

Environmental influences on stomatal traits of mangrove *Ceriops decandra* (Griffith) Ding Hou in the Sundarbans, Bangladesh

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Abstract

Quantifying how stomatal traits respond to multiple interacting environmental variables is crucial for understanding plant adaptations under changing environment. Based on trait and environmental data collected from the three salinity zones (less saline, medium saline and high saline zones), and using ANOVA and Generalized Additive Models (GAMs), we aimed to understand the effects of environmental variables (i.e., salinity, siltation, pH, light and soil nutrients) on stomatal morphology in an important shrubby mangrove *Ceriops decandra* in the Bangladesh Sundarbans. Specifically, we asked: (1) how do stomatal density (SD), stomatal pore length (SPL), stomatal pore width (SPW), guard cell length (GCL) and guard cell width (GCW) vary across the salinity zones? and (2) how do environmental variables influence stomatal traits in *C. decandra*? We found that the species forms stomata on the abaxial surface (hypostomaty) of leaves. Albeit SD, SPL and GCL did not vary significantly, SPW and GCW varied significantly across the salinity zones with wider SPW and narrower GCW in less saline areas. GAM models for SPW (49%) and GCW (44%) showed higher explanatory powers than other stomatal traits. Among the environmental traits salinity had the strongest effect on SPW (negative) and GCW (positive) and P and K had strong effects on SPW and GCW, respectively, although leaf area index (LAI) had less influence on the stomatal traits. The trade-off between SPW and GCW in regulating stomatal pore areas in response to fluctuating habitat conditions suggests that *C. decandra* can efficiently maintain its gas exchange capacity under stress, thus offering us an example of how plants may acclimatize under changing environments.

Introduction

Mangroves are carbon-dense intertidal ecosystems sustaining coastal livelihoods in 118 (sub) tropical countries (Donato et al. 2011; Románach et al. 2018). Despite providing numerous ecosystem services, mangroves are increasingly threatened by rapid environmental changes and human pressures (Spalding and Leal 2021). Moreover, climate change, in particular, rising sea level may have serious consequences on mangrove structure, functions, growth and productivity (Sarker et al. 2021; Friess et al. 2022).

Stomata are small pores that are bounded by two guard cells (GCs) on the leaf surfaces and stalks, and play a major role in regulating tree functioning, ecosystem productivity, hydric stress tolerance, and water and carbon cycles through gas exchange (Wang et al. 2014; Anderegg et al. 2017). By adjusting guard cell turgor pressure, trees can alter stomatal pore aperture, thereby regulating gas exchange (Henry et al. 2019). Synchronized stomatal pore movements allow trees to balance CO₂ influx under variable environmental conditions (Haworth et al. 2016). However, stomatal movements are sensitive to environmental cues at cellular level, such as temperature, light intensity, atmospheric CO₂ concentration, humidity, and soil moisture (Mott 2009; Chaves et al. 2016). In water limiting environment, trees reduce hydraulic conductivity and guard cell turgor pressure which results in reduced stomatal aperture and stomatal conductance (g_s) (Bertolino et al. 2019). These changes lead to an improved water conservation, however, often at the expense of carbon assimilation (Flexas and Medrano 2002). Hence,

tree species that developed strategies to regulate stomatal movements help them coping under variable hydrological conditions (Matthews and Lawson 2019).

Plants suffer from osmotic stress in mangrove ecosystems (Lovelock and Feller 2003) and many mangrove species have developed specialized adaptation mechanisms to ensure growth and development under environmental stress (Reef and Lovelock 2015). Stomata exhibits a diverse range of shapes, sizes, and numbers across different mangrove trees species (Das and Ghose 1993). Although stomatal behavior, patterning and morphology are important factors that contribute to gas exchange in different terrestrial ecosystems (Haworth et al. 2016; Liu et al. 2019; Matthews and Lawson 2019; Henry et al. 2019; Sun et al. 2021), relatively little is known about how stomatal traits vary in responses to different environmental variables (salinity, elevation, pH, soil texture, nutrients and light etc.) in mangroves (Ball and Farquhar 1984; Sobrado 2005; Rodríguez-Rodríguez *et al.* 2018; Lopes et al. 2019). Because in mangrove ecosystems, environmental variables have synergistic influence on trees (Twilley and Rivera-Monroy 2005), thus regulating tree growth, functions and ecosystem productivity (Sarker et al. 2021). Therefore, a clear understanding of how stomata respond to multiple environmental variables is critical for predicting species adaptation capacity in mangroves under future environmental changes.

Stomatal size and stomatal density, two key hydraulic traits, together determine g_s (Liu et al. 2019). GCs receive environmental signals and control stomatal pore width (SPW) to ensure appropriate stomatal function for trees. In general, SD is more responsive to environmental conditions than stomatal size (Premoli and Brewer 2007). Earlier studies reported that different species show different SD responses i.e., increasing (Kouwenberg 2007), decreasing (Wang et al. 2014), or stable (Holland and Richardson 2009) along environmental gradients. Stomatal traits may also vary within leaves, trees, and individuals of a single species (Al Afas et al. 2006). In addition, stomatal sensitivity and response to water stress also differ among the species, and sensitivity is higher in slow growing species (Aasamaa and Sober 2011). Therefore, variations of stomatal morphology in response to environmental conditions affect g_s (Matthews and Lawson 2019), and thereby affect carbon assimilation due to a close relation between photosynthesis and g_s (Henry et al. 2019).

Sundarbans is the largest mangrove ecosystem on Earth that harbors wide range of plant and animal species including IUCN red listed categories (Sarker et al. 2019). Considering its unique significance, the Sundarbans was declared as RAMSAR Wetland Site and World Heritage Site (Chowdhury et al. 2016). Apart from anthropogenic disturbances, this mangrove is also vulnerable due to climatic stresses including sea level rise (SLR) (Chowdhury et al. 2016; Rahman et al. 2020). *Ceriops decandra* (Griffith) Ding Hou, a shrubby shade tolerant evergreen mangrove species of the Rhizophoraceae family, is native to a narrower range in Australasia and Indo-Malesia, but not extensive to East Africa (Duke 1992). Being a local invasive species in the Sundarbans, it is rapidly expanding the range from high saline to less saline areas (Sarker et al. 2019). Das and Ghose (1993) and Das (2002) have eloquently described the stomatal morphology of this species. However, we have a limited understanding of how stomatal morphology behaves under variable environmental conditions, especially in the Sundarbans.

