (*N* = 23, of which two are *R. racemosa*). In bold, significant at *p* < 0.05.

Our soil analysis is consistent with the above expectations. The increase in organic matter in higher tidal zones lowers the pH of the superficial soil layers associated with humic and fulvic acids generated during organic matter decomposition (Chen et al., 2014; Nóbrega et al., 2013; Yücel et al., 2010). Concentrations of N and S follow the same pattern as the organic matter. They increased by 2.1 and 2.8, respectively, whereas P concentration decreased by 30%. P concentration is probably associated with sediment deposition from upriver sources (Fabre et al., 1999). The same occurs with the metals Al, Fe, and Mn, which are main components of clay and oxy-hydroxides in sediments (Gilkes & McKenzie, 1988; Marchand et al., 2006; Souza-Júnior et al., 2008), and decreased in concentration between 30 and 60%. The larger total sulfur concentration in higher tidal zones is possibly associated with organic matter and the minerals of the reduced forms of Fe (like pyrite) and Mn (Canfield et al., 1993; Nóbrega et al., 2013; Souza-Júnior et al., 2008; Turner et al., 2016).

Despite the input of organic matter in the upper tidal zone, the concentrations of Mg and K did not decrease, and Ca even increased. This result suggests the preferential uptake of those elements by the vegetation and their recycling in litterfall. Additionally, Ca concentration is probably associated with the mountain range's lithology at the northern border of the Paria gulf (Colonnello et al., 2009; Marquez et al., 2013). The pattern of Na accumulation in higher tidal zones probably is a consequence of the decreasing frequency of tidal flushing with distance from the coastline. These results support the idea that the gradient of element concentrations in the soil is related to the flushing and sediment deposition rate at the seaward fringe, and to the vegetation inputs inland. Also, soils at the lower intertidal sites may induce lower salt stress to the trees because of the lower Na and S concentrations, and their significantly higher K/Na and lower S/Ca values.

Finally, it is essential to note that we measured all soil characteristics at the beginning of the dry season in our study site. Soil characteristics, especially the salinity, may vary seasonally. For instance, our study on the Pacific coast of Panama found that salinity in soils was higher during the dry season than in the wet season over the study site. Moreover, at the dry season, the low intertidal zones had higher salinity than in high intertidal zones during the wet season. The dry season gradient was the opposite of the wet season, with higher salinity in the high intertidal zones (Cerón-Souza et al., 2014). In the Yaguaraparo site, seasonal changes in soil salinity and freshwater supply are less pronounced due to the combination of rivers input draining from the northern mountains and the macrotidal regime. These results highlight the complexity of soils in mangrove areas, their dynamism over seasons, and how they can vary across a few kilometers depending on the geology of the zone, especially the influence of the rivers surrounding the mangrove forest.

## 4.2 | Presence and zonation of *Rhizophora* taxonomic groups

A striking result from this study site in Atlantic Venezuela was the scarcity of pure *R. racemosa* compared with pure *R. mangle* and *R. × harrisonii*, which hinders identification of the determinants of local spatial distribution for each species. However, the abundance of hybrids in the study site, together with the ecological requirements of each parental species, particularly for salinity tolerance, provided a hypothetical explanation for the scarcity of pure *R. racemosa*.

Our previous study in the Pacific suggests that hybrids exhibit higher salinity tolerance than pure *R. racemosa* (Cerón-Souza et al., 2014). Therefore, wherever the salinity conditions are demanding (compared with upriver mangroves or Pacific mangroves with higher precipitation), selection against the less salinity-tolerant species (*R. racemosa*) could favor not only pure *R. mangle* but also hybrids. Consequently, it is possible that in mixed mangroves with salinity levels above the optimum for *R. racemosa*, the occurrence of this species is gradually displaced over time by both the hybrids and *R. mangle*.



