



Salinity responses of inland and coastal neotropical trees species

Alejandro De Sedas · Benjamin L. Turner · Klaus Winter · Omar R. Lopez

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Abstract Salinization of coastal lands by sea-level rise means that information on the response of tropical tree species to salinity is urgently required to effectively manage coastal systems under future climatic scenarios. While salinity represents a major selective abiotic stress, little is known about the underlying mechanisms determining salinity tolerance in tropical trees. We examined salinity responses in seedlings of eight neotropical tree species from Panama, including four *coastal* species (*Sterculia apetala*, *Pithecellobium ungi-cati*, *Terminalia cattapa*, and *Thespesia populnea*) and four *inland* species (*Minquartia guianensis*, *Apeiba membranaceae*, *Ochroma pyramidale*, and *Ormosia macrocalyx*). Three-month-old seedlings of each species were subjected to increasing concentrations of 80, 120, 200, and 300 mM of either NaCl or

KCl, while controls were irrigated with tap water. Overall, growth parameters such as leaf area (LA), leaf area ratio (LAR), stem height (SH), total dry mass (TDM), and relative growth rates (RGR) were reduced for all species as salinity increased, regardless of salt treatment. However, species from coastal environments outperformed *inland* species at high salinity. For example, seedlings of *coastal* species growing in 300 mM of NaCl or KCl, corresponding to ~ 50% seawater, survived and maintained LA, SH, and TDM between 50 and 90% compared with control plants. In contrast, *inland* species showed reductions in RGR, LA, and SH of up to 100%, at 120 mM of either salt. At the foliar level, K⁺ accumulation remained similar under NaCl and KCl treatments, and almost all species, with the exception of *Minquartia* from inland forests, maintained foliar Na⁺ accumulation across treatments when compared to controls. While species from coastal environments maintained foliar Cl⁻ under NaCl and KCl treatments, *inland* species such as *Ochroma* showed up 95% increase in foliar Cl⁻. Our results suggest that salinity tolerance among tropical trees is predetermined by habitat association and ultimately by the ability of species to manage toxicity associated to foliar Na⁺ and Cl⁻. While the ecological implications of sea-level rise in coastal vegetation require further examination, is foreseeable that adaptation strategies in tropical shorelines consider the use of *coastal* species (i.e., reforestation) as

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A. De Sedas · O. R. Lopez (✉)
Centro de Biodiversidad y Descubrimiento de Drogas,
Instituto de Investigaciones Científicas y Servicios de Alta
Tecnología, Apartado 0843- 01103, Edificio 219, Ciudad
del Saber, Clayton, Panamá
e-mail: prioria@yahoo.com

A. De Sedas
Acharya Nagarjuna University, Guntur,
Andhra Pradesh, India

B. L. Turner · K. Winter · O. R. Lopez
Smithsonian Tropical Research Institute, Apartado
0843-03092, Balboa, Ancón, Panamá

the best tool to ameliorate the impact of increased salinity.

Keywords Coastal · Inland · Salinity · Total ion toxicity · Relative growth rate

Introduction

Sea-level rise is predicted to increase over half a meter by the year 2100 (IPCC 2012; Nicholls and Lowe 2004). This will impact the biology and geochemistry of coastal ecosystems (Zhang et al. 2013), causing species displacement in estuaries (e.g., mangroves, IPCC 2014), reduce biological productivity and compromise ecosystem functioning (Nicholls 2002; Setia et al. 2012). As sea-level rise, salinization imposes different challenges for plant communities near the coast. For example, along the Meso American corridor, dry forests in the Pacific coast might endure greater salinization effects than wet forests in Caribbean, not only due to differences in the forecasted sea-level rise (see Hughes et al. 2003), but because of the inherent climatic (i.e., drought) and geomorphological (i.e., tidal variation) characteristics of both coasts (Lezcano and Lopez 2015). Therefore, examining tolerance and responses to salinity among neotropical tree species is key for drafting strategies for managing coastal systems and understanding the broader ecological implications of increased soil salinity in the face of sea-level rise and climate change for the tropics.

In plants, salinity tolerance is dichotomized into halophytes, which can complete their life cycle under high salinity > 200 mM of NaCl (Sairam and Tyagi 2004) and glycophytes, which cannot grow and survive under high salinity (Kozłowski 1997; Kumar-Parida and Das 2005). A crucial difference between glycophytes and halophytes is also that halophytes typically require low levels of NaCl for optimal growth (Flower and Colmer 2008; Ramos et al. 2004; Flower et al. 1977). In most plants, salinity impairs growth via water stress and the resulting cell injury caused by the inability of vacuoles to sequester Na^+ and Cl^- (Blum 2011). With increasing salinity, reductions in gas exchange capacity (i.e., stomatal conductance and photosynthesis) come by plant's inability to maintain osmotic adjustment and turgor

maintenance (Duarte et al. 2013; Lambers 1998) and increased respiration (Shekoofa et al. 2013). Salinity induces leaf senescence (Munns and Tester 2008), inhibits lateral buds growth, flower phenology and reproductive output (Buchanan and Balmer 2005). While studies have shown that some plant responses to salinity stress mimic the responses to drought (Fricke and Peters 2002), homeostasis under salt stress rests on the ability to manage high ion concentrations (Flowers et al. 2015). Therefore, preventing Na^+ uptake into the transpiration stream is the first mechanism employed to avoid salinity stress (Munns and Tester 2008). However, as salinity increases, the ability to manage Na^+ decreases, causing increased cytosolic concentrations of Na^+ (Møller et al. 2009). Increased Na^+ in the cytosol has a negative impact on osmotic balance similar to the effect caused by K^+ (Maathuis et al. 1997; Shabala and Cuin 2008), suggesting that plant salinity tolerance is intrinsically associated with the ability to use K^+ to maintain Na^+/K^+ ratios. Yet, salinity tolerance among glycophytes cannot be solely determined by plants ability to maintain Na^+/K^+ ratios given the inherent toxicity of Cl^- as entrance of this ion appears to be less regulated than Na^+ in some plants (Díaz-López et al. 2012).

