Ecophysiology of the Mangroves from Estuarine Sedimentary Islands, Southwest Coast of Kerala

A thesis

submitted in partial fulfilment of the requirements

of the degree of

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By

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CERTIFICATE

Certified that the work incorporated in the thesis entitled **"Ecophysiology of Mangroves from Estuarine Sedimentary Islands, Southwest coast of Kerala"** Submitted by **Abdulla Naseef S A**, was carried out by the candidate under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously to award any degree or diploma from any other University or.institution.

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DECLARATION

I hereby declare that the work presented in the thesis entitled "**Ecophysiology of Mangroves from Estuarine Sedimentary Islands, Southwest coast of Kerala**" is based on the original work done by me under the guidance of Dr Sreejith K A and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using "**iThenticate**" software at C.H.M.K. Library , University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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Chapter 1: Introduction.

Mangroves are a group of halophytic trees and shrubs that thrive in the coastal and estuarine habitats of tropical and subtropical regions across the globe. They are one of the crucial ecosystems on earth, as their ecosystem services contributions are disproportionately more significant than any other ecosystem. They support unmatched levels of biodiversity, human livelihood, carbon sequestration, water filtration, coastal protection, fisheries and many more. They are also facing unprecedented challenges from climate change, even though they have evolved multiple adaptions to surpass many stresses they face in their environment. It is predicted that the consequences of changing climate, such as drought, heat waves and cyclones, will intensify and become more frequent in the tropics, especially in the coastal regions. Yet this super important ecosystem has to receive much-needed attention in studying its response to different environmental factors. There has been some effort from Southeast Asia and Australia, but hardly anything has been done from Indian mangroves. This thesis comprehensively attempts to understand mangrove ecophysiology using a multi-trait and multi-stress approach. This thesis revealed the level and variation in mangroves in their tolerance to rising atmospheric temperatures, drought. It increased atmospheric CO_2 concentrations based on in-depth plant functional trait analysis of 13 true mangrove species at south west coast, India.

Chapter 2: Thermotolerance and its relationship with key functional traits in mangroves.

Increasing intensity of mean daily temperatures and intensity and frequency of summer heatwaves are highly threatening consequences of climate change. How mangrove systems will respond to future climates with more frequent and severe extreme temperatures due to changing climates has received little attention. Indeed, there is already evidence of the adverse effects of warming temperatures on these systems, and recent large-scale dieback of mangroves has occurred during periods of severe heat stress. To understand how vulnerable mangroves are to future warming compared to other terrestrial ecosystems, the study quantified leaf thermotolerance and estimated thermal safety margins (TSM) for thirteen mangrove species from the southwestern Indian coast. This attempt quantified thermotolerance as temperatures that resulted in a 50% reduction in photosystem II function (T_{50}) and TSM as the difference between T_{50} and estimates of maximum leaf temperatures. Additionally, it also tested if thermotolerance was related to leaf traits. T_{50} ranged from 48.5°C in *Avicennia marina* to 56°C in *Bruguiera gymnorhiza*, with a mean of 53.3°C for the thirteen species. Both thermotolerance and TSMs were exceptionally high compared to other plant communities. Contrary to other studies, the study observed no clear relationships between the leaf traits and thermotolerance. With their high thermotolerance and large safety margins, mangroves may be relatively less vulnerable to increased temperatures. More importantly, the study revealed that this mangrove is the most thermotolerant system globally, based on available data.

Chapter 3: Drought tolerance and its relationship with key functional traits in mangroves.

Even though the summer patchy dieback and large-scale events occur in the mangroves worldwide and alarming threats from changing climate, mangroves are overlooked mainly for climate change impacts. Only a few mangrove species have been studied for their drought tolerance traits, and hardly any have been studied from Indian mangroves. This chapter explores the physiological mechanisms underlying drought tolerance in mangrove species through pressure-volume curve analyses. It was aimed to understand how resistance to early dehydration (using Ψ_{TLP}, RWC_{TLP}) functions in mangroves. The study found that both the Ψ_{TLP} and RWC_{TLP} varied significantly between species, suggesting different strategies in turgor regulation to early dehydration. Current study also revealed their relationship to a) stomatal traits, b) the modulus of elasticity of the tissue, and c) leaf capacitance. Only the modulus of elasticity, cell wall rigidity, is positively correlated to RWC_{TLP}, suggesting that a rigid cell wall helps retain higher relative water content even at the turgor loss point. We also examined the relationship of early dehydration traits with other leaf morphological traits such as LMA, LDMC, SSD, and LA. Only Stem Specific Density positively correlated with Ψ_{TLP}; otherwise, none found any correlation with Ψ_{TLP} nor RWC_{TLP}. A dense stem supports the plant in retaining higher water potential at early dehydration and regulates turgor loss. Study also attempted if the resistance traits to early dehydration and the rate of water loss in these species correlate with their functionality in their natural habitat concerning leaf lifespan. Current study could not find any relationship between phenology traits and drought tolerance. These studied traits are ecologically important and will help better understand mangrove response to changing climate and its consequences on coastal ecosystems and blue carbon storage.

Chapter 4: Assimilation to elevated CO_2 and relationships with key functional traits in mangroves.

This study aimed to examine the response of mangroves to elevated CO_2 concentrations (A/Ci curves) in twelve true mangrove species from the southwestern Indian coast. It is also investigated the correlations between photosynthetic parameters (Amax, Vcmax, Jmax, Rd) and some key functional traits (LMA, LDMC, LA, stomatal attributes, SSD). Measurements of CO_2 response were performed on mature plants growing in mangrove islands in the Ashtamudy backwaters estuary using an Infra-Red Gas Analyser. Our experiments revealed considerable species-level variation in Amax, Vcmax, and Jmax, with *Avicennia marina* and *Sonneratia caseolaris* displaying the highest photosynthetic capacity, whereas *Sonneratia alba* exhibited the lowest. Nevertheless, no substantial associations were found between photosynthetic parameters and leaf morphological or stomatal traits across species. The results offer novel insights into the potential responses of mangroves to environmental alterations, including increased atmospheric CO_2, and underscore the necessity for more targeted research on mangrove ecophysiology. This research enhances the comprehension of mangrove carbon dynamics and its function in rapidly changing climate scenarios.

Chapter 5: Conclusions

This study revealed a comprehensive ecophysiology of any mangrove ecosystem together in a single attempt. It was found that mangroves from the southwest Indian coast have the highest thermotolerance values compared to any other ecosystem on earth. It also showed that leaves with bigger surface areas have higher leaf thermotolerance, and any other leaf functional trait could not explain the trend in thermotolerance values. This thesis also found that these mangroves are not good at drought tolerance from a turgor loss perspective, and turgor loss parameters were not correlated to any of the leaf functional traits examined, including phenology traits. Even though photosynthetic responses to elevated CO_2 were high in these mangroves, they did not correlate with any leaf functional traits. Overall, this study reports hopeful functional responses of mangroves from high temperatures and rising CO_2 levels to stress, but it also warns about drought's impact on them.

Chapter 6: Recommendations

Although this study attempted to examine mangrove ecophysiology from multiple perspectives comprehensively, it also points to the need for added exploration of the same perspectives. On-field live monitoring of peak summer leaf temperatures is unavoidable in quantifying actual leaf temperatures; hence, an in-situ leaf thermal safety margin calculation is needed. It is also needed to look at the xylem vulnerability and field level leaf water potential diurnal and annual trends to estimate hydraulic safety margins in mangroves. This study also recommends conducting temperature response experiments on CO_2 assimilation in mangroves to better understand the direct impact of global warming on photosynthesis.

CONTENT

Chapter 2 **Leaf thermotolerance in Mangroves and its relationship with other leaf traits**

Chapter 3 **Early dehydration traits and its relationships to other plant functional traits in Mangroves**

Chapter 4 **Mangrove leaf photosynthetic response to elevated Carbon dioxide (CO_2) and its relationship with other functional traits.**

Chapter 1

Introduction

Mangroves are salt-tolerant trees and shrubs that thrive in coastal intertidal zones. These distinctive ecosystems are located in tropical and subtropical areas globally, mostly in estuaries, deltas, and coastal shorelines where freshwater converges with seawater (Tomlinson, P. B. 1986, Hogarth, P. J. 1999, Chapman, V. J. 1976, Lugo, A. E., & Snedaker, S. C. 1974). In 2020, global mangrove forests covered 145,068 km², with 39.2% located in Asia. Indonesia had the highest number, followed by Brazil and Australia, while the largest mean patch size was recorded in the Sundarbans, which is shared by India and Bangladesh (Jia M et al., 2023). In India, mangroves encompass approximately 5,000 square kilometres, accounting for about 3.44% of the global mangrove area (Jia M et al., 2023). Nevertheless, the areal extent of mangrove forests has diminished by 30–50% over the past fifty years due to coastal development, aquaculture expansion, and over-exploitation (Alongi, 2002; Duke et al., 2007; Polidoro et al., 2010). A notable characteristic of mangrove forests is the species distribution, which exhibits a distinct zonation pattern perpendicular to the shore (Semeniuk and Wurm, 1987). India hosts around 46 species of mangroves along its eastern and western coasts, with the greatest diversity located in the Sundarbans (Naskar & Mandal, 1999; Ghosh et al., 2015). Kerala's southwest coast hosts approximately 15 mangrove species, albeit their distribution is significantly fragmented due to anthropogenic activities (Selvam, 2003; Kathiresan, 2010).

Mangrove ecosystems worldwide are exceptionally productive and serve vital functions in coastal and marine environments. Their productivity competes with some of the most productive ecosystems on Earth, offering nursery grounds for marine species and habitats for many organisms (Alongi, 2014; Barbier et al., 2011). Mangroves are recognised for their capacity to function as "blue carbon sinks," sequestering substantial quantities of carbon in their biomass and waterlogged soils, so playing a crucial role in climate change mitigation (Donato et al., 2011; Kauffman et al., 2017). Mangroves offer extensive ecosystem services, including the support of biodiversity, enhancement of fisheries, maintenance of water quality, and provision of a buffer against natural disasters (Kathiresan & Bingham, 2001; Ellison, 2015).

Mangrove refers to a diverse group of woody trees and shrubs that form the primary vegetation in tidal, saline marshes along tropical and subtropical coastlines (Semeniuk and Wurm 1987). Mangrove forests are located along tropical ocean coastlines and offer several ecological services, including fisheries production and nitrogen cycling. The economic value of mangrove ecosystem services is estimated at $194,000 per hectare per year, depending on location and the particular services provided (Costanza et al., 1997; Barbier et al., 2011). Mangroves are vital for coastal protection, alleviating the impacts of waves, storm surges, and erosion. Mangrove forests are acknowledged for their function in safeguarding lives and property during natural disasters, such as the 2004 Indian Ocean Tsunami and cyclones in

Tamil Nadu and Odisha, where shorelines with intact mangroves experienced considerably less damage than unprotected areas (Kathiresan & Rajendran, 2005; Danielsen et al., 2005).

Recent empirical studies on plant functional types and traits have advanced significantly, enhancing our comprehension of traits pertinent to local vegetation and ecosystem dynamics (Cornelisen et al. 2003). These enquiries focus on the responses of vegetation to environmental variations or changes, particularly in climate, atmospheric chemistry, land use, and natural disturbance regimes. Reciprocal enquiries focus on the effects of vegetation on extensive environmental parameters (Lavorel and Garnier 2002). A rapidly expanding scientific community has recognised that identifying plant species based on functional characteristics is a potential approach for addressing such enquiries, along with several other ecological concerns (Díaz et al 2002). Plant functional types are described as collections of plant species that exhibit comparable functions at the organismal level, analogous reactions to environmental stimuli, and/or similar roles within ecosystems or biomes (Westoby 1998).