Taxon	<i>R. mangle</i>	<i>R. mangle</i>	<i>R. racemosa</i> + <i>R. × harrisonii</i>	<i>R. racemosa</i> + <i>R. × harrisonii</i>	<i>p</i> -Values
Position	LI	HI	LI	HI	
No. of samples	7	30	13	10	
C	38130 <sup>b</sup>	38736 <sup>b</sup>	41213 <sup>a</sup>	38828 <sup>b</sup>	0.0009
N	1036 <sup>ab</sup>	1176 <sup>a</sup>	915 <sup>b</sup>	1058 <sup>ab</sup>	0.0008
S	77 <sup>b</sup>	152 <sup>a</sup>	73 <sup>b</sup>	135 <sup>a</sup>	<0.0001
P	28	33	29	33	0.0496
Na	752	789	624	719	ns
K	164 <sup>a</sup>	140 <sup>a</sup>	119 <sup>ab</sup>	73 <sup>b</sup>	0.0003
Mg	182 <sup>ab</sup>	224 <sup>a</sup>	133 <sup>b</sup>	204 <sup>a</sup>	<0.0001
Ca	247 <sup>b</sup>	315 <sup>ab</sup>	310 <sup>ab</sup>	375 <sup>a</sup>	0.0338
Al	11	5	5	6	ns
Fe	4.9 <sup>a</sup>	2.4 <sup>b</sup>	2.2 <sup>b</sup>	2.4 <sup>b</sup>	0.0039
Mn	11.2 <sup>ab</sup>	6.5 <sup>c</sup>	15.4 <sup>a</sup>	8.8 <sup>bc</sup>	<0.0001
%Ash	9.9 <sup>ab</sup>	11.1 <sup>a</sup>	9.0 <sup>b</sup>	10.4 <sup>ab</sup>	0.0006
C/N	38.9 <sup>ab</sup>	33.6 <sup>b</sup>	46.2 <sup>a</sup>	38.0 <sup>b</sup>	<0.0001
N/P	36.9	36	32.3	32.5	ns
K/Na	0.2	0.2	0.1	0.2	0.0424
Na/Ca	3.3	2.6	2.2	2	ns
K/Ca	0.7 <sup>a</sup>	0.5 <sup>a</sup>	0.4 <sup>ab</sup>	0.2 <sup>b</sup>	0.0005
Mg/Ca	0.8 <sup>a</sup>	0.7 <sup>a</sup>	0.5 <sup>b</sup>	0.6 <sup>b</sup>	<0.0001
S/Ca	0.3 <sup>bc</sup>	0.5 <sup>a</sup>	0.2 <sup>c</sup>	0.4 <sup>ab</sup>	<0.0001
δ <sup>13</sup> C ‰	-29.95 <sup>ab</sup>	-29.75 <sup>a</sup>	-30.88 <sup>b</sup>	-30.22 <sup>ab</sup>	0.0368
δ <sup>15</sup> N ‰	2.66 <sup>a</sup>	0.49 <sup>b</sup>	2.95 <sup>a</sup>	0.57 <sup>b</sup>	<0.0001

*p*-values for the one-way analysis of variance with three degrees of freedom. Significance of mean differences tested with the HSD Tukey–Kramer test, *p* = 0.05).

Other studies in the Atlantic are consistent with the hypothesis that *R. racemosa* requires even more freshwater supply than that reached in the study site. In Brazil, *R. racemosa* and the hybrids are only in high precipitation areas with continuous freshwater supply (Ximenes et al., 2016). Therefore, the establishment of *R. racemosa* in higher tidal zones is limited strongly by both water and high soil salinity (Jiménez & Sauter, 1991). In Venezuela's northeast Caribbean coastal areas, where *R. racemosa* is abundant, there is plenty of freshwater (Barreto & Barreto-Pittol, 2012). Similarly, in the upper reaches of French Guiana and Venezuelan Atlantic, *R. racemosa* also occurs in swamp forests with *Pterocarpus officinalis* and the mangrove fern *Acrostichum aureum* L. where freshwater availability is high (Colonnello et al., 2012; Fromard et al., 2004; Medina et al., 2007).

Future studies in the Atlantic or Pacific coast are however needed to confirm the association between the abundance of *R. racemosa* and low salinities. For example, a potential experimental design should sample *Rhizophora* spp. individuals along the river course, from the river mouth up to several kilometers upwards, combining the genetic identification of samples and the biochemistry of both soils and plants. We would expect increasing abundance of *R. racemosa*, with a peak of abundance where salinity levels are lowest