Apart from mangroves, understanding salinity tolerance in tropical woody tree species has proven difficult, given the concomitant effects of Cl^- in tropical woody plants. In the absence of Cl^- toxicity, most tropical woody tree species vary from sensitive to moderately sensitive to the increment of salt (NaCl) in the soil. For example, studies with different rootstocks and species of *Citrus* indicate a decrease in plant performance as salinity increased (Syversten and García-Sánchez 2014; Ruiz et al. 1995). However, little is known about salinity tolerance of tropical tree species and the extent to which Cl^- toxicity plays a role. Recently, De Sedas et al. (2019) evaluated over 25 tree species from *coastal* and *inland* tropical rainforests of Panama, finding that *coastal* species are significantly more salt tolerant than those from *inland* wet forests, although the mechanisms behind this tolerance remains unknown. Experiments with different salt solutions are required to disassociate the effects of these different ions (Na^+ , K^+) but in particular the role of Cl^- on salinity tolerance of tropical woody species.

Here we report the responses of eight common neotropical tree species to the addition of NaCl and

KCl salts to the soil. We compare growth responses to salinity among four tree species restricted to *coastal* and *inland* habitats common to Central America. We asked: (i) how does salinity, caused by either NaCl and KCl, affect the growth of eight tropical tree species? (ii) how does growth vary among *coastal* and *inland* rainforests tree species in response to increased NaCl and KCl concentrations? and (iii) what is the relationship between the foliar concentrations of K^+ , Na^+ and Cl^- ions and salinity sensitivity? We aim at addressing these questions with the objective to broaden our understanding of salinity tolerance mechanisms in tropical species and provide evidence for mitigation strategies in the face of sea-level rise in the tropics.

Materials and methods

Study site

We conducted all experiments in a greenhouse facility located in Gamboa (09°07' N, 79°42' W), about 25 km north of Panama City, operated and maintained by the Smithsonian Tropical Research Institute. We used an open-glass rain-sheltered greenhouse, which allowed full air circulation, receiving an average photosynthetic active radiation (PAR) of $31 \text{ mol m}^{-2} \text{ days}^{-1}$, a monthly average relative humidity of 86% and temperature of 27.1 °C.

Species selection and descriptions

The study species represent a subsample of common trees, native and naturalized, found along Pacific *coastal* habitats as well as in *inland* wet forests of Panama. Our eight focal species represent a subset of 26 species studied by De Sedas et al. (2019), to provide an in-depth understanding of salinity tolerance mechanisms between *coastal* and *inland* species in relation to foliar ionic concentration as salinity increase by the addition of NaCl or KCl solutions.

Among the *coastal* species, *Terminalia cattapa* L. “Almendro” (Combretaceae) is a tree native to Asia that now occurs as naturalized across all tropical seashores. This species can grow up to 25 m, forming a typical dogwood tree branching model (“pagoda-like”; Condit et al. 2010). Although is found in coastal areas, it can grow further *inland*, where produces fewer fruits (O.R.L. pers. observation). *Thespesia*

populnea (L.) Sol. ex Corrêa “Majaguillo” (Malvaceae), is a small tree or shrub probably native to India (Nelson 1994), now occurring pantropically in coastal areas, along estuaries or at the upper tidal range. It can grow up to 15 m tall but branches at low height. *Pithecellobium unguis-cati* (L.) Benth. “Espino carbon” (Fabaceae-Mim.), is a native tree 3–10 m tall with a low-branched trunk. This species can grow in thickets on arid limestone as well as in open woodland on the edges of salt marshes or savannas near the coast. It is common in areas near the Pacific coast of Panama, but rare or absent in the Caribbean, and is broadly distributed from southern United States to Venezuela and the Caribbean. *Sterculia apetala* (Jacq.) H. Karst. “Panama tree” (Malvaceae), is a native tree that grows up to 35 m tall, usually with a straight trunk and medium-sized buttresses. The Panama tree grows in dry or wet climates. Common in pastures and dry forests of the Pacific coast of Panama, it is rare or absent in the Caribbean rainforests. This species is distributed from Belize, to Brazil and Caribbean islands.

Of the *inland* species, *Ochroma pyramidale* (Cav. ex Lam.) Urb. “Balso” (Malvaceae) is a common fast growing-pioneer tree species, native to Central and South America. It can reach up to 35 m tall and is frequent in disturbed sites of lowland forests up to mid elevations (i.e. 800 m a.s.l.) in either dry or wet climates. *Ormosia macrocalyx* Ducke “Coralillo” (Fabaceae-Pap.) is a common native tree reaching 35 m tall, frequently found in lowland and mid elevation rainforests from Mexico to the Amazon. *Apeiba membranacea* Spruce ex Benth. (Malvaceae) is a native tree up to 30-m tall with a trunk with small buttresses at the base. *Apeiba* can be found from lowland to mid elevations (i.e. 800 m a.s.l.) wet forests from Honduras to Brazil. *Minquartia guianensis* Aubl. “Criollo” (Olacaceae), can grow up to 35-m tall and is common in Caribbean rainforests up to mid elevations (i.e. 800 m a.s.l.) in very wet habitats, but rare or absent in dry forests of the Pacific slope. This species is found from Nicaragua to Brazil.

Plant material

We collected seeds of our focal *coastal* and *inland* species from trees growing on beaches along the Pacific shoreline and from *inland* areas (c.a. 35 km from the coast) in Soberania National Park and La

Amistad International Park, in central and northwestern Panama, respectively. Seeds were properly cleaned or disaggregated (*Ochroma*, *Apeiba*) and kept in dry place until germination (2–3 weeks). For germination, we placed seeds in 2.3 L pots (Stuewe and Sons, Covallis, OR) filled with forest soil in a shaded greenhouse with 35% sun transmittance. After germination, seedlings with one to two fully developed leaves were transferred to the greenhouse where they receive full sunlight.

Experimental design and NaCl and KCl treatments

We conducted a full factorial experiment using solutions of sodium chloride (NaCl) and potassium chloride (KCl) at four different concentrations: 80, 120, 200, and 300 mM. Potassium chloride was employed to disassociate the role of chloride from sodium. Plants were irrigated once a week with 150 ml solution of each salt (NaCl and KCl), while controls received 150 ml of tap water (0 mM), using eight replicates (seedlings) per treatment, concentration by species. After salinity treatment irrigation, all plants were watered every two days with 100 ml of tap water to avoid drought dehydration. Salinity treatments were carried for about 60 days.