Plant functional traits are the morphological, physiological, and phenological characteristics that embody ecological strategies, dictate plant responses to external stimuli, impact other trophic levels, and shape ecosystem qualities. The variability in plant functional traits and trait syndromes has been useful in addressing significant ecological enquiries across various scales, leading to a need for standardised methods to assess ecologically relevant plant features (Perez et al 2013). Zoologists investigating pollinators, herbivores, frugivores, and granivores necessitate plant attribute data encompassing phenology, as well as leaf, flower, fruit, diaspore, and seed characteristics. Functional features encapsulate essential trade-offs that define the ecological responsibilities of organisms. A prime illustration is the trade-off between seed quantity and the size of each seed. Intense competition results in the generation of big seeds, which possess a higher likelihood of successful germination, albeit produced in limited quantities. Another technique involves producing numerous little seeds, so enhancing the likelihood of dispersal to transient locations with minimal competition and abundant resources; however, this approach is ineffective in the presence of intensive competition. Seed characteristics provide clues into a plant's dispersion and competitive capabilities. Additional functional features can provide significant insights into photosynthesis, insect defence, competitive capacity, and light acquisition. All essential functional features contribute to defining the ecological responsibilities of various plant species (Wright et al. 2002). The study by Westoby et al examines the four principal axes of variation among land plant species. The dimensions influence ecological strategy, specifically how species acquire carbon benefits during vegetative growth and facilitate gene transfer to future generations. The four principal dimensions identified by Westoby et al. (2002) are leaf mass per area–leaf lifetime (LMA-LL), seed mass–seed output (SM-SO), leaf size–twig size (LS-TS), and height.

Increased leaf mass per area signifies a higher expenditure for the plant. A prolonged leaf lifespan often necessitates leaves to possess structural integrity. Increased LMA safeguards against degradation and also inhibits herbivory. Species exhibiting slower leaf turnover ought to invest more resources to deter herbivores. Westoby et al. (2002) assert that LMA is the most valuable singular indication of leaf strategy for the following reasons. LMA represents the building cost of a unit leaf area, a crucial metric in leaf economics. The physical strength of a leaf is contingent upon its thickness and tissue density. Third, LMA typically exhibits a correlation with features such as leaf lifespan, nutrient residence time, and photosynthetic capability that is at least as significant as that of alternative markers (Wright et al 2002). Fourth, interior volumes are significantly more challenging to quantify than mass and area. The majority of workers would resist transitioning to expressing features based on volume instead of mass, except for species lacking internal gas volume, when the two measures are equivalent.

Investigations into mangrove physiology have demonstrated that these species exhibit many distinctive adaptations to environmental stressors such as temperature and drought (Ball, 1988; Krauss et al., 2008; Reef et al., 2016). Mangroves exhibit significant thermotolerance, allowing them to endure high temperatures in tropical and subtropical areas (Clough & Sim, 1989; Lovelock et al., 2006). This thermotolerance is partially attributable to their capacity to modulate leaf temperature via characteristics like as leaf area and water consumption efficiency (Sastry & Barua, 2017; Nguyen et al., 2017). Nonetheless, a substantial portion of this study has concentrated on a limited number of species, leaving considerable knowledge gaps about the thermotolerance of mangroves from various biogeographical locations (Li et al., 2022; Alongi, 2015).

Mangroves demonstrate drought resistance via several hydraulic adaptations, such as elevated tissue elasticity and the capacity to sustain turgor during water stress (Herppich & Willert, 1995; Choat et al., 2018). Analyses of pressure-volume curves have frequently been employed to evaluate water relations and drought resilience in mangroves; nevertheless, extensive research connecting these characteristics to their functional responses are few (Sack & Scoffoni, 2013; Zhang et al., 2019). Research indicates considerable interspecific heterogeneity in drought tolerance among mangroves; nevertheless, the correlation between plant functional features, such as leaf dry matter content (LDMC), and drought resistance is little comprehended (Beckett et al., 2023; Field et al., 2017; Poorter et al., 2009).

The photosynthetic responses of mangroves to environmental changes have been a primary focus of ecophysiological research. Mangroves are recognised for sustaining elevated photosynthetic rates, even in settings of increased CO_2 (Reef et al., 2016; Lovelock et al., 2006). The disparity in photosynthetic efficacy among species and its correlation with plant functional features, including leaf mass per area

(LMA) and stomatal density, remains inadequately examined (Ball, 1988; Lovelock et al., 2006). Although it is established that photosynthesis may be constrained by stomatal conductance, minimal research has investigated the influence of mangrove stomatal characteristics on CO_2 assimilation and water usage efficiency (Wong et al., 1979; Lawson & Blatt, 2014). Analysis of functional features in mangroves has predominantly concentrated on attributes such as leaf mass per area (LMA), leaf area, and wood density, which are frequently associated with ecological strategies (Wright et al., 2004; Reich, 2014). These characteristics have been utilised to comprehend how mangroves manage trade-offs among growth, reproduction, and survival in challenging situations (Poorter et al., 2009; Wright et al., 2004). Nonetheless, in contrast to terrestrial plants, trait-based studies of mangroves remain nascent, necessitating further investigation into the correlation between these features and physiological performance under stress conditions (Reich et al., 2014; Bahar et al., 2018).

The reviews indicated that prior researchers identified two cross-species trends. A thicker plant axis (stem) correlates with larger individual appendages (leaves, inflorescences, and fruits). The closer the branching, the thinner the final axes and the smaller the appendages. Halle et al. (1978) designated these patterns as Corner's Rules (White et al. 1983). The cross-sectional area of a twig is anticipated to correlate with the total leaf area it supports, due to both hydraulic and mechanical factors. Nonetheless, a specific total leaf area could conceivably consist of numerous little leaves or a limited number of huge leaves. Consequently, the alignment of twig cross-sectional area with the transpiration demands of the leaves or the necessity for mechanical support does not inherently establish a correlation between the dimensions of twigs and the size of individual leaves. It is thought that when twigs are in closer proximity, the leaves should be smaller to avoid inefficient overlap. According to Westoby’s review, leaf size generally diminishes in arid, illuminated, or nutrient-deficient settings; densely branched structures are more adapted to high-light conditions. The correlation between leaf size and twig size is strong with infructescence size and weak with the mass of individual seeds (Westoby et al. 2002).

Mangroves are vital biological resources essential to the coastal ecosystem. Mangroves, celebrated for their diverse ecosystem services such as fisheries and fibre production, sediment regulation, and protection against storms or tsunamis (Polidoro et al 2010), are experiencing rapid decline due to degradation from agriculture, aquaculture, tourism, pollution, urban development, and over-exploitation. India had a 40% reduction in its mangrove area over the past century. Therefore, it is essential to evaluate the condition and trends of mangroves and their conservation in India (Sahu et al 2015). Mangroves rank among the most carbon-dense forests in the tropics, averaging 1,023 Mg of carbon per hectare (Donato et al. 2011). For decades, scientists have examined the halophytic characteristics of intertidal plants, generally positing that the intrinsic distinction between obligatory and facultative halophytes is mostly linked to the plant's ability to endure freshwater conditions, with few additional factors (Krauss et al 2013).

Approximately 70 species and hybrids of mangroves have been documented worldwide (Duke et al 1998). Mangrove genera generate functionally unique aerial root types that serve as the principal interface between shallow, aboveground surface processes and soils (Tomlinson 1986). Functional root types encompass prop roots, big and small pneumatophores, knee roots, and plank roots, which emerge along diverse hydrological and geomorphological gradients in several forests. Significant variations in root morphology among these root types may also extend subterraneously (McKee 1996). The survival of mangrove forests in the marine intertidal zone is affected by regional sea-level rise and coastal geomorphology; however, vegetation also influences soil structure and alterations in surface elevation, which refers to the vertical displacement of the mangrove soil surface. Mangroves, along with potentially other vegetative communities, actively respond to environmental changes; they possess a robust capacity to alter their surroundings, inherently foster habitat resilience, and function as coastal adaptation engineers (Cheong et al 2013).

Lawong's research revealed that mangroves exhibited varying functional performance across different zonation bands. Species in the mean low tide (MLT) zone had the highest net maximum photosynthetic carbon assimilation, stomatal conductance rate, and leaf mass per area (LMA). The photosynthetic and foliar resources exhibiting strategic features demonstrated a decreasing trend inland. The photosynthetic properties of these leaves exhibited significant variation across distinct growth types. Mangroves exhibit extensive functional capabilities despite thriving within a limited ecological spectrum. A leaf-level investigation into photosynthetic functional performance revealed, for the first time, compelling evidence of significant variations in functional performance among mangrove species across zonation bands and distinct growth patterns. Certain animals demonstrated both extravagant and conservative resource utilisation behaviours (Lawong et al. 2011).

The accumulation of organic matter on the soil surface via litter fall (such as leaves, reproductive materials, and twig debris) can enhance elevation in certain mangrove forests. In circumstances when tidal flushing is restricted, the absence of leaf-shredding crabs and the slow rate of decomposition (Middleton & McKee 2001) might lead to the accumulation of leaf litter, hence contributing to soil accretion (McKee 2011). In comparison to leaves and wood, roots possess a significantly higher capacity to enhance soil volume and elevation due to their resilient characteristics and the anaerobic soil conditions that impede decomposition (McKee & Faulkner 2000).

A study on leaf thermo-tolerance in tropical trees from a seasonal dry forest. It was discovered that

species exhibiting elevated leaf mass per area (LMA) demonstrated increased thermo-tolerance. The correlations between thermo-tolerance and leaf characteristics were analogous when analysed independently for the wet and dry seasons, specifically a strong positive correlation for leaf mass per area (LMA) and an absence of correlation for leaf size. Sastry and Barua (2017) Sastry et al. examined leaf thermo-tolerance by measuring the temperatures that caused a 50% reduction in photosystem II function (T50) in experimentally cultivated saplings of 12 tree species from a seasonally dry tropical forest in the northern Western Ghats. Research indicated that dryness enhanced temperature tolerance, with species exhibiting greater drought tolerance simultaneously demonstrating better thermo-tolerance. Thermo-tolerance was strongly correlated with the essential leaf functional feature - leaf mass per area (LMA), and correspondingly, it was adversely correlated with photosynthetic rates. Guha et al. (2017) discovered that hydraulic anatomy influences genotypic diversity in plant water utilisation and exhibits organ-specific flexibility in response to drought in Sorghum bicolour. A distinct correlation was noted between hydraulics and leaf gas exchange characteristics, with genotypes exhibiting safety-efficiency trade-offs in hydraulic attributes. Drought stress prompted modular phenotypic plasticity in hydraulic anatomy, while leaf, stem, and root xylem properties shown varying degrees of plasticity in response to drought. Guha et al. (2018)
Notwithstanding the expanding corpus of study on mangroves, numerous knowledge gaps persist. A significant portion of the study on mangrove physiology has been undertaken in controlled settings or experimental gardens, which may not adequately reflect the conditions encountered by these plants in their native ecosystems (Slot et al., 2021; Krauss et al., 2008). Moreover, the emphasis has been placed on above-ground characteristics, while below-ground processes such as root functionality and its role in stress tolerance have received comparatively less scrutiny (Lovelock et al., 2006; Kauffman et al., 2017). This indicates a substantial disparity, as root characteristics are essential for water and nutrient absorption, particularly in saline conditions (Reef et al., 2016; Alongi, 2015).

A significant gap exists in the studies about the global variability of mangrove characteristics. Although research has focused on mangroves in areas such as Australia and Southeast Asia, there is limited data regarding the physiological responses of mangroves from other regions, including the southwest Indian coast (Nguyen et al., 2017; Reef et al., 2016). Addressing this deficiency is essential for comprehending the adaptation of mangroves globally to climate change and for formulating international conservation policies (Duke et al., 2007; Alongi, 2015). Another notable gap in the literature is the absence of extensive investigations connecting functional features to physiological performance in mangroves. Although research has examined individual variables such as leaf mass area (LMA), leaf area, and photosynthetic capability, there is a paucity of studies that synthesise these traits with physiological responses to several stressors, including temperature, drought, and increased CO_2 (Reef et al., 2016; Poorter et al., 2009). Comprehending these linkages is essential for forecasting mangrove responses to climate change and for recognising features that may provide resilience (Bahar et al., 2018; Reich et al., 2014).

Research has been constrained in comprehending the complete scope of mangrove response to environmental stresses. Research has predominantly concentrated on Australian and Southeast Asian mangroves, whereas other areas, including the southwest Indian coast, have received comparatively little scrutiny. This site exemplifies a unique ecosystem wherein mangroves encounter specific environmental problems, including variable salinity and high humidity, necessitating more research (Nguyen et al., 2017; Alongi, 2015; Saintilan et al., 2020). The ecophysiological study of mangroves is essential, especially in the context of climate change. Elevating sea levels, escalating temperatures, and more frequent and severe storms jeopardise mangrove ecosystems globally. Mangroves are particularly susceptible to temperature variations, drought conditions, and increased CO_2 levels, all of which can affect their physiological processes and long-term viability (Alongi, 2015; Lovelock et al., 2006). Due to their ecological significance and susceptibility, it is crucial to investigate the responses of mangroves to environmental stressors in order to formulate conservation and management strategies for the protection of these ecosystems in the future (Reef et al., 2016; Saintilan et al., 2020).