In this study, we evaluate the effects of environmental gradients (i.e., salinity, siltation, pH, light and soil nutrients) on stomatal morphology of *C. decandra* in the Sundarbans, Bangladesh. We addressed the following questions: (1) How do stomatal density (SD), stomatal pore length (SPL), stomatal pore width (SPW), guard cell length (GCL) and guard cell width (GCW) vary across the salinity zones? and (2) How do environmental variables influence stomatal traits in *C. decandra*? Considering strong influences of environmental variables on g_s (Buckley and Mott 2013), we assumed that *C. decandra* growing at stressed environmental conditions (high salinity zones) would have lower SD and smaller pore size to reduce water loss and thus reducing gas exchange. In addition, a positive relation between GCs and stomatal pore size is expected because GCs regulate stomatal pore opening and closure.

Materials and methods

Study site

The Sundarbans is located in the coast of Bay of Bengal (Fig. 1) spanning over Bangladesh and India. The Bangladesh part of the Sundarbans covers about 6000 sq. km with 69% land area and 31% water bodies (Siddique et al. 2021). The soil is grey in color, fine textured and composed of silty-clay-loam whereas the subsoil is stratified, compacted at greater depth (Sarker et al. 2019). The mean elevation is about 2 m above mean sea level, and however other geomorphic features such as tidal flats and estuarine beaches are also present (Payo et al. 2016). It is inundated twice in a day although relatively elevated sites in the northern (landward) region are inundated by spring tides during the monsoon.

Precipitation and upstream river discharges from the Ganges mainly influence the hydrology in the Sundarbans. The interactions of fresh water inputs from both sources and sea water influence regional salinity (Rahman et al. 2020). The Bangladesh Sundarbans is divided into three salinity zones: less saline zone (LSZ), medium saline zone (MSZ) and high saline zone (HSZ) (Sarker et al. 2019). The salinity within the forest varies across space and over time (Rahman et al. 2020). For example, salinity remains low in all three zones during the monsoon (June – September) because of higher precipitation and fresh water discharges in the river system while salinity reaches maximum during dry season (December–May) due to limited freshwater supply from the upstream (Chowdhury et al. 2016).

Sampling

Study sites with *C. decandra* species were selected in three salinity zones (Fig. 1). Twenty saplings (average DBH, 3.5 ± 0.8 cm and height, 3.8 ± 0.5 m, see Table 1) were selected for sampling in each zone based on accessibility and not prone to erosion. DBH (cm) and total height (m) were measured using diameter tape and height measuring pole, respectively. The third order fully expanded leaves pair from the apex of plagiotropic (lateral) branches was selected for sampling. We avoided leaves with obvious symptoms of pathogen or herbivore attack or with a substantial cover of epiphylls. If any symptom present, we measured the fourth order leaf of the branches. Stomatal size varies with stomatal opening

and closure in response to irradiance (Roelfsema and Hedrich 2005). Therefore, all leaf samples were collected at the same time (predawn) in a sunny day from each saline zone during the first quarter of January 2021. Afterward, the collected leaves were preserved in polythene bags for analyses. Three soil samples were collected from the surrounding of each sapling (within 0.5 m) to a depth of 15 cm using a cylindrical soil core sampler of 5 cm in diameter and preserved in polythene bags for analysis.

Environmental data

In mangrove systems, environmental regulators (i.e., non-resource variables) and resource variables (e.g., nutrients and light) together influence ecosystem functioning (Twilley and Rivera-Monroy 2005), and mangrove productivity (Sarker et al. 2021). In this study, we considered seven key environmental variables i.e., soil salinity, pH, silt concentration, LAI, N, P and K to understand their influences on stomatal morphology in *C. decandra*.

We measured LAI using a Handheld Photosynthesis System (CI -340, CID Bio-Science, Inc USA). Three soil samples for each *C. decandra* sapling were measured and then averaged. The hydrometer method was used for measuring silt % in soil (Gee and Bauder 1986). Soil salinity was measured as electrical conductivity (EC) using a digital electrical conductivity meter (Extech 341350A-P Oyster) in a dilution ratio 1: 2 distilled water following Monteleone (2016). Soil pH was measured in a soil suspension of distilled water to the soil: water ratio of 1: 2 (McClean 1982) using a digital pH meter. The glass electrode pH meter was calibrated with standard buffer solutions of pH 7.0 and pH 4.0 and pH 10.0. Soil total nitrogen concentration (N) was determined following the Kjeldahl method (Sáez-Plaza et al. 2013). Total phosphorus (P) was measured using the Tri-acid digestion method and potassium (K) was measured using the flame photometer method (Jadoon et al. 2015).

Leaf morphology and stomatal anatomy

Leaf area (include petiole) of the fresh leaves was measured using a leaf area meter. Leaf thickness was measured using a digital slide caliper. Specific leaf area (SLA) was calculated as ratio of fresh leaf area (cm^2) and dry mass (g). Nail varnish peels were taken from the abaxial surface of each leaf and observed under a light microscope (AmScope, FMA050) with a camera system to capture the images. The images were analyzed using an image analysis (ImageJ) software (Schneider et al. 2012) and measured stomatal density (SD, number of stomata permm^2), stomatal pore length (SPL, μm), stomatal pore width (SPW, μm), guard-cell length (GCL, μm) and guard-cell width (GCW, μm) following a protocol of Lawon *et al.* (1998). For each leaf sample, 100 measurements were taken for each stomatal trait and then averaged.

Statistical analysis

The normality of the data was tested by the Shapiro-Wilk test. For comparing different morphological traits, such as plant (i.e., height and DBH), leaf (i.e., leaf area, leaf thickness and SLA), stomatal (i.e., SD, SPL, SPW, GCL and GCW), and environmental (i.e., salinity, silt, pH, N, P and K) among the three study zones (LSZ, MSZ and HSZ), we used one-way analysis of variance (ANOVA) followed by a post-hoc test (Tukey). Pearson correlation analysis was conducted among plant, leaf and stomatal traits to check the relationships among them. These morphological traits have the potential to identify plants strategies in terms of water use efficiency, growth, and resource use (Kröber et al. 2015; Sarker et al. 2021).