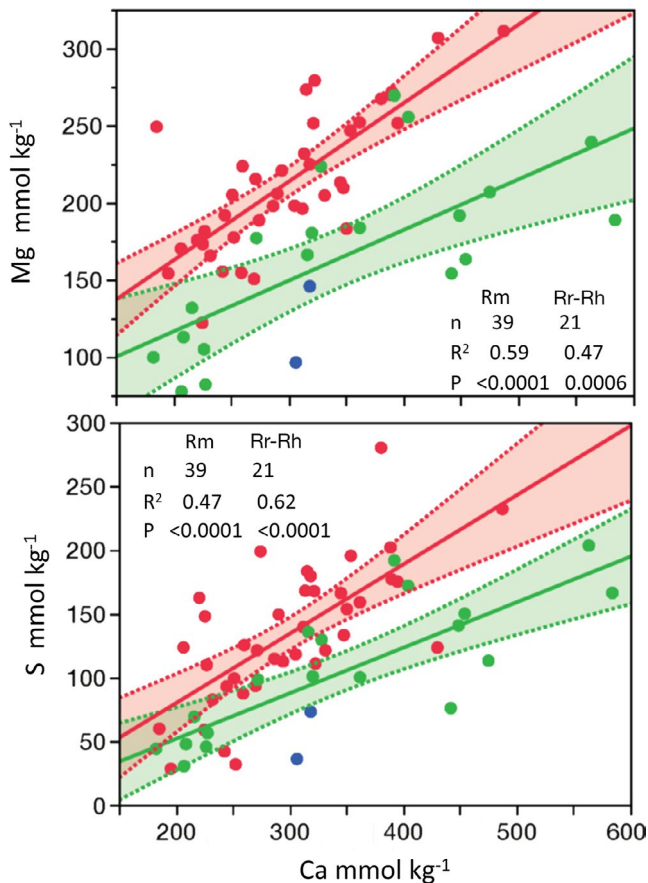
and closest to freshwater. Regarding *Rhizophora* hybrids, we would expect them to occur throughout such a sampling design, with different introgression levels toward either *R. mangle* or *R. racemosa*, depending on the soil salinity.

### 4.3 | Responses to nutrient and salinity in soil across *Rhizophora* taxonomic groups

In our previous study in the Pacific (Cerón-Souza et al., 2014), we found that N and P concentrations in the soil and leaf N, C/N ratios decreased with distance from the shoreline regardless of the taxonomic group. Thus, distance from the shoreline was the only factor that explained variation in foliar N and P concentrations, implying that those nutrients were not directly involved in the maintenance of *Rhizophora* spp. hybrid zones or in the spatial zonation along which the two species occur. In contrast, the results of the present study reveal a more complex pattern.

Here, we found that either intertidal position or taxonomic group or both were associated with differences in elemental leaf composition across the *Rhizophora* spp. hybrid complex. Both species behave as typical halophytes capable of growing in soils with

TABLE 3 Means of element concentrations in leaves comparing the two taxonomic groups, *R. mangle* vs. *R. racemosa* + *R. × harrisonii*, at the two intertidal positions (LI—low and HI—high)



**FIGURE 4** Magnesium–calcium (a) and inorganic sulfur–calcium (b) relationships in canopy leaves of 39 *R. mangle* (red dots), and two *R. racemosa* (blue dots) +19 hybrids *R. × harrisonii* (green dots) trees sampled across the intertidal zones. Linear regression and 95% confidence intervals depicted for *R. mangle* (red) and for *R. racemosa* + hybrids (green)

high concentrations of soluble Na and accumulating similar amount of this element in their leaf tissues. Tolerance to the high Na leaf levels is in part related to Ca availability, which is the second most abundant element in leaf tissues (Cramer, 2002). High levels of Mg and S brought about additional salinity effects in the root substrate, and the results showed that the intertidal zone influenced the leaf concentrations of these elements (Table 1). Differences in relative accumulation of Mg and S between the genetic types were visible when considering Ca as an indicator of cation uptake that is not retranslocated from leaves. The Mg-to-Ca ratios are significantly higher in *R. mangle* compared with the *R. racemosa* + *R. × harrisonii* group in both intertidal zones. A similar although less significant trend is observed in the S-to-Ca ratio (Table 3 and Figure 4). These values indicated that uptake of Mg and S per unit of Ca is higher in *R. mangle*, suggesting a higher tolerance to cellular concentrations of these ions compared with the *R. racemosa* + *R. × harrisonii* group.

Moreover, beyond leaf element concentrations related to intertidal position, *R. mangle* also differed in the accumulation of C, N, K, Mg, Fe, Mn, and  $\delta^{13}\text{C}$  from the *R. racemosa* + *R. × harrisonii*

group in specific intertidal positions. These results support our second hypothesis. However, the lack of differences in Na concentrations or K/Na ratios also suggests that at least in this study site, all *Rhizophora* trees, independent of their taxonomic group, were similarly capable of preventing Na uptake from interstitial water.

Comparing this result with what we found in the Pacific site (Cerón-Souza et al., 2014), it seems that there is not a single pattern regarding how pure *R. mangle*, *R. racemosa*, and their hybrids interact with salinity tolerance. Two factors may influence the differences between the two sites. First, the geological complexity of the estuary systems within each ocean basin could generate differences in salinity gradients in the intertidal zone and therefore different local adaptation patterns of *Rhizophora* spp. Second, it is also possible that hybridization levels and the direction of introgression differ in the two sites. In the Pacific, it seems that introgression mainly occurred toward *R. mangle* (Cerón-Souza et al., 2014). In contrast, our results in the Atlantic suggest that introgression mainly occurred toward *R. racemosa* (Figure 3).