An urgent necessity exists to establish effective management strategies grounded in a comprehensive understanding of the physiological and ecological mechanisms that govern the dynamics of the mangrove ecosystem. Comprehending these processes is crucial for the advancement of more thorough and scientific restoration, conservation, and management initiatives in mangrove ecosystems. The research objective of this study was to determine how mangrove species from the southwest Indian coast adapt to higher temperatures, drought, and CO_2 levels. How are photosynthetic capacity, water-use efficiency, and thermotolerance associated with essential functional qualities such as phenology? What are the physiological thresholds for temperature and drought stress in mangroves, and how do these thresholds differ among species?

Outline of the thesis:

This thesis employs a multi-species, multi-trait, and multi-stress response methodology to elucidate the ecophysiology of mangrove trees from the Ashtamudy backwaters in Kollam, Kerala. The enquiries examined in the three principal chapters are summarised as follows.

1) In what ways does the thermotolerance of mangrove leaves differ among species, and do essential functional features account for these variations? What is the impact of variations in leaf thermotolerance and leaf temperature during peak hours on the thermal safety margin in mangroves?

2) How does drought tolerance differ among mangrove species in relation to turgor loss? What is the relationship between pressure-volume interactions and essential functioning features in mangroves? Does leaf longevity affect them?

3) How do mangrove plants' assimilation responses vary with rising atmospheric CO2 concentrations? and how are these variances affected by plant functional traits?

References:

Alongi, D. M. (2002). Present state and future of the world's mangrove forests. *Environmental Conservation*, 29(3), 331-349. https://doi.org/10.1017/S0376892902000231

Alongi, D. M. (2014). Carbon cycling and storage in mangrove forests. *Annual Review of Marine Science*, 6, 195-219. https://doi.org/10.1146/annurev-marine-010213-135020

Alongi, D. M. (2015). The impact of climate change on mangrove forests. *Current Climate Change Reports*, 1(1), 30-39. https://doi.org/10.1007/s40641-015-0002-x

Bahar, N. H. A., Hocknull, M. S., McNamara, S., & Domec, J. C. (2018). Functional traits determine photosynthesis and water-use efficiency trade-offs in mangrove species: Importance of root-to-leaf hydraulic conductance. *Oecologia*, 187(2), 355-369. https://doi.org/10.1007/s00442-018-4114-5

Bahar, N. H. A., Ishida, F. Y., Weerasinghe, L. K., Guerrieri, R., O'Sullivan, O. S., Bloomfield, K. J., Asao, S., Lloyd, J., & Santiago, L. S. (2018). Leaf-level photosynthetic capacity in lowland Amazonian and Andean tropical moist forests of Peru. *Plant Physiology*, 178(2), 776-793. https://doi.org/10.1104/pp.18.00562

Ball, M. C. (1988). Ecophysiology of mangroves. *Trees*, 2(3), 129-142. https://doi.org/10.1007/BF00196018

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81(2), 169-193. https://doi.org/10.1890/10-1510.1

Beckett, H., Choat, B., Tissue, D. T., & Rymer, P. D. (2023). Coordination between hydraulic vulnerability and foliar drought tolerance in mangroves. *Functional Ecology*, 37(3), 592-603. https://doi.org/10.1111/1365-2435.14181

Chapman, V. J. (1976). *Mangrove Vegetation*. J. Cramer.

Cheong, S. M., Silliman, B., Wong, P. P., Van Wesenbeeck, B., Kim, C. K., & Guannel, G. (2013). Coastal adaptation with ecological engineering. *Nature Climate Change*, 3(9), 787-791. https://doi.org/10.1038/nclimate1854

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., Lopez, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, 558(7711), 531-539. https://doi.org/10.1038/s41586-018-0240-x

Clough, B. F., & Sim, R. G. (1989). Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapour pressure deficit. *Oecologia*, 79(1), 38-44. https://doi.org/10.1007/BF00378239

Cornelissen, J. H., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., ... & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), 335-380. https://doi.org/10.1071/BT02124

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., ... & van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387(6630), 253-260. https://doi.org/10.1038/387253a0

Danielsen, F., Sørensen, M. K., Olwig, M. F., Selvam, V., Parish, F., Burgess, N. D., ... & Rasmussen, M. S. (2005). The Asian tsunami: A protective role for coastal vegetation. *Science*, 310(5748), 643. https://doi.org/10.1126/science.1118387

Díaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H., Jalili, A., ... & Zarrinkamar, F. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, 15(3), 295-304. https://doi.org/10.1111/j.1654-1103.2004.tb02266.x

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011). Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience*, 4(5), 293-297. https://doi.org/10.1038/ngeo1123

Duke, N. C., Ball, M. C., & Ellison, J. C. (2007). Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology and Biogeography*, 7(1), 27-47. https://doi.org/10.2307/2997695

Duke, N. C., Meynecke, J. O., Dittmann, S., Ellison, A. M., Anger, K., Berger, U., ... & Dahdouh-Guebas, F. (2007). A world without mangroves? *Science*, 317(5834), 41-42. https://doi.org/10.1126/science.317.5834.41b

Ellison, J. C. (2015). Vulnerability assessment of mangroves to climate change and sea-level rise impacts. *Wetlands Ecology and Management*, 23(2), 115-137. https://doi.org/10.1007/s11273-014-9397-8

Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. E., ... & White, L. L. (2017). *IPCC, 2014: Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1-1132). Cambridge University Press.

Ghosh, A., Biswas, J. K., & Mandal, S. C. (2015). Diversity of mangrove species in Sundarbans, India. *Journal of Coastal Life Medicine*, 3(3), 202-205. https://doi.org/10.12980/JCLM.3.201514J69

Guha, A., Barua, K. K., & Baruah, K. K. (2018). Adaptive plasticity in response to drought: Hydraulic anatomy, leaf water relations and gas exchange characteristics in *Sorghum bicolour*. *Journal of Agronomy and Crop Science*, 204(5), 439-449. https://doi.org/10.1111/jac.12261

Guha, A., Parthasarathi, B., & Baruah, K. K. (2017). Hydraulic architecture and water use efficiency in *Sorghum bicolour* under drought. *Plant Physiology and Biochemistry*, 119, 249-256. https://doi.org/10.1016/j.plaphy.2017.09.004

Halle, F., Oldeman, R. A. A., & Tomlinson, P. B. (1978). *Tropical trees and forests: An architectural analysis*. Springer-Verlag.

Herppich, W. B., & Willert, D. J. (1995). Turgor and osmotic potential changes in mangrove leaves during and after stomatal closure. *Oecologia*, 103(2), 166-173. https://doi.org/10.1007/BF00329076

Hogarth, P. J. (1999). *The Biology of Mangroves*. Oxford University Press.

Jia, M., Wang, Z., Zhang, Y., Bai, Y., & Xie, X. (2023). Global distribution of mangroves and their patch sizes in 2020 based on satellite data. *Science of The Total Environment*, 878, 163084. https://doi.org/10.1016/j.scitotenv.2023.163084.

Kathiresan, K. (2010). Importance of mangrove ecosystems. *Journal of Coastal Environment*, 3(2), 120-135.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology*, 40, 81-251. https://doi.org/10.1016/S0065-2881(01)40003-4

Kathiresan, K., & Rajendran, N. (2005). Coastal mangrove forests mitigated tsunami. *Estuarine, Coastal and Shelf Science*, 65(3), 601-606. https://doi.org/10.1016/j.ecss.2005.06.022

Kauffman, J. B., Heider, C., Cole, T. G., Dwire, K. A., & Donato, D. C. (2017). Ecosystem carbon stocks of Micronesian mangrove forests. *Wetlands*, 31(2), 343-352. https://doi.org/10.1007/s13157-011-0153-6

Krauss, K. W., Lovelock, C. E., McKee, K. L., López-Hoffman, L., Ewe, S. M. L., & Sousa, W. P. (2008). Environmental drivers in mangrove establishment and early development: A review. *Aquatic Botany*, 89(2), 105-127. https://doi.org/10.1016/j.aquabot.2007.12.014

Krauss, K. W., McKee, K. L., Lovelock, C. E., Cahoon, D. R., Saintilan, N., Reef, R., & Chen, L. (2014). How mangrove forests adjust to rising sea level. *New Phytologist*, 202(1), 19-34. https://doi.org/10.1111/nph.12605

Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16(5), 545-556. https://doi.org/10.1046/j.1365-2435.2002.00664.x

Lawong, P., Parid, A. L., & Siregar, S. Z. (2011). Functional performance of mangrove species across zonation bands in Sumatra, Indonesia. *Wetlands Ecology and Management*, 19(3), 243-258. https://doi.org/10.1007/s11273-010-9204-9

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*, 164(4), 1556-1570. https://doi.org/10.1104/pp.114.237107

Li, Y., McDowell, N. G., Scott, R. L., & Goulden, M. L. (2022). High temperature and low humidity increase drought vulnerability in a semi-arid mangrove forest. *Global Change Biology*, 28(4), 1451-1464. https://doi.org/10.1111/gcb.15977

Lovelock, C. E., & Ellison, J. C. (2006). Vulnerability of mangroves and tidal wetlands of the Great Barrier Reef to climate change. *The Great Barrier Reef: Vulnerability Assessment*, 238-269.

Lovelock, C. E., Ball, M. C., Martin, K. C., & Feller, I. C. (2006). Variation in carbon assimilation and allocation among mangrove species along a nutrient gradient in Panama. *Journal of Ecology*, 94(5), 1132-1144. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Lugo, A. E., & Snedaker, S. C. (1974). The ecology of mangroves. *Annual Review of Ecology and Systematics*, 5, 39-64. https://doi.org/10.1146/annurev.es.05.110174.000351

McKee, K. L. (1996). Growth and physiological responses of neotropical mangrove seedlings to root zone hypoxia. *Tree Physiology*, 16(11-12), 883-889. https://doi.org/10.1093/treephys/16.11-12.883

McKee, K. L., & Faulkner, P. L. (2000). Mangrove peat analysis and interpretation of recent accretion history: a case study from Belize. *Ecological Applications*, 10(2), 347-353. https://doi.org/10.1890/1051-0761(2000)010[0347:MPAAIO]2.0.CO;2

McKee, K. L., & McGinnis, T. E. (2011). Ecosystem stability and resilience in coastal wetlands. In *Treatise on Estuarine and Coastal Science* (Vol. 8, pp. 49-70). Elsevier. https://doi.org/10.1016/B978-0-12-374711-2.00805-7

Middleton, B. A., & McKee, K. L. (2001). Degradation of mangrove tissues and implications for peat formation in Belizean island forests. *Journal of Ecology*, 89(5), 818-828. https://doi.org/10.1046/j.0022-0477.2001.00603.x

Naskar, K. R., & Mandal, R. N. (1999). *Ecology and Biodiversity of Indian Mangroves*. Daya Publishing House.

Nguyen, H. T., Stanton, D. E., Schilling, E. M., & Burgess, S. S. O. (2017). Mangrove responses to temperature increase: Whole-plant and leaf gas exchange responses in *Avicennia marina* and *Rhizophora stylosa* at ambient and elevated temperatures. *Frontiers in Plant Science*, 8, 1157. https://doi.org/10.3389/fpls.2017.01157

Nguyen, H. T., Stanton, D. E., Schmitz, N., Farquhar, G. D., & Ball, M. C. (2017). Growth responses of the mangrove *Avicennia marina* to salinity: Development and function of shoot hydraulic systems require saline conditions. *Annals of Botany*, 119(2), 275-288. https://doi.org/10.1093/aob/mcw227

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., ... & Cornelissen, J. H. C. (2013). New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), 167-234. https://doi.org/10.1071/BT12225

Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., ... & Yong, J. W. H. (2010). The loss of species: Mangrove extinction risk and geographic areas of global concern. *PLoS ONE*, 5(4), e10095. https://doi.org/10.1371/journal.pone.0010095

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565-588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Reef, R., Lovelock, C. E., & Feller, I. C. (2016). The impacts of elevated CO_2 and nutrient enrichment on photosynthesis and growth of C_3 and C_4 mangroves. *Plant, Cell & Environment*, 39(10), 2205-2214. https://doi.org/10.1111/pce.12768

Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275-301. https://doi.org/10.1111/1365-2745.12211

Sack, L., & Scoffoni, C. (2013). Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist*, 198(4), 983-1000. https://doi.org/10.1111/nph.12253

Sahu, M., Subudhi, H. N., & Kar, S. K. (2015). Mangrove conservation and restoration initiatives in India: A review. *Journal of Environmental Research And Development*, 9(3A), 1204-1211.