To determine the influence of environmental variables on the stomatal traits, we used generalized additive models (GAMs) with a Gaussian likelihood and an identical-link because of their capacity to handle complex, non-monotonic relationships between the response and the predictors. Moreover, non-parametric smoothing functions were used to depict response-predictors relationships without a priori knowledge of the functional form of these relationships using the package 'mgcv' version 1.8–38 (Wood 2011) in software R (R Core Team 2021). Model selection and model averaging were carried out using the 'MuMIn' version 1.43.17 package (Barton 2020).

We fitted all possible candidate GAMs considering stomatal traits as the response variables and using all possible combinations of environment variables. After that the resulting models were ranked using the second-order AIC (AICc) because the ratio between sample size and the number of covariates was < 40 . The relative support for each model was then calculated using the ΔAIC values (difference between the AIC value for the best model and the AIC value for each of the other models). We used the ' $\Delta AIC \leq 2$ ' criterion to select our confidence set of models. Akaike weights (AICw) were used to examine relative support for each model in the confidence set. To reduce model selection uncertainty and bias, we then used AIC-weighted model averaging on the parameter estimates of the models. These averaged parameter estimates were used to measure the goodness-of-fit of the models using the R^2 (coefficient of determination) statistic between the observed and fitted values. To determine the key variables, we ranked the variables based on their Relative Importance (RI) values. RI of each variable was calculated by summing the AICcw of the models in which the variable was included. RI values vary between 0 and 1, where 0 specifies that the target variable is not included in any of the competing models while 1 means that the variable is included in all competing models.

Results

Stomatal morphology variation among the saline zones

In all leaf samples of *C. decandra*, stomata were found on the abaxial leaf surface only. Guard cells (GCs) were placed within a substomatal chamber forming a distinct beak-shaped cuticular outgrowth either at outer side or both at outer and inner side of the stomatal pore. Considering all samples collected from the three salinity zones, the average stomatal density (SD), stomatal pore length (SPL), guard-cell length (GCL), guard-cell width (GCW), stomatal pore width (SPW) were $118 \pm 18 \text{ mm}^{-2}$, $30.64 \pm 1.62 \text{ }\mu\text{m}$, $36.39 \pm 2.58 \text{ }\mu\text{m}$, $15.79 \pm 1.86 \text{ }\mu\text{m}$ and $3.75 \pm 0.67 \text{ }\mu\text{m}$, respectively. SD, SPL and GCL did not

differ significantly among the salinity zones (Fig. 2A, B & D). However, SPW exhibited a significantly wider stomatal width in the LSZ compared to the MSZ and HSZ (Fig. 2C). Contrary to SPW, GCW had significantly smaller width in LSZ (Fig. 2E). In term of leaf traits, leaf area and SLA were higher in LSZ compared to MSZ and HSZ. However, the leaf thickness maximized in MSZ (Table 1).

Relations of stomatal traits with plant structural and leaf morphological traits

Correlation analysis revealed varying relations among tree, leaf and stomatal traits (Fig. 3). Even though DBH was independent in each case, height of *C. decandra* showed significant negative correlation with leaf area and SPW while the opposite correlation was found with GCW. In case of leaf traits, leaf area showed significant positive correlation with SLA and leaf thickness, similarly with GCW. The SD showed significant negative correlation with SPL. The SPL positively correlated with GCL whereas SPW related inversely with GCW (Fig. 3).

Influences of environmental variables on stomatal morphology

While soil salinity significantly varied across the salinity zones, silt concentration levels in soil were nearly similar in all zones (Table 1). Soil pH was significantly higher in LSZ (7.42 ± 0.06) compared to MSZ (5.70 ± 0.52) and HSZ (6.39 ± 0.31). PAR LAI was significantly lower in HSZ compared to LSZ and MSZ. In case of soil nutrients, P showed almost similar concentrations in all salinity zones. LSZ comprised the most N-rich ($0.87 \pm 0.22 \text{ mg g}^{-1}$) and K-rich ($9.67 \pm 1.23 \text{ mg g}^{-1}$) sites while HSZ comprised relatively the most N-poor sites.

GAM models for SPW (DE = 49%) and GCW (DE = 44%) showed a substantially higher explanatory power than the models for other stomatal traits (Table 2). The confidence set ($\Delta\text{AIC} \leq 2$) of GAM models implies that soil salinity, pH, silt, PAR LAI and nutrient variables had combined effects on stomatal traits (Table 2; Appendix S1). Among the variables, salinity had a strong negative influence on SPW and a positive effect on GCW (Table 2; Fig. 4). However, salinity was not included in the confidence set of models for SD, SPL and GCL. The pH and siltation also had less influence on stomatal traits (Table 2; Appendix S1). Partial plots, however, showed a slight increasing trend in GCW with increasing pH (> 6.5) and silt concentration ($> 45\%$) (Fig. 4). PAR LAI was the most important variable causing variations in stomatal traits with highest influence on SD (RI = 0.90) and least influence on GCW (RI = 0.27) (Fig. 4, Table 2). SD was higher in relatively N-poor sites ($< 0.7 \text{ mg g}^{-1}$) while increasing N ($> 0.8 \text{ mg g}^{-1}$) and P ($> 0.43 \text{ mg g}^{-1}$) contributed to higher SPL (Fig. 4). K had negative influence on both SPW and GCW with an increasing concentration ($> 8 \text{ mg g}^{-1}$).

Discussion

Stomatal morphology variation across the salinity zones

We found that stomata in *C. decandra* are located only on the abaxial surface (hypostomatous) in all salinity zones in the Sundarbans. An earlier study (Das and Ghose 1997) also observed hypostomatous leaves in this species in the Indian Sundarbans. *C. decandra* is a shade tolerant shrubby species, and the presence of hypostomatous leaves may be an adaptive feature to maximize water use efficiency in saline environment, where CO₂ is unlikely to limit photosynthesis (Peat and Fitter 1994). Guard cells in *C. decandra* are placed within a substomatal chamber (sunken) and show a distinct beak-shaped cuticular outgrowth either at outer side or both at the outer and inner side of the stomatal pore (Das and Ghose 1997). This structure provides an additional support to prevent water loss through the stomatal pore during transpiration, and thus helping this species to cope with water stress conditions in mangrove environment (Das and Ghose 1997; Das 2002).