Therefore, the hypothesis that soil nutrients are involved in the maintenance of the hybrid zone or in the spatial zonation of *Rhizophora* spp. does not have a simple answer. Our data suggest that responses of the red mangrove hybrid complex to nutritional soil conditions could differ depending on the study area. Consequently, we need to design controlled experiments to understand how plastic is this ability of each *Rhizophora* spp. to cope with different levels of nutrients, salinity, and light and how these factors combined could restrict or promote the hybrid zone's maintenance, the species zonation in the intertidal zones, and differences in the salinity tolerance of *Rhizophora* spp.

#### 4.4 | Isotopic signatures of N across *Rhizophora* taxonomic groups

The higher  $\delta^{15}\text{N}$  values found in leaves from the LI zone probably result from the loss of lighter N by leaching from the soil during the mineralization of organic N (Martinelli et al., 1999). A similar pattern in a narrow fringe mangrove was found much farther inland within the San Juan River floodplain at much lower salinity (Medina et al., 2008). Studies in *R. mangle* mangroves in the Caribbean have shown that low or negative  $\delta^{15}\text{N}$  values can also result from low P availability (McKee et al., 2002; Medina et al., 2010). However, this pattern does not occur in our study site since, despite the slightly decrease in soil total P from lower to upper tidal zones, the two taxonomic groups showed similar high P concentrations. The leaf N content could be an indicator of nutrient availability (Fry & Cormier, 2011; Fry & Smith, 2002; Gritcan et al., 2016), and higher N availability would increase fractionation leading to low  $\delta^{15}\text{N}$  values (McKee et al., 2002; Muzuka & Shunula, 2006). Our values of N concentration and  $\delta^{15}\text{N}$  are not correlated. Therefore, we conclude that the primary factor in fractionation at the LI plots was preferential export of lighter N supporting our hypothesis 3.

## 5 | CONCLUDING REMARKS AND FURTHER RESEARCH

- Unlike the previous study in the Pacific, we did not find a clear zonation of taxonomic groups and only two pure individuals of *R. racemosa*. This suggests that both the abundances of *R. mangle* and *R. racemosa*, as well as the zonation pattern of these two species and their hybrids, are not only influenced by their physiological tolerances but also influenced by the seasonal interaction of geomorphological, environmental, and soil factors. In the study site, the influence of the rivers surrounding the mangrove forest seems to be determinant.
- We cannot conclude that *R. mangle* is associated with more saline soils than *R. racemosa*, both because of the scarcity of *R. racemosa* in our study site and because the changes in soil salinity across the intertidal zones were small at the time of sampling. Under these conditions, all trees regardless of taxonomic group showed similar capacity for controlling Na transport to the leaves and water-use efficiency. This suggests that only when gradients of soil salinity are more pronounced can we expect a clear separation of the species in the intertidal zone.
- However, we found that Mg/Ca differ significantly between *R. mangle* and *R. racemosa* + *R. × harrisonii* regardless of the intertidal position. Moreover, *R. mangle* differed in the accumulation of C, N, K, Mg, Fe, Mn, the molar ratios of C/N, K/Ca, Mg/Ca and  $\delta^{13}\text{C}$  from the *R. racemosa* + *R. × harrisonii* group in specific intertidal positions. These results suggest that taxonomic groups of *Rhizophora* spp. cope differently with saline environments.

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### CONFLICT OF INTEREST

The authors have no conflict of interest.

### AUTHOR CONTRIBUTIONS

I.C.S., G.N.F., & E. M. conceived the ideas and goals of this study. I.C.S., M.B.B., E.B.P., A.S., & E.M. made the investigation. I.C.S., M.B.B., & E.M. performed the formal analysis. I.C.S., E.M., M.B.B., & G.N.F. wrote and edited the manuscript. All authors reviewed and approved the final manuscript.

### DATA AVAILABILITY

Data are available from the Dryad Digital Repository (Cerón-Souza et al., 2020). <https://doi.org/10.5061/dryad.zkh18938z>

### ORCID

Iviana Cerón-Souza  <https://orcid.org/0000-0002-2731-0362>

María Beatriz Barreto  <https://orcid.org/0000-0002-2906-4951>

Gonzalo N. Feliner  <https://orcid.org/0000-0002-7469-4733>

Ernesto Medina  <https://orcid.org/0000-0001-5557-5782>

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