Saintilan, N., Wilson, N. C., Rogers, K., Rajkaran, A., & Krauss, K. W. (2020). Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global Change Biology*, 26(6), 3054-3065. https://doi.org/10.1111/gcb.15022

Sastry, A., Barua, D (2017). Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. Sci Rep 7, 11246 . https://doi.org/10.1038/s41598-017-11343-5

Sastry, A. et al., (2016) Leaf thermotolerance in dry tropical forest tree species: relationships with leaf traits and effects of drought. AoB PLANTS, Volume 10, Issue 1, https://doi.org/10.1093/aobpla/plx070

Selvam, V. (2003). Environmental classification of mangrove wetlands of India. *Current Science*, 84(6), 757-765.

Semeniuk, V., & Wurm, P. A. S. (1987). Zonation and stratification of mangrove communities in northwestern Australia. *Journal of Vegetation Science*, 16(4), 259-266. https://doi.org/10.1111/jvs.12142

Slot, M., Winter, K., & Kitajima, K. (2021). Mangrove photosynthesis: Response to increasing temperatures and implications for resilience to climate change. *New Phytologist*, 231(5), 2008-2020. https://doi.org/10.1111/nph.17515

Tomlinson, P. B. (1986). *The botany of mangroves*. Cambridge University Press. https://doi.org/10.1017/CBO9780511753440

Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 199(2), 213-227. https://doi.org/10.1023/A:1004327224729

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33(1), 125-159. https://doi.org/10.1146/annurev.ecolsys.33.010802.150452

White, P. S., & Harper, J. L. (1983). Corridors of ecological displacement: Contemporaneous high-light and long-distance dispersal within vegetation patches. *Annual Review of Ecology and Systematics*, 14(1), 281-312. https://doi.org/10.1146/annurev.es.14.110183.001433

Wong, S. C., Cowan, I. R., & Farquhar, G. D. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature*, 282(5737), 424-426. https://doi.org/10.1038/282424a0

Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, 15(4), 423-434. https://doi.org/10.1046/j.0269-8463.2001.00542.x

Wright, I. J., Reich, P. B., & Westoby, M. (2002). Strategy shifts in leaf physiology, structure and nutrient content between species of high-and low-rainfall and high-and low-nutrient habitats. *Functional Ecology*, 16(3), 423-434. https://doi.org/10.1046/j.1365-2435.2002.00650.x

Wright, I. J., Reich, P. B., & Westoby, M. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827. https://doi.org/10.1038/nature02403

Zhang, Y., Ward, M. J., & Silander, J. A. (2019). Trait–environment relationships differ between tropical and temperate tree species across a global rainfall gradient. *Functional Ecology*, 33(6), 1056-1067. https://doi.org/10.1111/1365-2435.13309

Chapter 2.

Leaf thermotolerance in Mangroves and its relationship with other leaf traits

Abstract:

How Mangrove systems will respond to future climates with more frequent and severe extreme temperatures from changing climate has received little attention. Indeed, there is already evidence of the adverse effects of warming temperatures on these systems, and recent large-scale dieback of mangroves has occurred during periods of severe heat stress. To understand how vulnerable mangroves are to future warming compared to other terrestrial ecosystems, we quantified leaf thermotolerance and estimated thermal safety margins (TSM) for thirteen mangrove species from the southwestern Indian coast. We quantified thermotolerance as temperatures that resulted in a 50% reduction in photosystem II function (T_{50}) and TSM as the difference between T_{50} and estimates of maximum leaf temperatures. Additionally, we tested if thermotolerance was related to other leaf traits. T_{50} ranged from 48.5°C in *Avicennia marina* to 56°C in *Bruguiera gymnorhiza,* with a mean of 53.3°C for the thirteen species. Both thermotolerance and TSMs were exceptionally high compared to other plant communities, globally. Contrary to other studies, we observed no clear relationships between the leaf traits and thermotolerance. With their high thermotolerance and large safety margins, mangroves may be relatively less vulnerable to increased temperatures.

Introduction:

Heat stress, including heat waves, will be more intense in the 21st century than ever before and the average temperature increase by 2040 is predicted to be about 1.5 °C (IPCC AR6). It has already been reported from tropical forests that climate change has caused serious impacts on biodiversity as species loss, range shift and migration (Allen C D et al., 2010; Anderegge et al., 2013; Kueppers et al. 2005; Law B E 2014 & Zettlemoyer MA and Peterson ML 2021). A few years back, Australian mangroves also reported large-scale dieback due to climate change (Ball M C et al., 2017; Doughty C.E et al., 2023 & Duke N C et al., 2017).

Knowledge of the upper limits of plant functions like temperature tolerance is essential to understanding how plants will respond to global warming (Sastry and Barua 2017, Krause et al ., 2013). Photosynthesis is the fundamental physiological process and it is heavily temperature-dependent (Berry & Björkman, 1980) and the knowledge of its functional limits will enable us to understand the depth of the impact of global warming and to predict the vulnerability of the species to the future warming events. Although there is an increased interest across the globe in studying the high-temperature tolerance of plants from different biomes, very few literature available on mangrove ecosystems(Li et al., 2022).

Mangrove ecosystems face extremely high light intensities, high temperature, humidity and wind throughout the day time and it makes them the survivors on one of the most challenging environments on the planet (Cheesman et al., 1997, Ball 1988, Lovelock & Ball 2002,) The conservative water use behaviour and low stomatal conductance that is typical for mangroves result in low evapotranspiration rates that are unlikely to be sufficient to keep leaf temperatures at or below ambient air temperatures, and previous work has shown that leaf temperatures experienced by mangroves were often 4-15 °C degrees above ambient temperatures, i.e. reaching temperatures of 34-45°C at ambient air temperatures of 30°C (Ball 1988, Andrews 1985, Cheeseman 1997, Lovelock 1991).

Given the tropical environments that mangroves are abundant in with high radiation and high humidity and given the limited ability to effectively cool themselves, it is likely that these species may have higher tolerance to high temperatures (Li et al., 2022). Few studies have examined this: Mangroves studied temperatures that resulted in leaf necrosis, and showed that LT50 for mangroves in Puerto Rico ranged from 51-54 °C as compared to 46-53 °C for other co-occurring woody species(Biebl, 1964). In more recent time, mangroves have been studied for thermotolerance in Panama ranged from 49-54 °C as compared to 45-54 °C for other co-occurring woody species - red mangroves had the highest thermotolerance measures in the woody 147 species examined(Slot et al., 2021).

Thermotolerance is positively related to the leaf mass per area (LMA), and negatively related to photosynthetic rates. Thus, more productive species with lower LMA and higher photosynthetic rates may be more vulnerable to heat stress and more likely to be negatively affected by future increases in extreme climatic events (Sastry et al., 2017, Sastry and Barua 2017, Silva and Rosatto 2022). while they have found no relationship between leaf area and thermotolerance (Sastry and Barua 2017). Thermotolerance was found to have positively associated with leaf life span in woody savanna species (Zhang et al., 2017). In a recent study from 147 tropical low land and montane plant species have found that the T50 is positively correlated with leaf mass per area (Slot M et al., 2021). The leaf mass per area is a key leaf functional trait which is related to the trade-off between resource acquisition and leaf lifespan and which defines the leaf economic spectrum (Wright et al., 2004). Study found savanna tree leaves with lower specific leaf area, higher thickness, and higher T50 values than semi-deciduous forest plants (Silva and Rosatto 2022). In a recent study LMA and LA are reported to be positively correlated with T50 in mangroves(Li et al., 2022).

In almost all contemporary studies, temperature tolerance of plants is assessed by using techniques based on Photosystem II quantum efficiency; chlorophyll *a* (Chl *a*) fluorescence (Araújo et al., 2021; Li et al., 2022; Michaletz et al., 2015; Sastry et al., 2018; Slot et al., 2021). The temperature tolerance is higher for tropical species and lower for the temperate species and the woody savanna species with leaf flushing in the hot-dry season was greater than that of those with leaf flushing in the rainy season (Cunningham & Read, 2006; Zhang et al., 2012). The temperature tolerance increased by about 8 °C from poles to the equator and the thermal safety margin between the upper limits of the tolerance level and the atmospheric higher temperature is lower at the equatorial region and higher towards the poles as also shown for arid regions plants from Australia (O Sullivan et al., 2016, Curtis et al., 2016). In a study of four different biomes from Yunan province of China has reported to have the following order; subtropical broad-leaved forest > tropical evergreen forest > tropical rain forest > savanna (Nawatbhrist et al., 2021). The thermal safety margin Evergreen trees from Amazonia have showed higher thermotolerance and a significant seasonal acclimation of the T50 also reported (Rakesh et al., 2020). In study of 40 tropical plant species, it is demonstrated that the upper thermal limits are closer to the high temperatures experienced at the site and the thermal safety margin range from 3.5 °C to 8 °C. It is also showed the seasonal acclimation and also evergreen species has a higher thermotolerance compared to deciduous species (Sastry and Barua 2017, Sastry et al., 2017).

The objectives of this study are: 1) To characterize the temperature tolerance (T50) and thermal safety margin of mangroves. 2) Find out the relationship between the thermotolerance and key leaf functional traits in mangrove plants. 3) How mangroves differ in thermotolerance with other ecosystem, globally.

Materials and Methods:

This research concentrated on sedimentary islands in Ashtamudi Lake, Kerala, India (8°59'N 76°36'E, Supplementary Fig. S1). This enormous interior saltwater lake, covering 32 km², is situated approximately 2.5 km from the western coast of peninsular India, however, it is linked to the Arabian Sea. The average annual precipitation in this location is around 2398 mm, with almost one-third occurring between June and September (Supplementary Fig. S2). The mean yearly temperatures varied from 25.5°C to 27.5°C. The interval from December to May signifies comparatively arid months characterised by elevated mean and maximum temperatures. This study encompasses all 13 real mangrove species identified on the sedimentary islands of Ashtamudi Lake, out of the 18 species reported from the southwestern coast of India (Sreelekshmi et al. 2018).

Leaf Trait Measurements:

We sampled thirteen true mangrove species (Table 2.1S) from five estuarine sedimentary mangrove islands in the Ashtamudi lake (backwaters), at the southwest coast of India. We followed the protocols from Perez et al[3]., 2013 for the leaf trait measurements. Leaf weight was measured using a high precision microbalance (AUW220D, Shimadzu, Kyoto, Japan), and leaves were scanned (CanoScan LIDE 110- Canon) to quantify leaf area and leaves were dried at 70°C for 72 hours using a hot air oven (ENG-24 HAO, SAMCO Instruments, Nagpur, India). Fevicol; a synthetic resin adhesive, was used to make stomatal impressions, and they were taken off the leaves using cello tape and mounted on a glass slide and photographed with the help of a microscope (DFC 280, Leica Microsystems, Wetzlar, Germany) and the images were analysed using Image analysing software Image J (2.0.0-rc-43/1.42n, National Institute of Health , USA). We followed protocol from Perez et al. 2016, for the measurements of all functional traits used in this study. The One-sided area of the leaf is quantified as Leaf Area(LA), it is divided by the Oven Dried Mass to calculate the Leaf Mass per Area (LMA), The ratio of the Leaf's Dry Mass to the Fresh Mass is calculated as the Leaf Dry Matter Content(LDMC), The number of stomates per unit area of the leaf is quantified as the Stomatal Density (Stom Den), The vertical length of the Stomatal Guard Cells is measured as the Guard Cell Size(Guard Size), Vertical length of the pore opening is measured as the Stomatal Pore Size (Pore Size), Stomatal Pore Index is calculated the product of Stom Den*(Pore Size) and Leaf Vein Density is measured as the total length of the vein spread below secondary order per unit area of the leaf.

Temperature tolerance assay:

Sampling:

We followed the method from Sastri & Barua, 2017 for the temperature tolerance assay. All available mangrove species were used for this study. Healthy, fully grown, mature, sun-exposed leaves were collected from three healthy individuals of all species for the assay. The 80-100 cm long branches were collected in the predawn and kept in a water-filled bucket such that the cut end was immersed in the water. Leaves were detached from the stem just before the assay.