It is expected that *C. decandra* leaves in higher salinities would have lower SD and smaller stomatal size in order to reduce water loss. In contrast, we observed non-significant variation in SD and stomatal length (SPL) including GCL. However, leaves in LSZ are characterized by significantly wider SPW and narrower GCW compared to higher salinity zones (Fig. 2). Earlier study of Lovelock and Feller (2003) reported that nonsignificant SD variation in two other mangrove species, *Laguncularia racemosa* (L.) C.F. Gaertn. and *Avicennia germinans* L. On the other hand, presence of the highest SD is reported for *Rhizophora mangle* L. in the highest salinity sites at Yucatan Peninsula, Mexico (Peel et al. 2017). Because of similar SD in Sundarbans' three salinity zones, wider SPW and larger leaf area in the LSZ (Table 1), *C. decandra* would have higher potential leaf surface area to ease CO₂ movement into the leaf. Therefore, a strong positive correlation between leaf area and SD is expected. However, we found a weak relation between them ($r = 0.01$; $p > 0.05$, $n = 60$). In contrast to this finding, as expected, SD in *R. mangle* growing in the Mexican mangroves shows a strong positive relation with leaf area (Peel et al. 2017). The invariability of SD across the salinity zones may indicate SD's limited role in maintaining water-use efficiency in *C. decandra* growing in the Sundarbans. Instead, the species might maintain physiological metabolisms in high saline environments, thus allowing stomatal movement to ensure minimal water loss relative to carbon gain, as found in other mangrove species (e.g., *A. marina*) growing in Australia (Ball and Farquhar 1984).

Even though SD and SPW had a weak relation ($r = 0.07$, $p > 0.05$, $n = 60$), SPL showed a significant negative relation with SD (Fig. 3), as commonly described for other mainland species (Wang et al. 2014; Liu et al. 2019). The trade-off between SD and SPL is assumed to maximize carbon gain and minimize water loss (Franks and Beerling 2009). Guard-cell length (GCL) or width (GCW) was negatively correlated with SD although the relationship was not significant (Fig. 3).

In terms of relationships between plant structural traits and stomatal traits in *C. decandra*, DBH did not show any significant relation with any of the stomatal traits (Fig. 3). However, plant height showed a significant negative relation with SPW ($r = -0.35$, $p < 0.05$, $n = 60$), and a significant positive relation with GCW ($r = 0.34$, $p < 0.05$, $n = 60$). In terms of relationships between plant structural, leaf and stomatal

traits, taller *C. decandra* tends to have smaller leaf area and smaller stomatal width (Fig. 3) with nearly constant SD (Fig. 2) that may together reduce stomatal pore area for gas exchange. However, in many species, SD increases with plant height that is likely to be related to increasing crown exposure, because irradiance increases with plant stature (Norby et al. 2003). Being a shrubby and shade-tolerant species, greater light availability may contribute to decreasing stomatal pore area which may reduce water loss in *C. decandra*. In addition, GCW increased significantly with leaf thickness (Fig. 3). The wider GCW in higher salinity zones (Fig. 2E) may be useful for stomatal opening (Outlaw 2003) and to adjust the pore area in response to the changing environment.

Our results show that *C. decandra* growing in the Bangladesh Sundarbans have lower average SD ($118 \pm 18 \text{ mm}^{-2}$), SPL ($30.64 \pm 1.54 \text{ }\mu\text{m}$), and SPW ($15.79 \pm 1.76 \text{ }\mu\text{m}$) than that of the Indian Sundarbans (Das and Ghose 1997). Such smaller size stomata in Bangladeshi *C. decandra* may help the species responding quickly under environmental perturbations through rapid stomatal opening and closing (Bertolino et al. 2019) and by providing a reduction in total pore area that can facilitate faster aperture response (Lawson and Blatt 2014). In addition, smaller and faster stomata of this species could minimize the effects of excessive water-potential gradients in saline environment that might help to avoid the risks of xylem embolisms (Franks and Beerling 2009).

Effects of environmental variables on stomatal traits

The interactions of seawater and freshwater discharges from the upstream river flows along with climate seasonality result in high spatial and temporal variability in fine-scale habitat conditions in the Sundarbans (Sarker et al. 2019). Such variability may influence stomatal conductivity that could further affect carbon assimilation in trees. Our GAM analysis revealed that salinity has a strong negative effect on SPW and a positive effect on GCW (Table 2; Fig. 4). However, salinity had a weak influence on other stomatal traits (Appendix S1). Usually, stomatal conductance (g_s) shows a negative response to salinity under hydric stress conditions in mangroves although the magnitude of the response may vary across different species (Rodríguez- Rodríguez *et al.* 2018). Earlier studies (Bertolino et al. 2019) reported that stomatal morphological adjustments, such as SD and stomatal size can modify the range of g_s by altering the maximum stomatal conductance (g_{smax}) in a wider range of species. Our results indicate that declining SPW with increasing salinity might reduce the influx of CO_2 in leaves which can ultimately limit carbon assimilation in *C. decandra*. pH and silt concentration, in general, have limited influence on stomatal variables (Table 2, Appendix S1) although we observed a slightly increasing trend in GCW with increasing pH (> 6.5) and silt ($> 45\%$) (Fig. 4). Wider guard cell pair in stressful conditions usually offers efficient solute accumulation that forces the guard cells to bow outward through changing turgor pressures and enlarging the SPW (Outlaw 2003). Therefore, our above results guide us to assume that under stress (e.g., high salinity), trade-offs between SPW and GCW may help the species in stomatal opening.

LAI (leaf area per unit ground area) is considered a key trait driving the exchange of CO_2 , water vapor and energy between canopy and atmosphere (Norby et al. 2003). LAI is assessed using data on

photosynthetically active radiation (PAR) transmittance (Norman and Campbell 1989) which is highly variable and can differ as a function of vegetation type, climatic and soil conditions (Iio et al. 2014). GAM analysis revealed a strong relative influence of LAI on SD (RI = 0.90) and SPL (RI = 0.83), although models' deviance explanatory power is low (Table 2). SPL increased while SD decreased up to the LAI value 4.0 and after that both variables showed an opposite trend (Fig. 4), indicating LAI's influence on stomatal pore area to maintain gas exchange.