Growth parameters

During the experiment, we measured each plant stem height (SH) as the distance from the ground to the distal apical bud and counted and measured the size (width and length) of all leaves, every 15 days. At the end of the experiment, all plants, as per treatments and species, were harvested to determine total leaf area, total dry mass and leaf weight ratio. After harvest, plant material was rinsed with water and divided into leaves, stems (including leaf petioles) and roots. Leaf area (LA) was quantified measuring all fresh leaves using a leaf area meter (Li-3100, Li-Cor Instruments, Lincoln, Nebraska, USA). Total dry mass represents the sum of leaves, stems and roots dry weight obtained after drying plant material for 72 h at 70 °C. The leaf weight ratio (LWR) was estimated as the proportion of leaf dry weight to the sum of roots and stem dry weight. Additionally, we harvested five plants prior to the initiation of the experiment to obtain the initial dry mass for each species. Relative growth rate (RGR) was

estimated using each species average initial dry mass and the final dry mass of each seedling, defined as:

$$\text{RGR} (\text{g g}^{-1}\text{days}^{-1}) = (\ln W_2 - \ln W_1)/t,$$

where W_1 is the initial dry mass and W_2 is the total dry mass at final harvest, and t is the time of the treatment in days.

Na⁺, K⁺ and Cl⁻ ion concentration in leaf tissue

Foliar Na⁺, K⁺, and Cl⁻ concentrations were determined in dried leaves of three individuals subsampled from each salinity treatment. Foliar Na⁺ and K⁺ were measured by nitric acid digestion and detection by inductively coupled plasma optical-emission spectrometry (ICP-OES). In short, 200 mg of ground leaf tissue were placed into a polytetrafluoroethylene (PTFE) digestion vessel with 2 ml of concentrated (70%) nitric acid. The samples were digested for 6 h at 180 °C, diluted to 50 mL, and analyzed by ICP—OES (7300DV, Perkin Elmer, Inc., Shelton, CT, USA).

The concentration of foliar Cl⁻ was determined by titration using a Salt Analyzer Titrator following the manufacture's protocol (SAT-500, DKK-TOA Corp. Tokyo, Japan). One hundred milligrams of ground leaf tissue were diluted into 20 ml of deionized water and set aside for 30 min to solubilize. The solution was then filtered through a cellulose filter at neutral pH and Cl⁻ measured with an electrode in the Titrator. Chloride concentrations were then calculated in relation to the weight of NaCl in the weight of the leaf tissue (wt/wt).

Statistical analysis

We used a general linear mixed model (GLMM) to quantify the effects of salts (NaCl or KCl), concentration (0, 80, 120, 200, or 300 mM of salt solution) in growth parameters (leaf area, leaf weight ratio RGR, and stem height) and foliar ion content (Na⁺, K⁺, and Cl⁻) across all study species. The effect of habitat (*coastal* vs. *inland*) has been highlighted as an explanatory factor in the response of these species to seawater addition (see De Sedas et al. 2019); therefore, we conducted separate GLMM's to gain insight at how growth parameters and foliar ion content across species within each habitat responded to salt treatments. Additionally, we took an in-depth look at how

species within habitat varied (e.g., multiple comparison) by conducting a HSD Tukey test on RGR response to the 120 mM of both salt together (see Supplementary Material). We conducted regression analysis on the relationship between growth parameters; leaf area, leaf weight ratio and stem height and relative growth rate, as well as the foliar ionic concentration of Na, K, and Cl with relative growth rate. A principal component analysis (PCA) was used to determine species grouping patterns with respect to all growth parameters and foliar ion content in response to salinity at 120 mM of either salt solution (NaCl or KCl). Statistical analyses were performed using IBM SPSS Statistic v. 25 (IBM Corporation, Armonk, NY). Principal component analysis were conducted using a JMP v. 13 (SAS Institute, Cary, NC) and all graphs, except the ordinations, were done in Sigma Plot v. 12.3 (Systat Software, San Jose, CA).

Results

Growth parameters

Overall, growth responses to salinity vary in relation to species regardless of salt type (NaCl or KCl) and the concentration used ($P < 0.0001$, see Table 1, Fig. 1). Similarly, when growth responses are analyzed taken together all species and type of salt, seedlings were significantly affected as the concentration of either salt increased (see Table 1, Fig. 1). Not surprisingly, the interaction of treatment concentration by species significantly reduced growth (see Table 1). However, when growth responses were analyzed separately in regard to habitat, the species by treatment interaction effect was maintained in the *inland* species and only significant for LA and LWR in *coastal* species (see Table 1).

Irrespective of species and salt used (NaCl or KCl), LA decreased as treatment concentration increased (see Table 1, Fig. 1). However, differences in LA as treatment concentration increased were associated with species habitat. When compared to controls, seedlings of *Ochroma* treated with 200 mM of either salt experienced near 85% reduction in LA (Fig. 1). In contrast, seedlings of *Pithecellobium*, a *coastal* species, showed no variation in LA across salt concentrations. Despite the consistent differences in growth responses to salinity treatment associated with

habitat, with the exception of RGR in coastal areas (Table 1, Figs. 1 and 2), the treatment by species interaction among the *coastal* species was only significant for LA and LWR (see Table 1, Fig. 1). Similarly, as the concentration of either salt increased, the effects in LWR become significant (Fig. 1). Yet, *inland* species appear to be more sensitive than those from coastal areas. For example, in comparison with control, seedlings of *Ormosia* under 200 mM of NaCl experienced a nearly 100% reduction in LWR (Fig. 1). In contrast, *Sterculia*, the most affected species among those from coastal areas, showed near 25% decrease in LWR (Fig. 1). The increased LWR observed in *Ochroma* as concentration increase might result from root loss and not due to greater leaf area.

Across species, plant stature, quantified as stem height (SH), responded similar to LA. We found significant differences in SH across species and treatments regardless of the salt employed. However, differences in the sensitivity between *coastal* and *inland* species, as concentration increased, were significant. Among the *inland* species, SH was significantly affected when the concentration of NaCl and KCl increased ($P < 0.0001$, see Table 1), *coastal* species also showed a significant reduction in SH under NaCl or KCl ($P = 0.035$, see Table 1), but it appears that sensitivity of the *inland* species was greater under the NaCl than the KCl treatment (Fig. 1). While *coastal* species maintained SH throughout salt concentrations, the *inland* species *Minuartia* showed between 22 and 25% reduction in SH at 80 mM of either salt (Fig. 1).