Temperature treatment:

Leaf discs were taken using a cork borer of 1 cm mouth diameter, and then they were immediately transferred into muslin cloth pouches and covered with aluminium foil to lightproof them. Then, these packs were kept in industrial-grade polythene zip-lock bags with wet tissue to ensure they didn't dry out. These packs then were treated for different temperatures ranging from room temperature to 35 °C, 40°C, 42.5°C, 45°C, 47.5°C, 50°C, 52.5°C, 55°C, 57.5°C and 60°C for half-hour time in a refrigerated water bath (KEMI CRYOSTAT KUCB 3, KEMI MOOSA INSTRUMENTS, INDIA). The zip-lock bags were kept inside a wire mesh to avoid free-floating in the water bath. Then, the temperature-treated leaf discs were removed from the packing and transferred to a petri dish with wet tissue to keep moisture and kept overnight in the dark.

Fv/Fm measurement:

The discs were then transferred to Hansatech leaf clips and dark-adapted for 30 minutes, then Photosystem II photochemical efficiency (chlorophyll a fluorescence: Fv/Fm) was measured using chlorophyll fluorometer (Figure 31. Handy PEA, Hansatech Instruments, Norfolk, UK) with a saturating light pulse of 2500 umol/m^2/s.

Figure 2.1 Chlorophyll Flourometer

Data analysis:

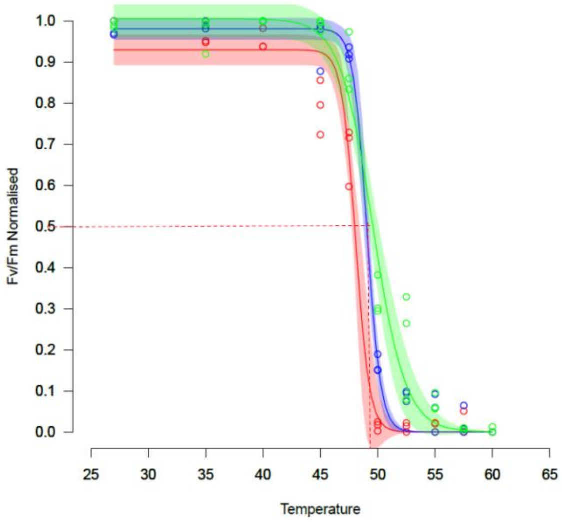


Figure 2.2 Determination of **T50** (Temperature at which 50% reduction in Fv/Fm), **Tcrit** (Temperature at the point where a significant drop in Fv/Fm begins) and **Tleth** (Temperature where 95% of Fv/Fm drops) from Fv/Fm curve.

A four-parameter logistic sigmoid curve was fitted to the chlorophyll *a* fluorescence (F_v/F_m) values along the range of temperatures examined using the R package 'drc'. The four-parameter model with the lower asymptote set to zero were used to generate appropriate curves. T_{50} is estimated as the temperature at which reduction in chlorophyll *a* fluorescence (F_v/F_m) was 50% of the upper asymptote of the curves, whereas Tcrit (Critical temperature) is estimated as the temperature point at which Fv/Fm drops significantly and Tleth (Lethal temperature) as the temperature where 95% of the Fv/Fm drops. We used 3 independent leaves from an individual at each of the temperatures to generate an F_v/F_m response curve from which we estimated T_{50}, Tcrit and Tleth for that individual. We repeated this for 3 individual plants per species. We tested the statistical significance of the interspecific variation in T_{50} using ANOVA and did a correlation matrix to see the relationship between thermotolerance (T_{50},Tcrit and Tleth) and other leaf traits, using Pearson correlation. All statistical analyses were done using R Studio, version 1.4.1717. Also collected T_{50} data for different ecosystems across the globe from published literature and compared with that of mangroves. Pearson correlation has been performed to test the relationship of leaf functional traits with the T50 and the TSM.

Thermal Safety Margins

Maximal leaf temperature is more appropriate to calculate the thermal safety margin of a leaf rather than air temperature given how leaf temperature affects physiological processes like photosynthesis and respiration and how leaves of different traits like size can have varied temperatures under the same environment (Jones, 2013). The upper thermal limit of the leaf is taken to be T50 which is the temperature at which the activity of photosystem II reduces to 50% (Sastry & Barua, 2017). TSM is calculated as the difference between T50 and maximal leaf temperature. The temperature of a leaf is determined by the energy leaving and entering the leaf at a given point in time. These energy fluxes are radiative flux R_n, sensible heat or convective flux C, and latent heat or transpiration flux λE (Jones, 2013, Michaletz et al., 2015). The radiative flux includes solar radiation or the shortwave from the Sun, and thermal radiation, or the longwave emitted by the surroundings and the leaf itself. The sensible heat flux, which depends on the leaf-to-air temperature difference, is the convective energy transfer between the leaf and the atmosphere through a thin layer of nearly stagnant, and turbulent air surrounding the leaf surface called the boundary layer. The latent heat flux, on the other hand, is the heat loss via transpiration through the stomata and the boundary layer. It depends on the amount of water that escapes the leaf, defined by the stomatal conductance g_s, the amount of water in the leaf and the surrounding air can carry, represented using the vapour pressure deficit D, and the rate of water transfer through the boundary layer, defined by the boundary layer conductance to water vapour g_{bw}.

When a leaf is in a steady state, or when the leaf's temperature does not vary with time, the total energy leaving the leaf should match the energy entering the leaf, called the leaf energy balance (Eq. 1) (Jones, 2013, Michaletz et al., 2015). During a sunny day, net radiation would be positive whereas, on a clear night, the leaf would lose energy via net radiation. The thermal radiation is calculated from the Stefan-Boltzmann equation using the emissivity ϵ and temperature T of the object (Jones, 2013). The thermal radiation emitted by the leaf introduces non-linearity to the model and there are several ways to work around it (Jones, 2013, Kyaw Tha Paw U, 1987, McColl, 2020). Jones (2013) proposes the use of isothermal net radiation R_{ni}, which is defined as the net radiation when the leaf is at the air temperature under the same environment. Therefore, R_{ni} is independent of the leaf temperature (Table 2) and this allows us to write R_n as a function of R_{ni} and the leaf T_l to air T_a temperature difference: $(T_l - T_a)$ (Eq. 2). The convective flux can also be written as a function of $(T_l - T_a)$ (Eq. 3). For the latent heat flux, we use a linearization called Penman linearization (Eq. 4) which will also equate the latent heat flux as a linear function of $(T_l - T_a)$. These four equations (Eq. 1 – 4) can be combined and solved to get an analytical solution for T_l (Eq. 5).

$$R_n - C - \lambda E = 0 \tag{1}$$

$$R_n = R_{ni} - \epsilon_l \sigma\left(T_l^4 - T_a^4\right) \approx R_{ni} - 4\epsilon_l \sigma T_a^3 (T_l - T_a) \tag{2}$$

$$C = \rho_a c_{pa} g_{bh} (T_l - T_a) \tag{3}$$

$$\lambda E = \frac{\rho_a c_{pa} g_w}{\gamma}\left(e_{sat}(T_l) - e_a\right) \approx \frac{\rho_a c_{pa} g_w}{\gamma}\left(D + s(T_l - T_a)\right) \tag{4}$$

$$T_l = T_a + \frac{R_{ni}\,\gamma\left(\frac{G_{HR}}{g_w}\right)}{\rho_a c_{pa} g_{HR}(s+\gamma(\frac{g_{HR}}{g_w}))} - \frac{D}{s+\gamma(\frac{g_{HR}}{g_w})} \tag{5}$$

To calculate leaf temperatures, we require several leaf traits and climate variables as given in Table 2. The climate variables were taken from ERA5 produced by the European Centre for Medium-Range Weather Forecasts (ECMWF, Copernicus Climate Data Store, Hersbach et al., 2020), except for the air temperature which was measured in the field using Hobo data loggers for the period of September 26, 2021, to August 24, 2022, excluding January 2022). Accessing and setting up the ERA5 dataset was done using ecCodes CLI and Python3 while the rest of the analysis was done using R (R Core Team, 2020). Assuming that the leaves, on a given day, achieved their maximum temperatures when the air hit its daily maximum temperature, we determined the daily maximum air temperature for the days where air temperature data were available. For all those time points of the day where the air reached its maximum, climatic variables like solar radiation were taken from ERA5, and leaf temperatures were calculated assuming 10% of maximum stomatal conductance (Franks and Beers 2009) estimated for each species (Eqn. 6).

$$\boldsymbol{g_{wmax} = \frac{d}{v}\, D\, a_{max}\, /(l + \frac{\pi}{2}\, \sqrt{a_{max}/\pi})}$$

(6)

Results:

We found that the thermotolerance; T50 (Table S2) for the mangroves are generally high and was above 50 °C for all species except *Avicennia marina*, which showed the lowest thermotolerance values; T50, Tcrit and Tleth were 48.9 ± 0.75, 47.15±.62 and 50.64±1.6. *Bruguiera sexangula* showed highest thermotolerance with a T50 value of 55.32±0.97, Tcrit value of 53.15±0.57 and Tleth 57.49±2.01 °C. Overall T50 was averaged 53.37±1.71, Tcirt averaged 50.23±1.63, and Tleth averaged 56.51±2.5.

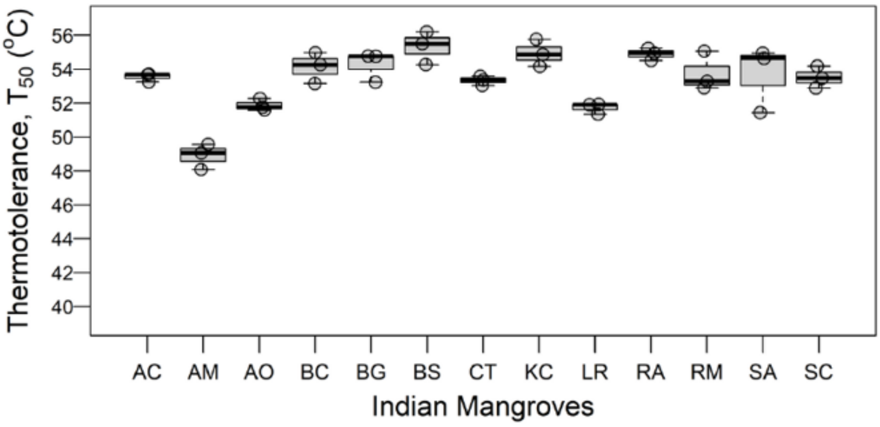
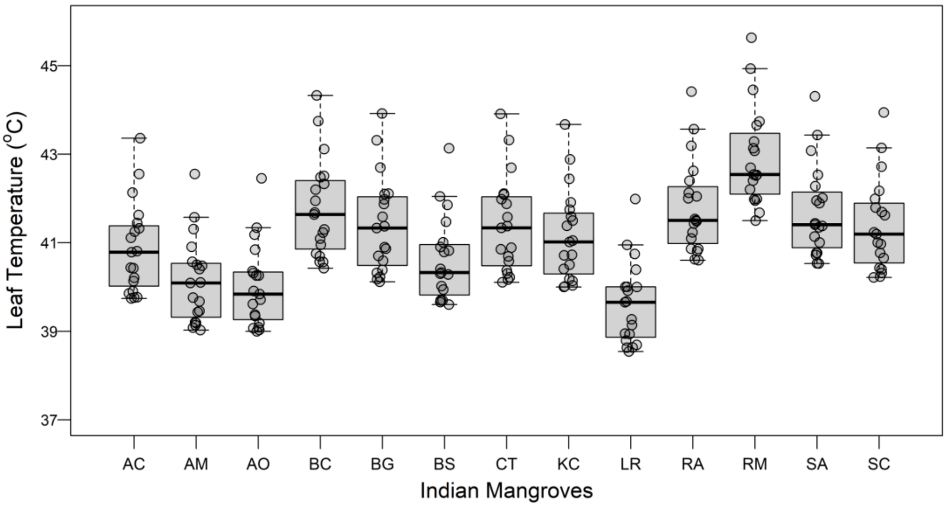
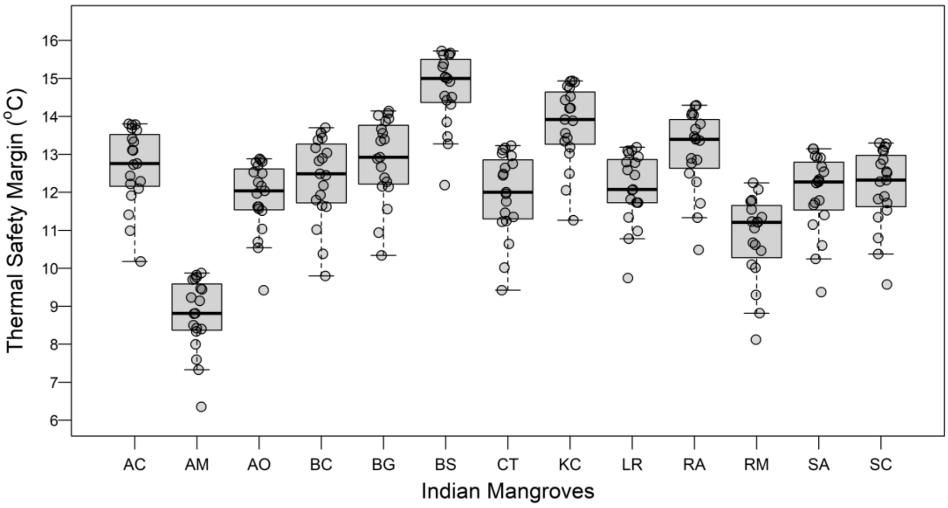


Figure 2.3: Species level leaf thermotolerance (T50 in °C) in mangroves from Ashtamudi lake. Species abbrevations and expansion in Table 3.1

Modelled maximum leaf temperatures (Figure 3.4) were ranging from 38.53° C in *Luminitzera racemosa* to 41.45 °C in Rhizophora mucronate while *Avicennia marina* showed the lowest TSM (Figure 3.5) with 9.87 °C and *Bruguiera sexangula* with the highest value 15.72 °C (Table S2). Overall maximum leaf temperature averaged across the species with low variation to 39.89±0.77 °C while the TSM average across the thirteen species also varied less; 13.41±1.39°C.