Even though mangrove is considered a nutrient limited ecosystem (Reef et al. 2010), the nutrients vary among and within the ecosystem (Feller et al. 2003; Sarker et al. 2021). In this study, we observed a substantial spatial variability in several soil nutrients: a significantly lower N ($3.73 \pm 0.43 \text{ mg g}^{-1}$) and K ($7.72 \pm 0.78 \text{ mg g}^{-1}$) concentrations in the HSZ compared to LSZ and MSZ in the Sundarbans (Table 1). However, the variation was not significant in P concentrations. GAM models revealed that P had higher relative importance (RI) in SPW (0.87) variation in *C. decandra* while K had higher RI in GCW (0.99). Even though N had higher RI (0.80) in SPL deviance, GAM combinedly explained low variability (29%) of SPL (Table 2 and S2). The partial plots (Fig. 4) showed a decreasing SD and an increasing SPL with increasing N ($> 0.7 \text{ mg g}^{-1}$). An earlier study showed that *C. decandra* is able to grow abundantly in the N-poor habitats in the Sundarbans (Sarker et al. 2021). The SPL and SPW increased up to 0.43 mg g^{-1} of P and then SPL remained constant whereas SPW exhibits a decreasing trend after crossing same P concentration. The GAM models also revealed that K has higher relative importance for SPW and GCW to explain maximum variations (Table 2). The flanking guard cells accumulate K + salts to increase turgor pressure (Outlaw 2003) that may help in stomatal opening (Roelfsema and Hedrich 2005) in higher saline conditions.

The GAM models showed that environmental variables can moderately explain variabilities in SPW (DE = 49%) and GCW (DE = 44%) compared to other stomatal traits (Table 2). Among the variables, salinity had the strongest negative effect on SPW and positive effect on GCW (Fig. 2). Moreover, an inverse relationship between SPW and GCW ($r = -0.30$, $n = 60$, $p < 0.05$) indicate that wider GCW might be helpful to open smaller SPW in (salinity) stressed habitats for gas exchange. Stomatal pore area is dynamically adjusted by changes in pore width, because pore length is rather rigid during opening and closure of stomata (Lawson et al. 1998). Osmotic H₂O influx causes increased guard cell turgor, asymmetric guard cell enlargement, and consequently increase in stomatal pore size while in stomatal closure, solutes are dissipated (Outlaw 2003). These plastic modulations of both stomatal traits might help to adjust the stomatal pore area in response to the changing environment that ultimately affecting the gas exchange in *C. decandra*.

The leaf economic spectrum (LES) (Wright et al. 2004) is increasingly used for understanding plant adaptation mechanisms under stress (Liu et al. 2019; Henry et al. 2019). Although LES incorporates important chemical, structural and physiological traits, stomatal traits have received less attention (Kröber et al. 2015). Stomal traits are also lacking in a recent trait-based study (Sarker et al. 2021) quantifying trait-environmental relationships to predict Sundarbans' biomass productivity. In addition,

stomatal traits are underrepresented in mangrove trait databases (e.g., Quadros and Zimmer 2017). Our findings that mangrove stomatal traits are affected by variability in fine-scale environmental variables suggests for integrating stomatal traits with LES to better understand mangrove growth dynamics and productivity under changing environment. However, this study focused only on a single species and limited sampling sites in three salinity zones. High spatial variability in environmental conditions even within a shorter distance in the Sundarbans and disproportionate responses of different mangrove species to different environmental variables (e.g., salinity, siltation, soil nutrients) (Sarker et al. 2021), suggest for future studies incorporating more species and more sampling sites. In addition, inclusion of important ecophysiological traits (e.g., stomatal conductance, transpiration rate, photosynthesis rate etc.) may provide a mechanistic understanding of how mangroves will respond under changing environment.

Conclusions

This study demonstrates how multiple environmental variables influence stomatal traits in *C. decandra* also quantify the relationships between different stomatal morphological traits. Several stomatal traits (i.e., SD, SPL and GCL) did not vary significantly across the salinity zones. However, SPW and GCW varied significantly across the salinity zones with substantially wider SPW and narrower GCW in less saline areas. In addition, an inverse relationship was observed between SPW and GCW, and SD and SPL. GAM models for SPW (49%) and GCW (44%) showed higher explanatory powers than other stomatal traits. Salinity had a strong negative influence on SPW and a positive influence on spatial variation in GCW. We observed a trade-off between SPW and GCW regulating stomatal pore areas in *C. decandra* in response to fluctuating habitat conditions, particularly salinity. This suggests that with increasing salinity stress this species maintains its gas exchange efficiency through allocating less pore space which provides an insight into how plants may acclimatize under changing environment in mangroves and elsewhere.

Declarations

Conflict of interest

The authors declared that they have no conflict of interest.

Authors contributions

MIHI: Conceptualization, Methodology (sampling, Lab analysis), Data analysis, Writing - Original Draft & Editing, Editing; MQC: Conceptualization, Methodology (sampling), Writing - Original Draft & Editing; SKS: Methodology (sampling), Data Analysis, Review & Editing the original Draft; AD: Methodology (sampling, Lab Analysis), Editing; RS: Methodology (sampling, Lab Analysis); RK: Methodology (Lab analysis); MSRS: Methodology (sampling); MMH: Methodology (Lab analysis).

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Tables

Table 1 Plant, leaf, regulators and resources variables variation among the salinity zones. Low saline zone (LSZ), medium saline zone (MSZ) and high saline zone (HSZ).

Variables		LSZ	MSZ	HSZ
Tree	DBH (cm)	3.8 ± 0.9	3.4 ± 0.7	3.3 ± 0.6
	Plant height (m)	3.5 ± 0.4	4.0 ± 0.4	3.9 ± 0.5
Leaf	Leaf area (cm ²)	46.02±8.21 ^a	37.67±8.61 ^b	36.12±11.32 ^b
	Leaf thickness (mm)	0.22±0.05 ^a	0.60±0.03 ^b	0.23±0.02 ^a
	SLA (cm ² g ⁻¹)	77.39±19.73 ^a	73.92±11.66 ^a	68.42±27.42 ^b
Regulators	Salinity (dSm ⁻¹)	2.20 ± 0.20 ^a	5.55±0.66 ^b	6.23±0.37 ^c
	pH	7.42 ± 0.06 ^a	5.70± 0.52 ^b	6.39±0.31 ^b
	Silt (%)	42.44 ± 7.53 ^a	41.66±4.59 ^a	43.62±2.54 ^a
Resources	LAI	4.50 ± 0.62 ^a	4.31 ± 0.31 ^a	3.73 ± 0.43 ^b
	N (mg g ⁻¹)	0.87±0.22 ^a	0.71 ± 0.16 ^b	0.55±0.10 ^c
	P (mg g ⁻¹)	0.43±0.09 ^a	0.44 ± 0.04 ^a	0.42±0.03 ^a
	K (mg g ⁻¹)	9.67±1.23 ^a	6.37 ± 0.53 ^b	7.72±0.78 ^c

Different letters indicate significantly different at $p < 0.05$.