Irrespective of the salt used (NaCl and KCl) significant differences in total dry mass were explained by concentration and species (Table 1). Yet, TDM at the end of the experiment was significantly affected by the interaction of species by treatment concentration (see Table 1). However, this interaction effect on TDM was only detectable for the *inland* species (see Table 1, Fig. 1). We did observe a slight increment in total dry mass when seedlings were exposed to low salt concentrations e.g., 80 and in some cases 120 mM (see Fig. 1), but such differences were only significant for the *coastal* species exposed to 120 mM ($P = 0.033$, Table 1, Fig. 1).

Relative growth rate (RGR) showed no significant response differences in relation to NaCl or KCl. However, across species significant differences emerge in RGR as salinity concentration of either salt

Table 1 Effect test of species, solution (NaCl or KCl), treatment (concentration), and their interactions on growth parameters (leaf area (LA), leaf weight ratio (LWR), stem height (SH), and relative growth rate (RGR)), for all samples and for *coastal* and *inland* species using a GLMM analysis

Source	Variable	All samples				<i>Coastal</i> species				<i>Inland</i> species			
		SS	df	F	Sig	SS	df	F	Sig	SS	df	F	Sig
Species	LA	2,416,527.2	6	159.38	***	1,890,654	3	601.66	***	525,873.27	3	39.7	***
	LWR	5.89	6	35.38	***	0.29	3	125.83	***	5.59	3	30	***
	SH	6132.71	6	245.75	***	2215.27	3	1222.92	***	3917.44	3	150.14	***
	Dry mass	471.31	6	313.65	***	429.53	3	525.63	***	41.78	3	62.62	***
	RGR	20.37	6	108.15	***	0.09	3	1850.07	***	20.29	3	94.64	***
Solution	LA	3185.75	1	1.26	n.s	36.78	1	0.04	n.s	6240.87	1	1.41	n.s
	LWR	0.11	1	3.96	0.047	0	1	1.88		0.22	1	3.5	n.s
	SH	14.24	1	3.42	n.s	1.2	1	1.99	n.s	34.4	1	3.96	0.048
	Dry mass	0.02	1	0.10	n.s	0.07	1	0.27	n.s	0	1	0	n.s
	RGR	0.08	1	2.66	n.s	0	1	0.42	n.s	0.14	1	2.02	n.s
Treatment	LA	1,363,659	4	134.91	***	10,875.74	4	2.6	0.037	2,336,702.2	4	132.29	***
	LWR	6.09	4	54.87	***	0.02	4	6.14	***	11.57	4	46.55	***
	SH	7020.59	4	422	***	6.36	4	2.63	0.035	12,838.57	4	369.03	***
	Dry mass	83.89	4	83.75	***	3.11	4	2.85	0.024	145.69	4	163.75	***
	RGR	5.41	4	43.08	***	0	4	0.55	n.s	9.44	4	33.04	***
Species × solution	LA	12,627.16	6	0.83	n.s	3453.76	3	1.1	0.350	9173.4	3	0.69	n.s
	LWR	0.55	6	3.29	0.003	0	3	1.61	n.s	0.54	3	2.92	0.035
	SH	44.88	6	1.80	n.s	4.1	3	2.26	n.s	40.78	3	1.56	n.s
	Dry mass	0.23	6	0.15	n.s	0.03	3	0.04	n.s	0.2	3	0.3	n.s
	RGR	0.22	6	1.18	n.s	0	3	2.17	n.s	0.22	3	1.04	n.s
Species × treatment	LA	2,000,176.5	24	32.98	***	24,161.01	12	1.92	0.032	1,976,015.5	12	37.29	***
	LWR	6.37	24	9.57	***	0.04	12	4.05	***	6.33	12	8.49	***
	SH	3983.56	24	39.91	***	7.42	12	1.02	n.s	3976.14	12	38.1	***
	Dry mass	47.41	24	7.89	***	3.46	12	1.06	n.s	43.95	12	16.47	***
	RGR	17.97	24	23.85	***	0	12	1.09	n.s	17.97	12	20.96	***
Solution × treatment	LA	7979.84	4	0.79	n.s	8556.42	4	2.04	n.s	7348.2	4	0.42	n.s
	LWR	1.06	4	9.53	***	0	4	1.33	n.s	1.86	4	7.5	***
	SH	51	4	3.07	*	9.14	4	3.79	**	69.03	4	1.98	n.s
	Dry mass	0.59	4	0.59	n.s	0.25	4	0.23	n.s	0.61	4	0.69	n.s
	RGR	0.99	4	7.87	***	0	4	0.92	n.s	1.52	4	5.33	***

Table 1 continued

Source	Variable	All samples				<i>Coastal</i> species				<i>Inland</i> species			
		SS	df	<i>F</i>	Sig	SS	df	<i>F</i>	Sig	SS	df	<i>F</i>	Sig
Species × solution × treatment	LA	55,612.94	24	0.92	n.s	18,275.27	12	1.45	n.s	37,337.67	12	0.71	n.s
	LWR	4.62	24	6.95	***	0.01	12	0.5	n.s	4.62	12	6.19	***
	SH	206.13	24	2.07	n.s	6.05	12	0.84	n.s	200.08	12	1.92	0.034
	Dry mass	3.22	24	0.54	n.s	0.66	12	0.2	n.s	2.57	12	0.96	n.s
	RGR	1.35	24	1.79	*	0	12	0.49	n.s	1.35	12	1.57	n.s

Significance levels: * = $P < 0.01$; ** = $P < 0.001$ and *** = $P < 0.0001$. For values approaching $P = 0.05$ the entire number was left