2.4: Maximum Leaf Temperature(°C) among the thirteen mangrove species. See table 3.1 for the expansions of the species abbreviations.



2.5: Thermal Safety Margin (TSM °C); The difference between the T50 and the maximum leaf temperature. See table 3.1 for the expansions of the species abbreviations.

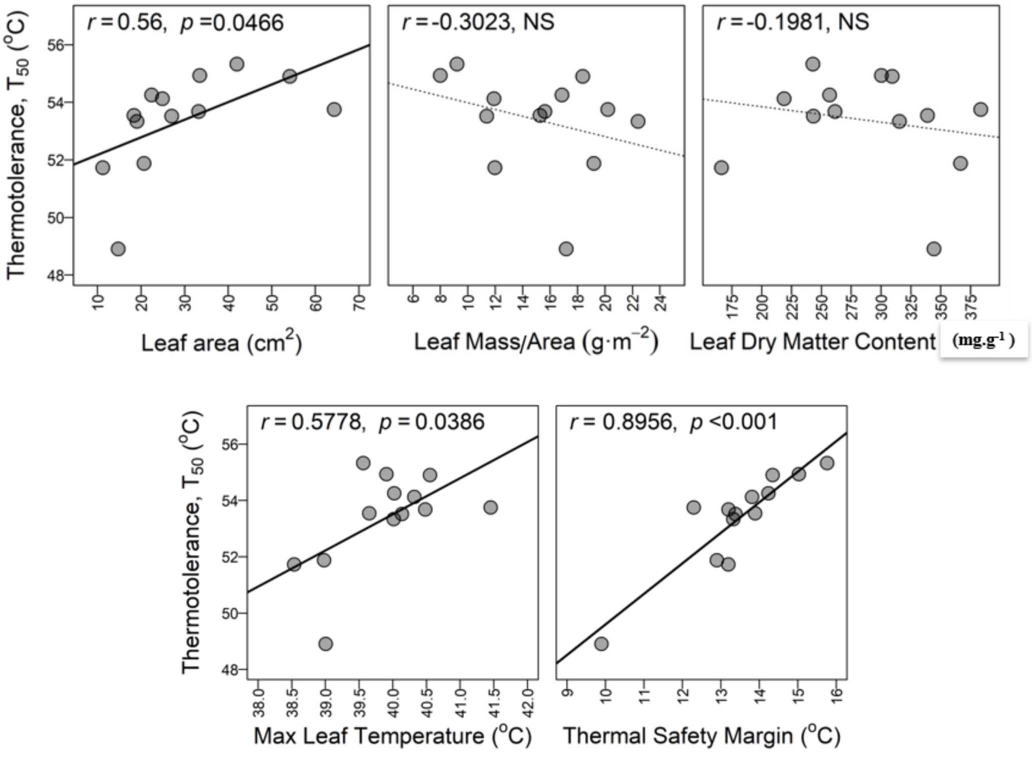


Figure 2.6 – Relationship between heat tolerance (T_{50}) and leaf traits: a) Leaf area, b) Leaf mass per area, c) Leaf dry matter content. Lower panel relationship between T50 with Maximum leaf temperatures, and e) Thermal Safety Margin. Results for Pearson's correlation coefficient (*r*) and *p*-value are presented. NS represents *p*-values greater than 0.05, and dashed lines represent relationships that were not significant.

T50 is positively correlated with Leaf Area (r=0.56, p=0.0466), Maximum Leaf Temperature (r=0.5778, p=0.0386) and Thermal Safety Margin (0.8956, p<0.001). None of the other key functional traits found significantly correlated neither to T50 nor to TSM (Figure 2.6).

The maximum leaf temperature is not at all correlated to the leaf traits that were not directly used in the leaf energy budget model including the traits that define the upper thermal limits.

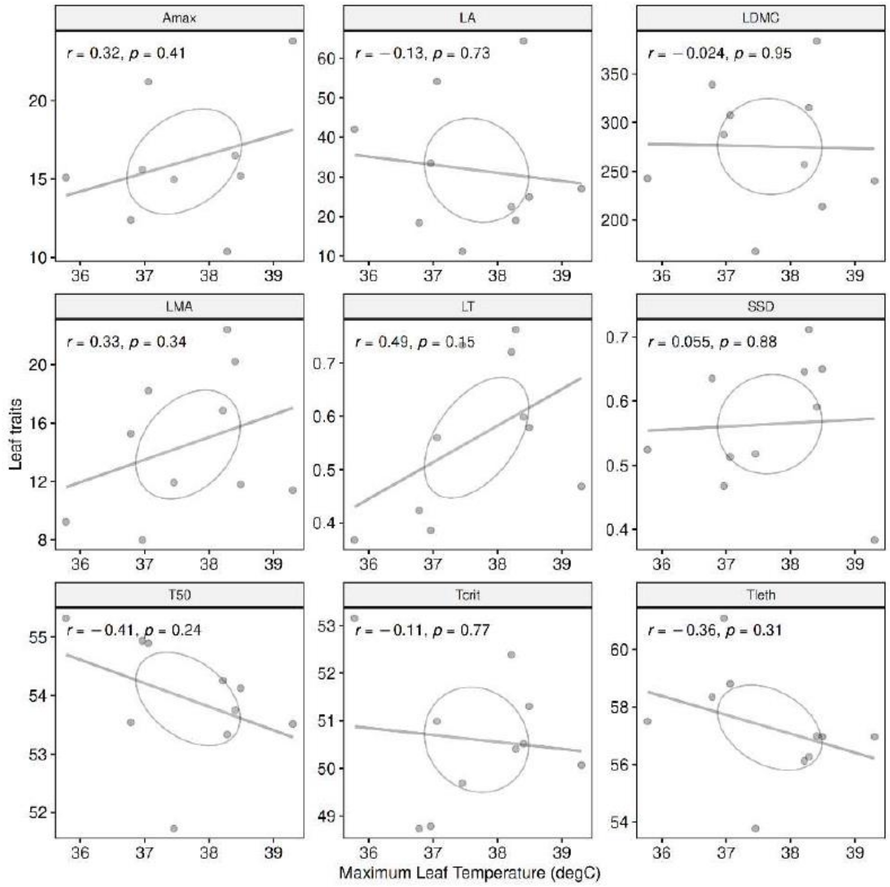


Figure 2.7: Pair wise Pearson’s correlations between maximum leaf temperature and leaf traits. Amax is the maximum Co2 assimilation rate, LA is the one-sided Leaf Area, LDMC is the Leaf Dry Matter Content, LMA is the Leaf Mass per Area, LT is Leaf Thickness and SSD is Stem Specific Density.

Discussion:

Our study brought novel information on thermotolerance (T50, Tcrit, Tleth, TSM) and maximum leaf temperature on mangroves. Mean T50, Tcrit and Tleth recorded for the mangroves in our site was 53.37°C, 50.23°C and 56.51°C while the Chinese mangroves reported to have a mean T50 value of 49.1°C and a mean Tcrit value of 40.7°C, much lower values compared to our results. *Avicennia marina* was found to be the most susceptible species to thigh temperature stress with a T50 value of 48.9±0.75°C, among the thirteen species studied and *Bruguiera sexangula* as the most tolerant species with a T50 value of 55.32±0.97°C. This is consistent with the findings from the Chinese mangroves that the most susceptible species to high temperature stress was A.marina and *Kandelia candel*. In contrary to that, our results on K.candel is the second most tolerant species with a with a T50 value of 54.93±0.79 °C, while Chinese reported 49.5°C (Li et al., 2022). Three mangrove from Panama was reported an average T50 value 51.53°C, as A.marina as the most susceptible species with a T50 of 49.3 °C and *Rhizophora mangle* as the most tolerant with 53.7 °C (Slot et al., 2021).

Mangroves in general showed a very high T50 value than other ecosystems around the world with a median of 52°C while other ecosystems showed around 50°C (Figure 3.8). Mangroves from our site (Red circles in Figure 3.8) reported much higher T50 values than the other mangroves (Black circles in Figure 3.8) probably because our system is closer to the equator and might have exposed to higher temperatures.

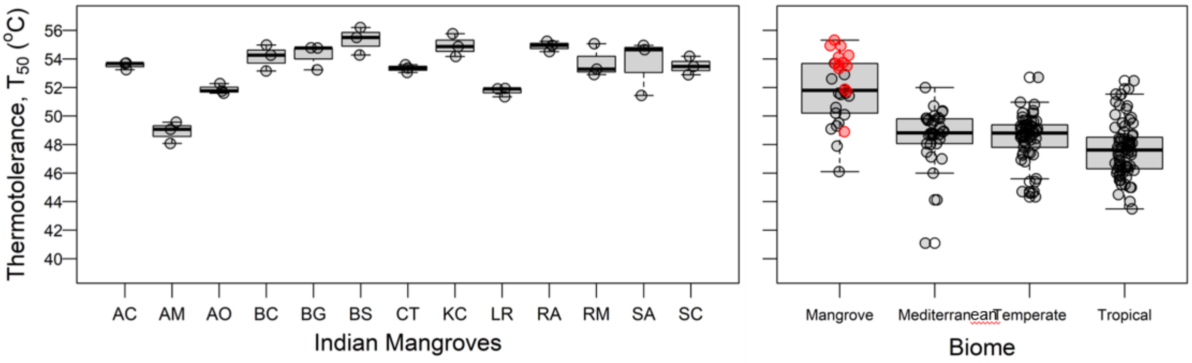


Figure 3.8: Mangrove T50 in comparison with the T50 values for other ecosystems around the world. We have collected data from published literatures with similar treatment methods, references for the data given separately as supporting information.

Plant from different tropical ecosystems have been studied for thermotolerance including western ghats. 41 tropical tree species were studied from northern western ghats and found that he T50 values ranged from 45.5°C to 50.5 °C; among that evergreen species showed higher thermotolerance than the deciduous species (Sastry & Barua, 2017). Seasonally dry tropical forest was also studied for the thermotolerance; a study from northern western ghats with 12 tree species reported T50 ranged from 44.5°C to 48.1°C and drought treatments further increased their thermotolerance(Sastry et al., 2018).

Thermal safety margin has been estimated in different ways over the time. We estimated as the difference between T50 and the maximum leaf temperature and it ranged from 9.87 °C to 15.72 °C with an average of 13.41±1.39 °C, which is much higher than that of any other ecosystems. TSM ranged from 0.5 to 11.5 in tropical montane forests from Rowanda (Tarvainen et al., 2022), North American *Populus fremontii* were found to decrease the TSM with an increasing altitude with a range from 8.5 °C at 149 meter above mean sea level to 4.8°C at 1230 meters (Moran, 2022). 19 tropical woody plant species grown in Fairchild tropical botanic garden in Florida were also studied and found to have TSM ranging from 3.03°C to 11.88°C. TSM are also estimated as the difference between T50 and Maximum air temperature at that region, but it will not be an appropriate estimate as the leaf temperature and the air temperature vary often times (Jones, 2014).