Table 2 Results of GAMs for the stomatal attributes of *C. decandra* growing in the Sundarbans mangrove ecosystem. Summaries of model fit in rightmost three columns are only shown for the best model (DE = deviance explained). Numbers in the main part of the table (enclosed in box) represent the Relative Importance (RI) of each covariate. Dark-shaded cells highlight covariates that were retained in the best model for each stomatal attribute. Light-shaded cells represent covariates retained in other models within the candidate set. Dashed boxes indicate no participation of that covariate in any of the candidate models. The covariate short-hands are: soil salinity (Salinity, dS m⁻¹), Photosynthetically Active Radiation

(PAR) Leaf Area Index (LAI), soil acidity (pH), silt concentration (%), soil total nitrogen (N, mg g⁻¹), soil total phosphorus (P, mg g⁻¹), and soil potassium (K, mg g⁻¹).

Stomatal Attributes	Salinity	PAR-LAI	pH	Silt	N	P	K	ΔAIC_{cw}	Adj. R^2	DE (%)
Stomatal density	-	0.90	0.28	-	0.41	-	-	0.35	0.18	17.10
Stomatal length	-	0.83	-	0.24	0.80	0.59	-	0.40	0.29	29.00
Stomatal width	0.80	0.33	-	-	-	0.87	0.42	0.34	0.48	49.00
Guard cell length	-	0.28	0.25	-	-	-	-	0.41	0.05	2.86
Guard cell width	0.37	0.27	0.33	0.62	-	-	0.99	0.34	0.43	44.20

Figures

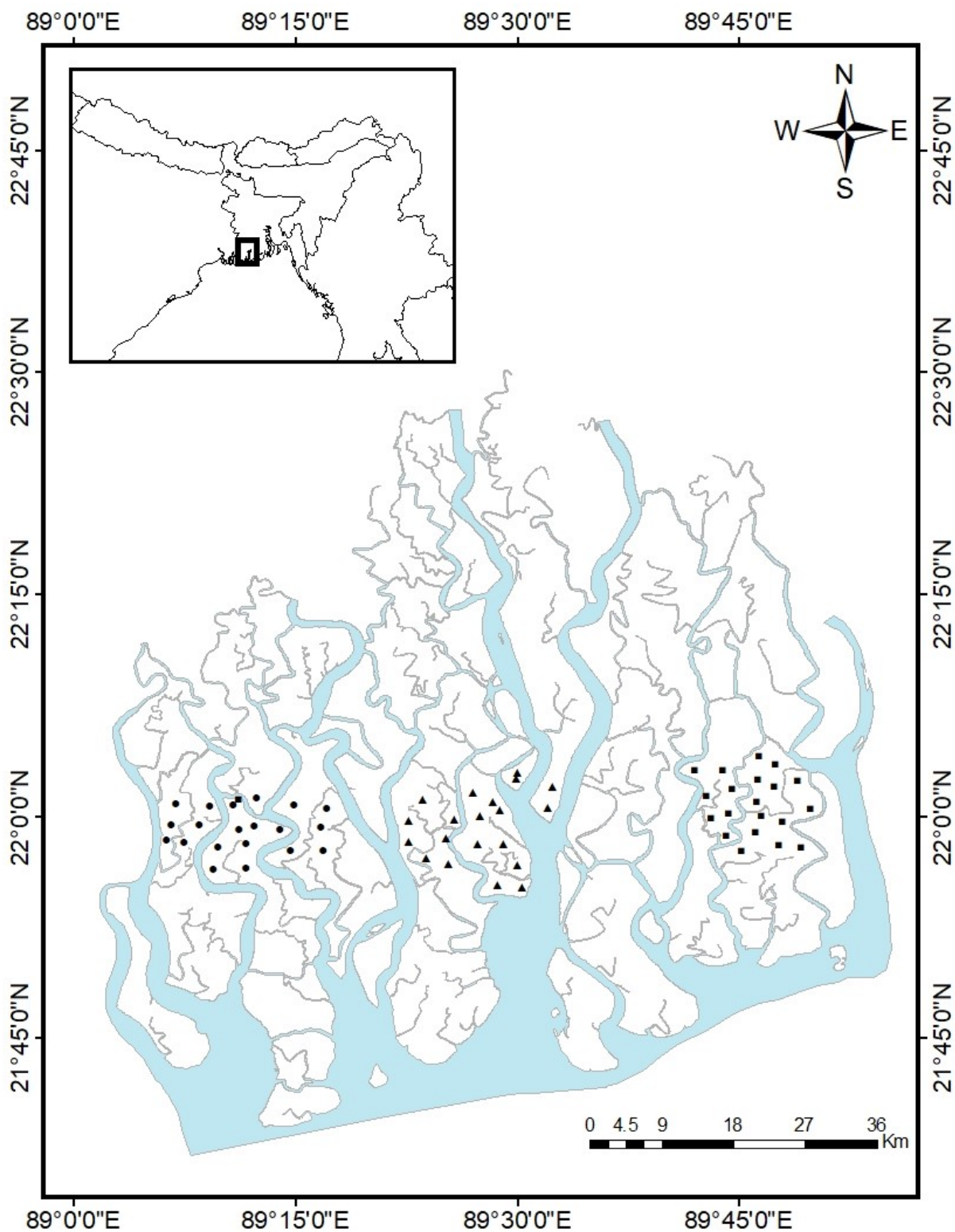


Figure 1

Sampling location in three salinity zones. Individual square indicates each sample plant in less saline zone (LSZ), triangle is in medium saline zone (MSZ) and circle is in high saline zone (HSZ).