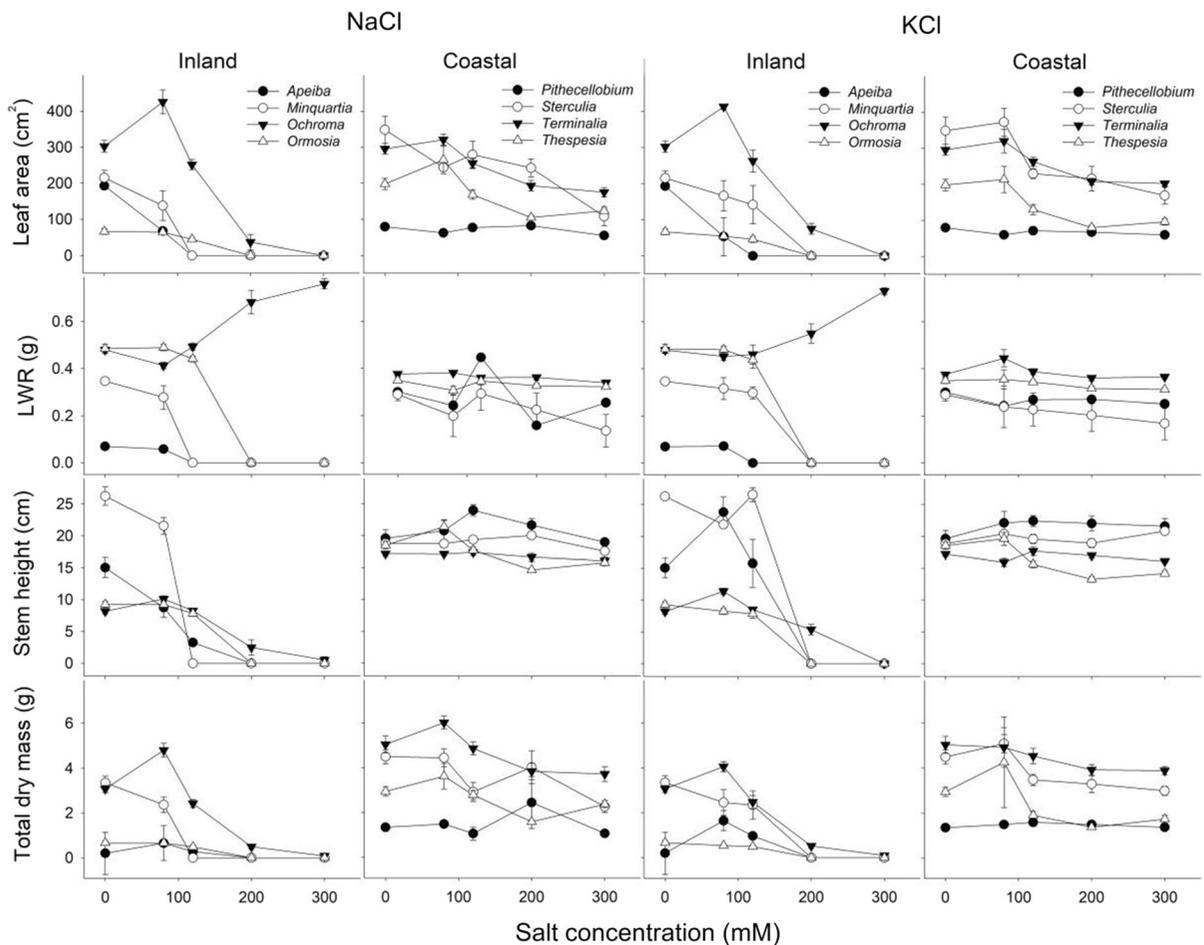
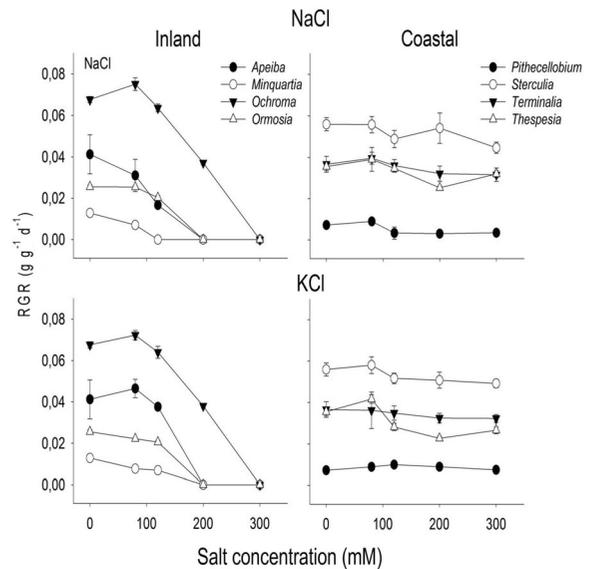


Fig. 1 Salinity effects on growth parameters for *inland* and *coastal* species under NaCl and KCl treatments. Leaf area was measured at the end of the experiment in controls (0 mM) and

treatments. LWR was estimated as the proportion of LA biomass over roots and stems. Plants were harvest after 60 days and dried at 70 °C per three days ($n = 8$ plants per treatment)

Fig. 2 Effect of salinity on RGR for *inland* and *coastal* species under NaCl and KCl treatments. Initial dry mass was 0.102 g (*Apeiba*), 1.548 g (*Minquartia*), 0.053 g (*Ochroma*), 0.144 g (*Ormosia*), 0.870 g (*Pithecellobium*), 0.156 g (*Sterculia*), 0.560 g (*Terminalia*), and 0.349 g (*Thespesia*) see methods for details on RGR estimations. Plants were harvest after 60 days and dried at 70 °C per three days ($n = 8$ plants per treatment)



increased (Table 1). In agreement with other growth parameters, RGR response depended on the species and the concentration of salt (species*treatment $P < 0.0001$, Table 1). Further, *coastal* species showed no significant reduction in RGR under either salt, while *inland* species grew significantly less as salinity concentration of NaCl or KCl increased beyond 80 mM (Fig. 2).

To assess the relationship between the response parameters: LA, LWR, SH, and RGR for *coastal* and *inland* species, we calculated growth responses and RGR as mean values over the entire experiment. Among these, LA explained between 73 and 56% of the variation in RGR for *inland* species under NaCl and KCl treatments ($r^2 = 0.56$ and $r^2 = 0.73$, respectively, $P < 0.0001$, Fig. 3). Similarly, nearly 70% of the differences in RGR among the *coastal* species was explained by LA under both treatments ($r^2 = 0.65$ and $r^2 = 0.70$ under NaCl and KCl treatments, respectively, $P < 0.0001$, Fig. 3). While LWR and SH did not explain variation in RGR in *coastal* and *inland* species under NaCl and KCl treatments, this appear to be due to a disruption of growth after 120 mM of salt concentration (Fig. 3).

Sodium, potassium, and chloride accumulation in leaves

Species had a significant effect on foliar concentration of Na^+ , K^+ and Cl^- regardless of concentration or type

of salt (Table 2). As expected, Na^+ foliar content was significant between solutions (NaCl or KCl). However, treatment effects were significant on Chloride ion foliar concentration, and consistent across whether species came from *coastal* or *inland* habitats (Table 2, Fig. 4). However, how much these ions were quantified in the leaves of the species varied drastically. Among the *inland* species, *Minquartia* showed great increased concentration of foliar Na^+ , while *Ochroma* showed a consistent increment in foliar Cl as the concentration of either salt increased (Fig. 4). Yet, in general, K^+ and Cl^- foliar content showed no significant difference between NaCl and KCl treatments (Table 2). (Fig. 4).