We estimated maximum leaf temperatures for each species using climatic variables and leaf traits. Mean maximum leaf temperature estimated was 39.89±0.77 °C. Tropical tree species from Fairchild tropical botanical gardens have already reported observed maximum leaf temperatures higher than this; with a mean value of 42.4 °C (Perez & Feeley, 2020). In a study of 20 species from Australian tropics have reported mean maximum leaf temperatures of 40.77 °C(Marchin et al., 2022). 15 plants from Chinese subtropics observed to have 37.4 °C; a mean of maximum leaf temperatures (Kitudom et al., 2022).

Leaf Thermotolerance and thermoregulation maybe related to key leaf functional traits such as leaf mass per area, leaf dry matter content and assimilation rate (Michaletz et al., 2016). We found a positive correlation of thermotolerance with maximum leaf temperature and leaf area, but not related to any other leaf traits, which further confirmed the report from Chinese mangroves(Li et al., 2022). Leaf mass per area is positively correlated to thermotolerance (T50) and negatively related to photosynthetic rate and Leaf area was not related to thermotolerance (Sastry et al., 2018; Sastry & Barua, 2017). However 24 woody species from a dry subtropical savanna showed no relationship with the LMA and a strong positive relationship with leaf lifespan (Zhang et al., 2012). In a study on 200 woody species across the globe has also found no relationship between thermotolerance and LMA (O'sullivan et al., 2017).

Conclusion:

Mangroves were found to have higher leaf thermotolerance comparing to the available results from other ecosystems across the globe as it can be expected for the plants that are already exposed to higher temperatures near the equatorial region. They have already been exposed to higher levels of humidity; wind reflected radiation from the water where they grow; all these might have preconditioned them to have a higher thermotolerance. The leaf temperatures were estimated using leaf energy balance model and found to have a relatively higher leaf temperatures for all the species. Even though Leaf area was positively correlated to the thermotolerance, we could not find any relationship for any other leaf functional traits with neither thermotolerance nor maximum leaf temperature. However, leaf thermotolerance and maximum leaf temperature were positively correlated. Species level data on leaft thermotolerance have practical implication in large scale restoration programms, especially in predicted increase in atmopheric temperature

References:

Araújo, I., Marimon, B. S., Scalon, M. C., Fauset, S., Marimon Junior, B. H., Tiwari, R., Galbraith, D. R., & Gloor, M. U. (2021). Trees at the Amazonia-Cerrado transition are approaching high temperature thresholds. *Environmental Research Letters*, *16*(3), 034047. https://doi.org/10.1088/1748-9326/abe3b9

Biebl, R. (1964). Temperaturresistenz tropischer Pflanzen auf Puerto Rico: Verglichen mit jener von Pflanzendergemigten Zone. *Protoplasma*, *59*(1), 133–156. https://doi.org/10.1007/BF01247858

Cunningham, S. C., & Read, J. (2006). Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. *Tree Physiology*, *26*(11), 1435–1443. https://doi.org/10.1093/treephys/26.11.1435

Doughty, C. E., Keany, J. M., Wiebe, B. C., Wolf, A., Tedder, A. J., Muir, C. D., Perez, C. I., Yañez, J., Buermann, W., & Wright, S. J. (2023). Tropical forests are approaching critical temperature thresholds. *Nature, 621*(7962), 105–111. https://doi.org/10.1038/s41586-023-06391-z

Jones, H. G. (2014). *Plants and microclimate: A quantitative approach to environmental plant physiology* (Third edition). Cambridge University Press.

Kitudom, N., Fauset, S., Zhou, Y., Fan, Z., Li, M., He, M., Zhang, S., Xu, K., & Lin, H. (2022). Thermal safety margins of plant leaves across biomes under a heatwave. *Science of The Total Environment*, *806*, 150416. https://doi.org/10.1016/j.scitotenv.2021.150416

Li, X., Wen, Y., Chen, X., Qie, Y., Cao, K.-F., & Wee, A. K. S. (2022). Correlations between photosynthetic heat tolerance and leaf anatomy and climatic niche in Asian mangrove trees. *Plant Biology*, *n/a*(n/a). https://doi.org/10.1111/plb.13460

Marchin, R. M., Backes, D., Ossola, A., Leishman, M. R., Tjoelker, M. G., & Ellsworth, D. S. (2022). Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. *Global Change Biology*, *28*(3), 1133–1146. https://doi.org/10.1111/gcb.15976

Michaletz, S. T., Weiser, M. D., McDowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2016). The energetic and carbon economic origins of leaf thermoregulation. *Nature Plants*, *2*(9), 16129. https://doi.org/10.1038/nplants.2016.129

Michaletz, S. T., Weiser, M. D., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2015). Plant Thermoregulation: Energetics, Trait–Environment Interactions, and Carbon Economics. *Trends in Ecology & Evolution*, *30*(12), 714–724. https://doi.org/10.1016/j.tree.2015.09.006

Moran, M. (2022). *Range in the Southwestern United States*. Arizona State University.

O'sullivan, O. S., Heskel, M. A., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., Zhu, L., Egerton, J. J. G., Bloomfield, K. J., Creek, D., Bahar, N. H. A., Griffin, K. L., Hurry, V., Meir, P., Turnbull, M. H., & Atkin, O. K. (2017). Thermal limits of leaf metabolism across biomes. *Global Change Biology*, *23*(1), 209–223. https://doi.org/10.1111/gcb.13477

Perez, T. M., & Feeley, K. J. (2020). Photosynthetic heat tolerances and extreme leaf temperatures. *Functional Ecology*, *34*(11), 2236–2245. https://doi.org/10.1111/1365-2435.13658

Sastry, A., & Barua, D. (2017). Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. *Scientific Reports*, *7*(1), 11246. https://doi.org/10.1038/s41598-017-11343-5

Sastry, A., Guha, A., & Barua, D. (2018). Leaf thermotolerance in dry tropical forest tree species: Relationships with leaf traits and effects of drought. *AoB PLANTS*, *10*(1). https://doi.org/10.1093/aobpla/plx070

Slot, M., Cala, D., Aranda, J., Virgo, A., Michaletz, S. T., & Winter, K. (2021). Leaf heat tolerance of 147 tropical forest species varies with elevation and leaf functional traits, but not with phylogeny. *Plant, Cell & Environment*, *44*(7), 2414–2427. https://doi.org/10.1111/pce.14060

Tarvainen, L., Wittemann, M., Mujawamariya, M., Manishimwe, A., Zibera, E., Ntirugulirwa, B., Ract, C., Manzi, O. J. L., Andersson, M. X., Spetea, C., Nsabimana, D., Wallin, G., & Uddling, J. (2022). Handling the heat – photosynthetic thermal stress in tropical trees. *New Phytologist*, *233*(1), 236–250. https://doi.org/10.1111/nph.17809

Zhang, J.-L., Poorter, L., Hao, G.-Y., & Cao, K.-F. (2012). Photosynthetic thermotolerance of woody savanna species in China is correlated with leaf life span. *Annals of Botany*, *110*(5), 1027–1033. https://doi.org/10.1093/aob/mcs172

Supporting Figures and Tables:

Table S1: List of the species used in this study along with family names and abbreviations used.

	Species	Family	Code
1.	*Aegiceras corniculatum*	Myrsinaceae	AC
2.	*Avicennia marina*	Avicenniaceae	AM
3.	*Avicennia officinalis*	Avicenniaceae	AO
4.	*Bruguiera cylindrica*	Rhizophoraceae	BC
5.	*Bruguiera gymnorrhiza*	Rhizophoraceae	BG
6.	*Bruguiera sexangula*	Rhizophoraceae	BS
7.	*Ceriops tagal*	Rhizophoraceae	CT
8.	*Kandelia candel*	Rhizophoraceae	KC
9.	*Lumnitzera racemosa*	Combretaceae	LM
10.	*Rhizophora apiculata*	Rhizophoraceae	RA
11.	*Rhizophora mucronata.*	Rhizophoraceae	RM
12.	*Sonneratia alba*	Sonneratiaceae	SA
13.	*Sonneratia caseolaris*	Sonneratiaceae	SC

Figure S1: Location of Ashtamudi Lake, Kerala, India (8°59'N 76°36'E). The study site in the sedimentary islands in this lake are shown in red and encircled by the red outline.

Table S2: Thermotolerance table: T50 values for all species used in this study with standard deviation values.

Species	T50	sd
Aegicera corniculatum	53.54056	0.261035
Avicennia marina	48.90293	0.754599
Avicennia officinalis	51.87757	0.347765
Bruguiera cylindrica	54.12702	0.917823
Bruguiera gymnorhiza	54.25521	0.886463
Bruguiera sexangula	55.32441	55.32441
Ceriops tagal	53.33474	0.273468
Kandelia candel	54.93401	0.798768
Luminitzera racemosa	51.72819	0.329298
Rhizophora apiculata	54.89838	0.368307
Rhizophora mucronata	53.75012	1.154724
Sonneratia alba	53.67949	1.944981
Sonneratia caseolaris	53.51498	0.652884

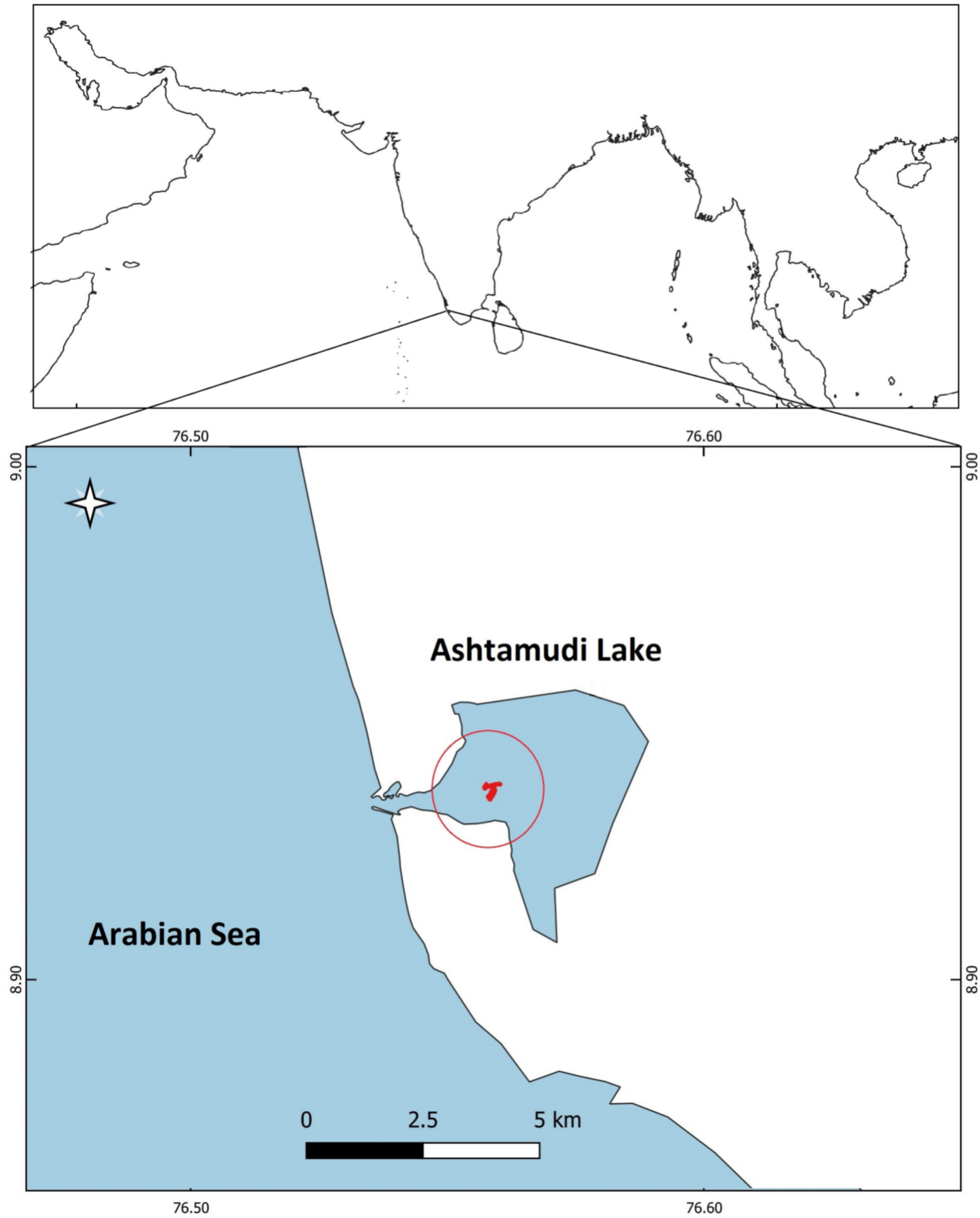


Figure S1: Location of Ashtamudi Lake, Kerala, India (8°59’N 76°36’E). The study site in the sedimentary islands in this lake are shown in red and encircled by the red outline.