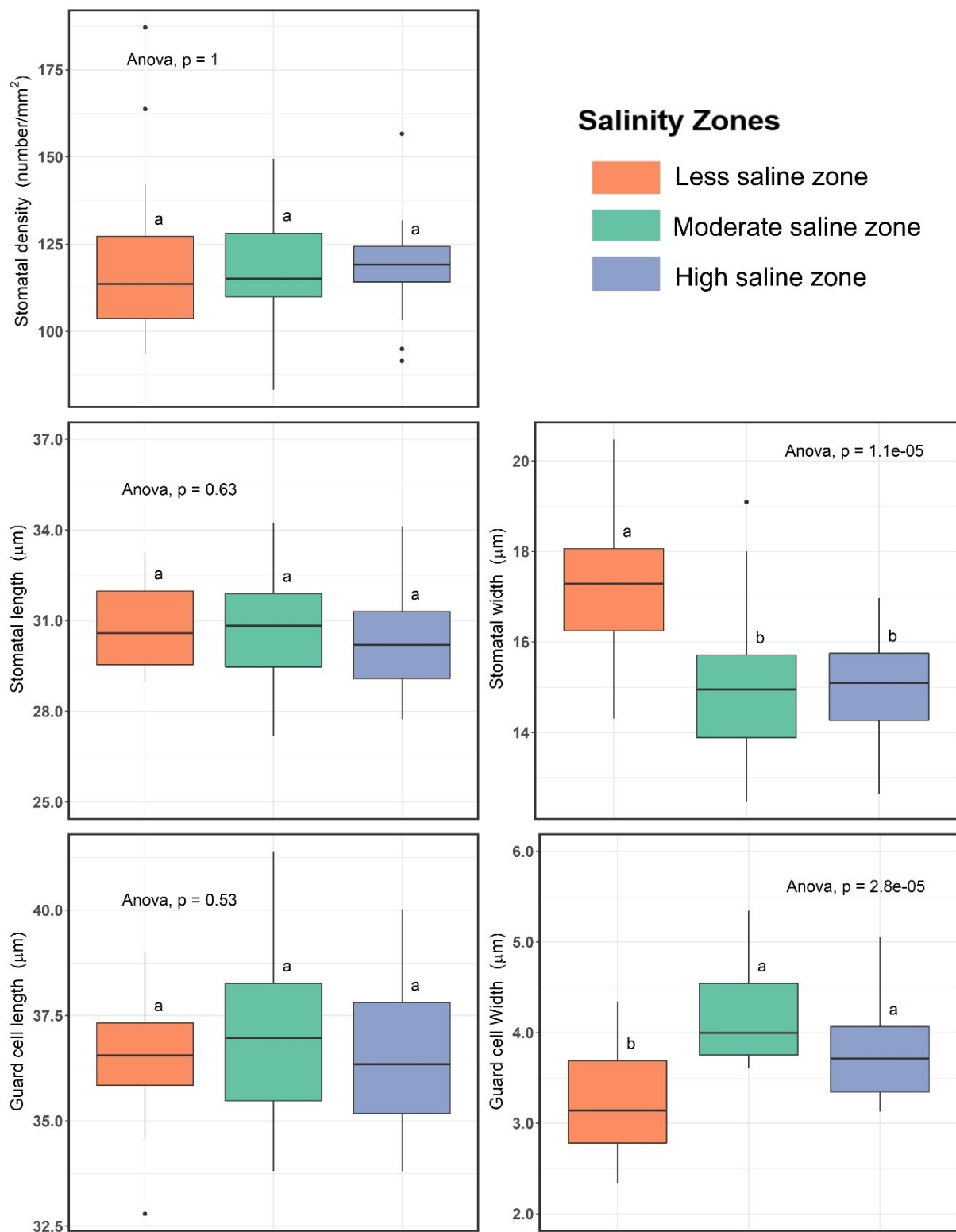


Figure 2

Stomatal traits variation among three salinity zones.