We examined how RGR was explained by Cl^- foliar concentration among *coastal* and *inland* species, and while there is indication that as Cl^- foliar content increased, RGR decreased, this negative relationship could not be supported by statistical inference giving the limitations of the data (Suppl. Materials Fig. 1).

Species ordination (PCA) in relation to growth parameters and foliar ion concentration

Principal component analysis (PCA) condenses growth and foliar chemistry responses into meaningful explanatory dimensions. The PCA produced two robust axes that together, accounted for 75 and 62% of the total variation under 120 mM NaCl and KCl solutions, respectively (Fig. 5). Under the 120 mM

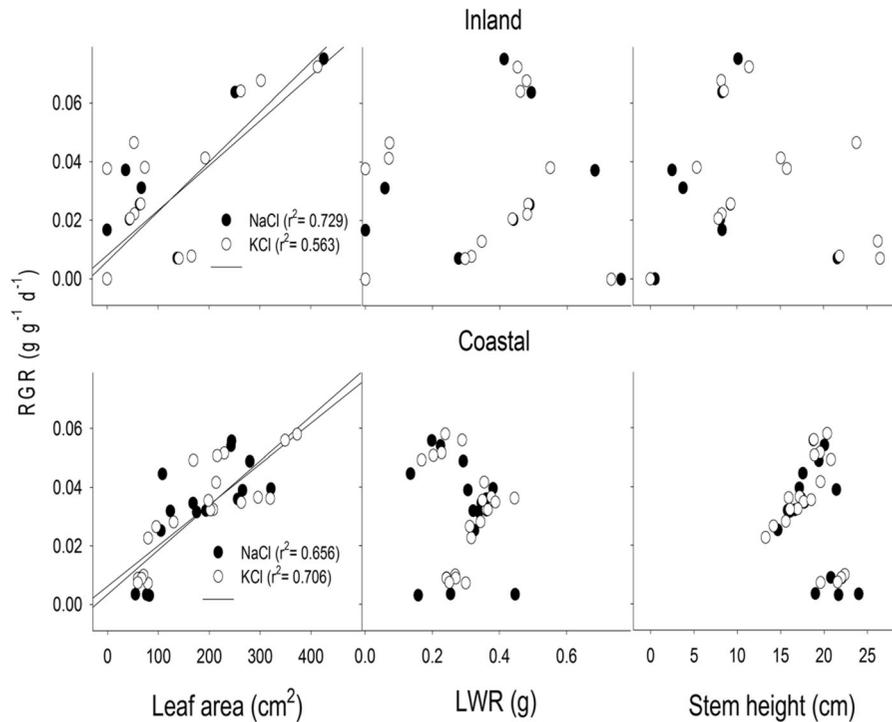


Fig. 3 Relative growth rate (RGR) in relation to growth parameters (*LA* leaf area, *LWR* leaf weight ratio and *SH* stem height) for *inland* and *coastal* species under NaCl and KCl treatments. Leaf area growth explains between 56 and 73% of

the variation in RGR for *inland* and *coastal* species (*Inland* $r^2 = 0.73$ and $r^2 = 0.56$; *Coastal* $r^2 = 0.65$ and $r^2 = 0.70$ under NaCl and KCl treatments, respectively). Plants were harvest after 60 days and dried at 70 °C over 72 h

NaCl solution, species' responses along the PC axis 1 were related to growth parameters such as leaf area and RGR, with eigenvalues of 0.41 and 0.36, respectively, while along PC axis 2 was dominated by the foliar concentrations of Cl^- , K^+ , and Na^+ , with eigenvalues of 0.49, 0.23, and 0.22, respectively. In the case of the 120 mM KCl solution, similarly the PC axis 1 was driven by growth parameters including RGR and leaf area with eigenvalues of 0.46 and 0.35, respectively; however, the PC axis 2 was directed by stem height and Na^+ ion foliar concentrations, with eigenvalues of 0.48 and 0.17, respectively.

Discussion

Overall growth responses

Seedlings of *coastal* species showed no reduction in growth even when exposed to the maximum salt

concentration (300 mM, ~ 50% seawater). On the contrary, growth of *inland* species was reduced at lower salt concentration (120 mM, ~ 20% seawater). The inability of *inland* species to grow under increased salinity appears to rest on the impact that this has on leaf growth (see Figs. 1 and 3). These findings are similar to salinity response reported in previous studies of tropical woody trees. For example, *Acca sellowiana* “pineapple guava”, a highland species from South America, showed up to 31% reduction in leaf growth at 80 mM NaCl (Casierra-Posada 2006). Similar results were found in *Citrus*, *Casuarina*, *Eucalyptus*, and *Persea americana* (Ruiz et al. 1997; Sun and Dickinson 1995; Kozłowski 1997). However, the ability of *coastal* species to maintain greater growth than *inland* species under salinity suggests that salinity response among common tropical tree species is highly variable and that those species exposed to some degree of salinity might be

Table 2 Effect test of species, solution (NaCl or KCl), treatment (concentration), and their interactions on foliar Na⁺, K⁺, and Cl⁻ content for all samples and for *coastal* and *inland* species using a GLMM analysis

Source	Variable	All samples				Coastal species				Inland species			
		SS	df	F	Sig	SS	df	F	Sig	SS	gl	F	Sig
Species	Na ⁺	1613.32	6	1671.09	***	3.51	3	12.70	***	1609.81	3	965.01	***
	K ⁺	4983.89	6	6.14	***	2624.19	3	27.45	***	2359.71	3	1.08	n.s
	Cl ⁻	7558.03	6	64.36	***	1869.16	3	56.67	***	5688.87	3	27.52	***
Solution	Na ⁺	263.33	2	818.27	***	5.88	2	31.88	***	307.49	1	552.98	***
	K ⁺	31.42	2	0.12	n.s	400.13	2	6.28	*	224.36	1	0.31	n.s
	Cl ⁻	10.50	2	0.27	n.s	20.43	2	0.93	n.s	1.91	1	0.03	n.s
Treatment	Na ⁺	1.30	4	2.01	n.s	1.84	4	4.98	**	0.20	3	0.12	n.s
	K ⁺	1730.66	4	3.20	*	1043.09	4	8.18	***	866.03	3	0.40	n.s
	Cl ⁻	11,265.21	4	143.88	***	6116.13	4	139.06	***	6075.59	3	29.39	***
Specie* solution	Na ⁺	391.42	6	405.44	***	1.95	4	5.28	**	1.99	1	3.58	n.s
	K ⁺	3851.72	6	4.75	***	438.19	4	3.44	*	0.63	1	0.00	n.s
	Cl ⁻	232.93	6	1.98	n.s	7.52	4	0.17	n.s	37.63	1	0.55	n.s
Specie × treatment	Na ⁺	2.86	11	1.61	n.s	0.42	9	0.51	n.s	2.44	2	2.19	n.s
	K ⁺	1623.57	11	1.09	n.s	921.06	9	3.21	*	702.51	2	0.48	n.s
	Cl ⁻	1679.82	11	7.80	***	782.4	9	7.91	***	897.42	2	6.51	*
Solution × treatment	Na ⁺	3.19	4	4.96	**	2.38	3	8.60	***	389.04	3	233.21	***
	K ⁺	372.70	4	0.69	n.s	334.99	3	3.50	0.020	3516.73	3	1.61	n.s
	Cl ⁻	37.17	4	0.48	n.s	80.76	3	2.45	n.s	152.17	3	0.74	n.s

Significance levels: * = $P < 0.01$; ** = $P < 0.001$ and *** = $P < 0.0001$. For values approaching $P = 0.05$ the entire number was left

able to better handle future salinization of coastal areas.

Compartmentalization of Na⁺ and K⁺

Foliar Na⁺ and K⁺ concentrations response to concentrations of either salt was idiosyncratic. This was consistent for *coastal* as well as for *inland* species. *Inland* species, for example, did not show differences in K⁺ in relation to both salts, while *coastal* species do (Table 2). However, this result could be confounded by the fact that most leaves of *inland* species, particularly above 120 mM, became chlorotic and fell thus limiting our inference on the effect of treatments and solution on ionic foliar content. While it is difficult to discern whether the increase in foliar Na⁺ concentration in the *inland* species *Minquartia* beyond 120 mM NaCl is due to more ions coming through their roots or via vacuole limitations, this species showed increased foliar Na⁺ concentration accompanied with increases in Cl⁻, suggesting a potential higher influx of Cl⁻ into the transpiration stream. In

Sorghum, *Gossypium*, *Citrus*, and *Hordeum*, an increment of Na⁺ accompanied with Cl⁻ is explained by vacuolar membrane permeability (Eaton 1942). Taken together, maintenance of foliar Na⁺ among the studied species reaffirms the notion of threshold responses. In the case of the *inland* species, this threshold is maintained up to 120 mM of NaCl, while the *coastal* species maintain foliar Na⁺ concentrations up to 300 mM of salt. Our results highlight the possibility that salinity tolerance among tropical trees might be dictated by “preadaptation” to prevalent environmental conditions experienced in habitats where the plant grows (Munns and Tester 2008; Zhang and Blumwald 2001).

Foliar Cl⁻ and its implications

Chloride is an essential micronutrient for higher plants (White and Broadley 2001) and a major osmotically active solute involved in osmoregulation (Fixen 1993). Chloride has been proposed to be relatively nontoxic at ‘normal’ concentrations between 2 and

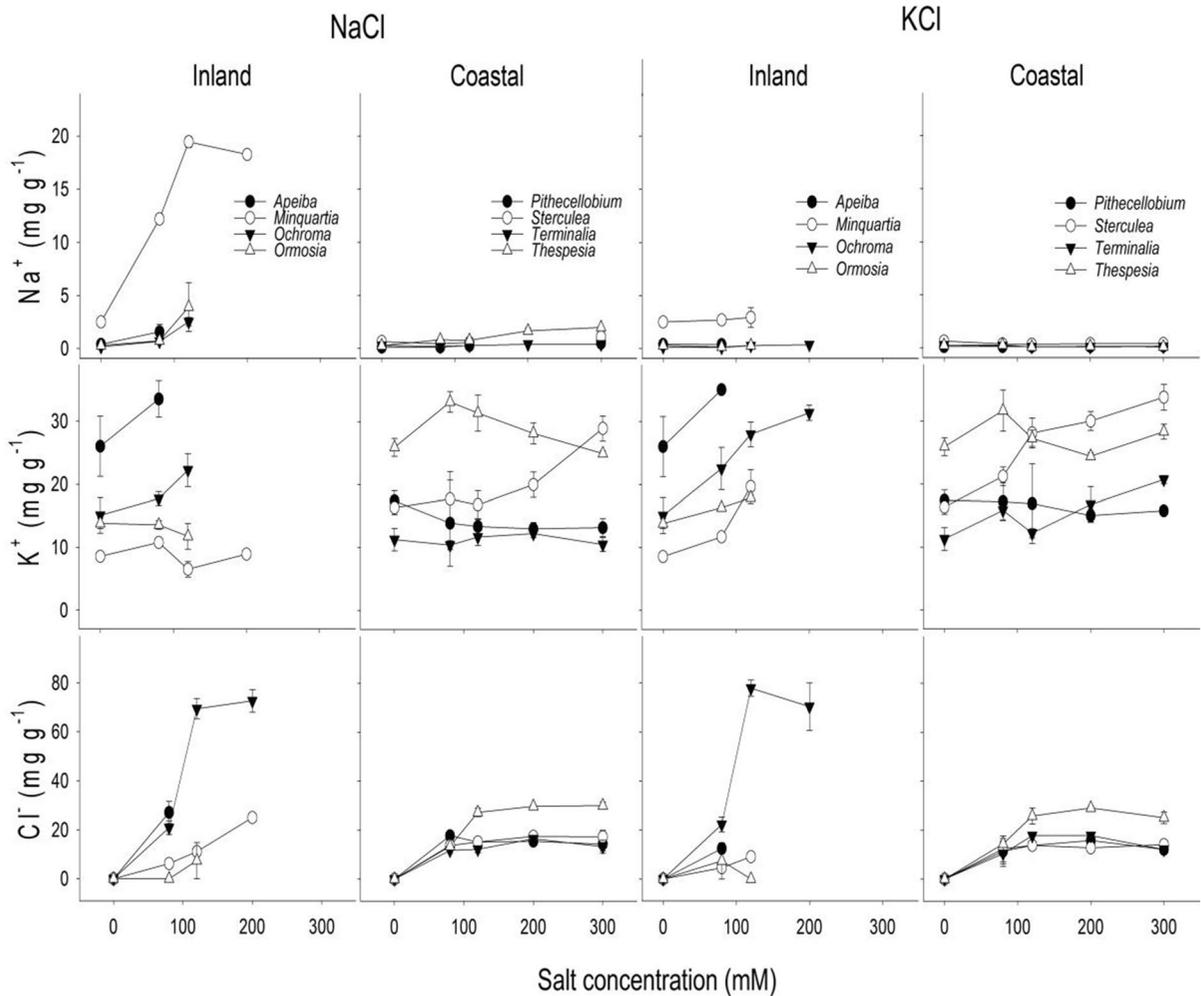


Fig. 4 Foliar sodium, potassium, and chloride ion content of *inland* and *coastal* species as mg·g of dry mass in relation to NaCl and KCl concentration. Note the response in Na⁺ ion