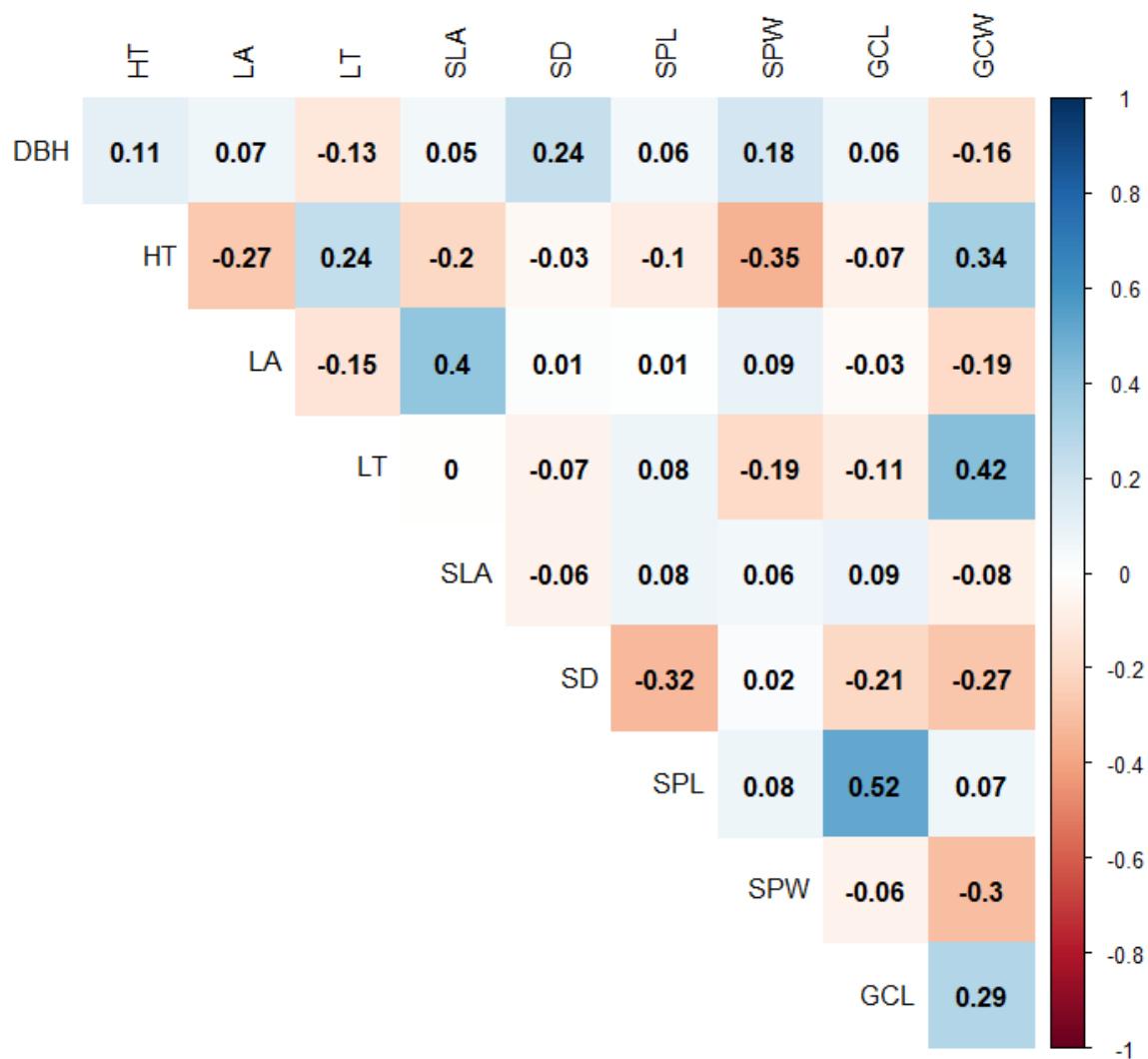


Figure 3

Correlation among plant, leaf and stomatal traits. Significance level is shown at $p < 0.05$. HT, plant height (m); DBH, diameter at breast height (cm); LA, leaf area (cm^2); LT, leaf thickness (mm); SLA, specific leaf area ($\text{cm}^2 \text{g}^{-1}$); SD, stomatal density (number mm^{-2}); SPL, stomatal length (μm); SPW, stomatal pore width (μm); GCL, guard cell length (μm); GCW, guard cell width (μm).

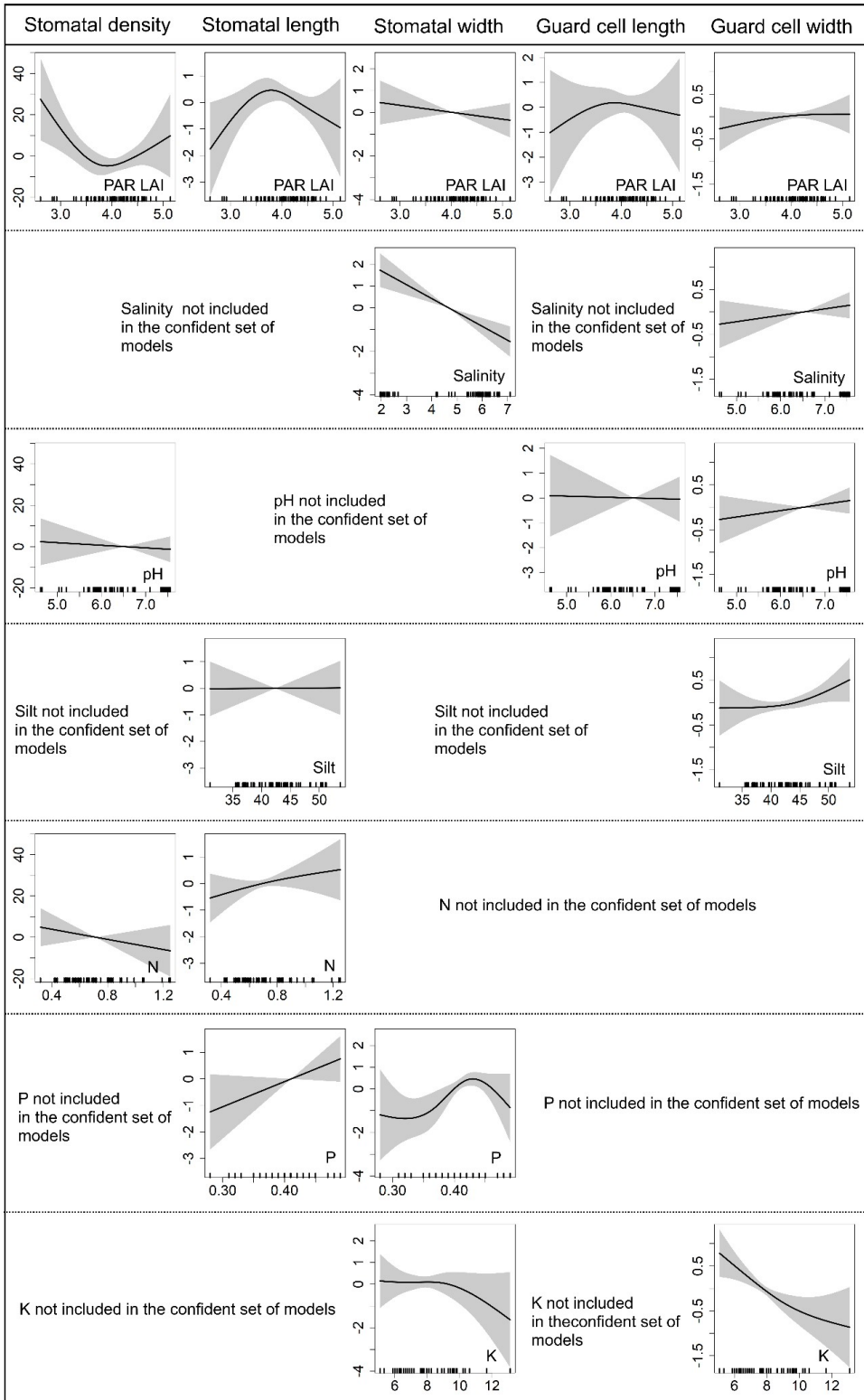


Figure 4

Effects of covariates inferred from the best GAMs fitted to the five stomatal variables of the mangrove *C. decandra*. The solid line in each plot is the estimated spline function (on the scale of the linear predictor) and shaded areas represent the 95 % confidence intervals. Zero on the y-axis indicates no effect of the covariate on stomatal attributes. Covariate units: soil salinity = dS m⁻¹, Photosynthetically Active

Radiation (PAR) Leaf Area Index (LAI), soil acidity (pH), silt concentration = %), N = mg g⁻¹), P = mg g⁻¹), and K = mg g⁻¹).

Supplementary Files

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