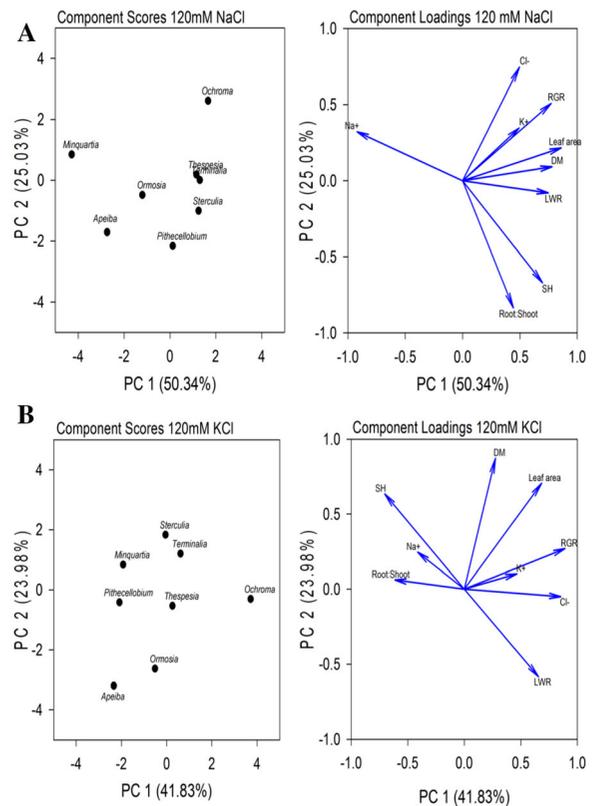
content for the *inland* species *Minquartia*, while *Ochromoa* appear to unmanage Cl⁻. Plants were harvest after 60 days and dried at 70 °C per three days

20 mg per gram of plant dry mass (Fixen 1993). This result was consistent for species of *coastal* and *inland* habitats in control treatments and showed that Cl⁻ accumulated in leaves of *coastal* and *inland* species as salt concentration increased. Foliar Cl⁻ concentrations in *inland* species appear more variable than in *coastal* species. Our results show a 3.5 times greater (71 mg g⁻¹) than in control plants, when salinity increases in the *inland* species *Ochroma*. Allen et al. (1994) highlighted the importance that excluding Cl⁻ from leaves and shoots has in woody trees, and therefore a plausible explanation for the decreased leaf area, and thus RGR, observed in *inland* species might be related to Cl⁻ toxicity in our study (Fig. 5). Mineral

toxicity is defined as a 90% reduction of growth, although such threshold has not been established for ionic salts making salinity tolerance in terms of chloride toxicity difficult to define among glycophytes (Flowers et al. 2015; Flowers and Yeo 1986). High foliar Na⁺ and Cl⁻ might be related to the observed reduced growth in the *inland* species. We believe, the ability to manage increased Na⁺ and Cl⁻ ion concentrations at the foliar level may define plant survival among tropical woody glycophytes, as *inland* species that showed about > 85% reduction in growth also showed greater mortality.

The ability of *coastal* species to maintain foliar ionic balances at relatively high concentrations of

Fig. 5 Principal component analysis (PCA) for all studied species under 120 mM of NaCl and KCl solution. Under NaCl species responses along PC axis 1 are weighted primarily by leaf area and RGR, while the PC axis 2 is dominated by the foliar concentrations of Cl^- , K^+ , and Na^+ . In the case KCl, PC axis 1 is driven by growth parameters and the PC axis 2 by stem height and Na^+ foliar concentrations



NaCl and KCl revealed some plasticity in tolerance to salinity among glycophytes and further suggests that tree species associated with littoral areas are better adapted to handle greater salinity concentrations. This is supported by the dimensional representation of *coastal* and *inland* species in the PCA ordination. At 120 mM NaCl, the *coastal* species appear to cluster while maintaining growth (e.g., RGR and leaf area), while the *inland* species, in particular *Ormosia* and *Minuartia*, ordinate in relation to the foliar ion content (i.e., chloride and sodium, respectively; Fig. 5a). This is less clear under the KCl solution, as the *coastal* species cluster along growth parameters, but the *inland* species appear to respond differently. *Ochroma* appears to respond particularly to foliar Cl^- content, while *Minuartia* responds to high levels of foliar Na^+ (Fig. 5b). The contrasting sensitivity of *inland* species to ionic concentration suggests that salinity tolerance among glycophytes cannot be solely assessed as the ability to maintain Na^+/K^+ ratios, but also as the capacity to manage chloride (Diaz-López et al. 2012).

Concluding remarks

Increased salinity in coastal tropical areas due to future sea-level rise scenarios will limit plant productivity and shift plant community composition. According to previous studies, 200 mM of NaCl is the threshold salinity concentration to distinguish salt tolerant species. In our study, *coastal* species showed sustained growth above this threshold, but *inland* species appear sensitive to concentrations below the threshold. This suggests that salinity tolerance among common tropical tree is highly variable and might be habitat-dependent. The ability of *coastal* species to maintain ionic balance at high concentrations of NaCl and KCl needs to be further explored as our results suggest a predisposition of tree species associated with littoral areas to be better adapted to handle salinity.

More broadly, increased salinity in coastal tropical areas due to sea-level rise will change the ecology of littoral areas. Coastal salinization therefore not only threatens a reduction in species diversity and habitat degradation, but will compromise the resilience of tropical coastal communities to other impacts of

climate change. While salinity research has focused on crop plants and some halophilic perennial herbs in the Amaranthaceae, efforts must also evaluate the responses of tropical woody trees to provide useful information for reforestation in coastal areas under increased salinity.

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