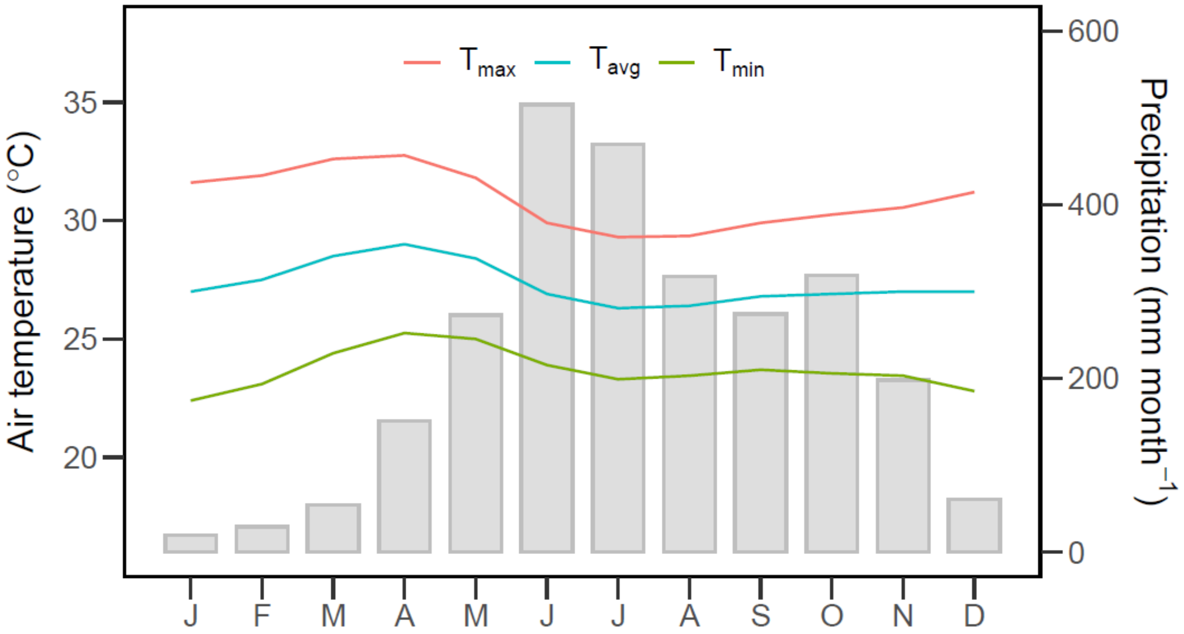


Figure S2: Climatological normals for the study site, Ashtamudi Lake, Kerala, India (8°59'N 76°36'E). Grey bars depict average monthly rainfall; the red, blue and green lines depict average monthly maximum (T_{max}), mean (T_{avg}) and minimum (T_{min}) temperatures, respectively. Data from Climate Research Unit (CRU) (New, M., Lister, D., Hulme, M., & Makin, I. (2002) A high-resolution data set of surface climate over global land areas, Inter-Research Science Publisher, http://dx.doi.org/10.3354/cr021001).

Supplementary Information S2

Table 1: Leaf energy balance and energy fluxes

Symbol	Variable	Units	Equation	Ref.
$\boldsymbol{S}$	Steady state energy balance	Wm^{-2}	$R_n - C - \lambda E = 0$	a,b,c
$\boldsymbol{R_n}$	Net radiative flux	Wm^{-2}	$R_{abs,solar} + R_{abs,therm}$	a
$\boldsymbol{C}$	Sensible heat flux	Wm^{-2}	$\rho_a\, c_{pa}\, g_{bh}\, (T_l - T_a)$	ab
$\boldsymbol{\lambda E}$	Latent heat flux	Wm^{-2}	$\frac{\rho_a\, c_{pa}\, g_w}{\gamma} (e_{sat}(T_l) - e_a)$	ab

Table 2: Solving for leaf temperature: Penman-Monteith Equation

Symbol	Variable	Units	Equation	Ref.
$\boldsymbol{R_{ni}}$	Isothermal net radiation	Wm^{-2}	$R_{abs,solar} + R_{abs,thermal} - \epsilon_l\,\sigma\,T_a^4$	a
$\boldsymbol{R_n}$	Net radiative flux	Wm^{-2}	$R_{ni} - 4\,\epsilon_l \sigma T_a^3 (T_l - T_a)$	a
$\boldsymbol{e_{sat(T_l)} - e_a}$	Vapor pressure deficit	kPa	$D + s(T_l - T_a)$	a
$\boldsymbol{T_l}$	Leaf temperature	K	$T_a + \frac{R_{ni}\gamma\left(\frac{G_{HR}}{g_w}\right)}{\rho_a c_{pa} g_{HR}\left(s + \gamma\left(\frac{g_{HR}}{g_w}\right)\right)} - \frac{D}{s + \gamma\left(\frac{g_{HR}}{g_w}\right)}$	a,b

Table 3: Leaf traits used in the model

Symbol	Variable	Units	Value/source	Ref.
$\boldsymbol{L}$	Effective leaf width	m	Measured for each species	d
$\boldsymbol{g_{s,max}}$	Maximum stomatal conductance	ms^{-1}	Calculated from stomatal traits	e
$\boldsymbol{\alpha_s}$	Leaf absorptivity to solar radiation	-	0.5	a
$\boldsymbol{\epsilon_l}$	Thermal emissivity of leaf	-	0.98	b
$\boldsymbol{\alpha_l}$	Leaf absorptivity to thermal radiation	-	ϵ_l	a

Table 4: Climate parameters

Symbol	Variable	Units	Value/ Source	Ref.
$\boldsymbol{T_a}$	Air temperature	K	Measured (see Methods)	
$\boldsymbol{Rh}$	Relative humidity	%	ERA5 (id: r)	f
$\boldsymbol{I_s}$	Global solar radiation on horizontal surface	Wm^{-2}	ERA5 (id: msdwswrf)	f
$\boldsymbol{I_{l,down}}$	Thermal radiation from the Sky	Wm^{-2}	ERA5 (id: msdwlwrf)	f

$\boldsymbol{T_g}$	Ground temperature	K	ERA5 (id: stl1)	f
$\boldsymbol{\epsilon_g}$	Thermal emissivity of the ground	-	0.97	
$\boldsymbol{U_{10}}$	Wind at 10m	ms^{-1}	ERA5 (ids: u10 and v10)	f
$\boldsymbol{fsr}$	Surface roughness	m	ERA5 (id: fsr)	f

Table 5: Radiation terms

Symbol	Variable	Units	Equation	Ref.
$\boldsymbol{R_{abs,solar}}$	Total absorbed solar radiation	Wm^{-2}	$\alpha_s I_s$	a
$\boldsymbol{R_{abs,therm}}$	Total absorbed thermal radiation	Wm^{-2}	$\alpha_l\left(I_{l,up} + I_{l,down}\right)$	a
$\boldsymbol{R_{em,therm}}$	Total emitted thermal radiation	Wm^{-2}	$2\ \epsilon_l\ \sigma\ T_l^4$	a,c
$\boldsymbol{I_{l,up}}$	Thermal radiation from the ground	Wm^{-2}	$\epsilon_g \sigma T_g^4$	a

Table 6: Conductance terms

Symbol	Variable	Units	Equation/value	Ref.
$\boldsymbol{g_{bh}}$	Boundary layer conductance to heat	ms^{-1}	$0.007\sqrt{\frac{U}{L}}$	ab
$\boldsymbol{g_{bw}}$	Boundary layer conductance to water vapor	ms^{-1}	$0.007\sqrt{\frac{U}{L}}$	bg
$\boldsymbol{g_w}$	Total water vapor conductance	ms^{-1}	$\frac{g_s\, g_{bw}}{g_s + g_{bw}}$	ab
$\boldsymbol{g_s}$	Stomatal conductance	ms^{-1}	$factor\ \cdot\ g_{s,max}$	a
$\boldsymbol{g_{HR}}$	Total thermal conductance	ms^{-1}	$g_{bh} + \frac{4\epsilon_l \sigma T_a^3}{\rho_a c_{pa}}$	a

Table 7: Standard equations and constants

Symbol	Variable	Units	Equation /value	Ref.
$\boldsymbol{U}$	Wind at 2 m	m	$U_{10}\frac{\log_e\left(\frac{2}{fsr}\right)}{\log_e\left(\frac{10}{fsr}\right)}$	h

$\boldsymbol{e_{sat}(T)}$	Saturation vapor pressure at temperature T (Kelvin)	kPa	$610.8\ \exp\left(\frac{17.27\ (T-273.15)}{T-35.85}\right)$	i
$\boldsymbol{D}$	Vapor pressure deficit	kPa	$e_{sat}\cdot\left(1-\frac{Rh}{100}\right)$	j
$\boldsymbol{s(T)}$	Slope of $e_{sat(T_l)} vs\ T$ at temperature T (Kelvin)		$2504484\ \frac{\exp\left(17.27\cdot\frac{T-273.15}{T-35.85}\right)}{(T-35.85)^2}$	a
$\boldsymbol{\rho_a}$	Density of air	kgm^{-3}	$1.2898-(T_a-273.15)\cdot 0.0041-(0.0001-(T_a-273.15)\cdot 0.0008)\cdot\frac{Rh}{100}$	a

Table 7: continued

Symbol	Variable	Units	Equation /value	Ref.
$\boldsymbol{\rho_a}$	Density of air	kgm^{-3}	$1.2898-(T_a-273.15)\cdot 0.0041-(0.0001-(T_a-273.15)\cdot 0.0008)\cdot\frac{Rh}{100}$	a
$\boldsymbol{\gamma}$	Psychrometric constant	PaK^{-1}	$64.904+0.0644\ \cdot(T_a-273.15)$	a
$\boldsymbol{\sigma}$	Stefan-Boltzmann Constant	$\frac{W}{m^2K^4}$	$5.670344\cdot 10^{-8}$	a,j
$\boldsymbol{c_{pa}}$	Specific heat of air	$\frac{J}{kgK}$	1010	a

[a]Jones, 2013; [b]Michaletz et al., 2016; [c]Campbell & Norman, 1998; [d]Leigh et al., 2017; [e]Franks & Beerling, 2009; [f]Hersbach et al., 2018; [g]Bonan, 2016; [h]Fleagle & Businger, 1980; Tennekes, H., 1973; [i]Murray, 1967; [j]Janka et al., 2016

References

Bonan, G. B. (2016). Ecological climatology: Concepts and applications (Third edition). Cambridge University Press.

Campbell, G. S., & Norman, J. M. (1998). Introduction to environmental biophysics (2nd ed). Springer.

Fleagle, R. G., & Businger, J. A. (1980). An introduction to atmospheric physics (2d ed). Academic Press.

Franks, P. J., & Beerling, D. J. (2009). Maximum leaf conductance driven by CO 2 effects on stomatal size and density over geologic time. Proceedings of the National Academy of Sciences, 106(25), 10343–10347. https://doi.org/10.1073/pnas.0904209106

Hersbach, H., Bell, B., Berrisford, P., Biavati, G., Horányi, A., Muñoz Sabater, J., Nicolas, J., Peubey, C., Radu, R., Rozum, I., Schepers, D., Simmons, A., Soci, C., Dee, D., & Thépaut, J.-N. (2018). ERA5 hourly data on single levels from 1940 to present [Computer software]. Copernicus Climate Change Service (C3S) Climate Data Store (CDS). 10.24381/cds.adbb2d47

Janka, E., Körner, O., Rosenqvist, E., & Ottosen, C.-O. (2016). A coupled model of leaf photosynthesis, stomatal conductance, and leaf energy balance for chrysanthemum (Dendranthema grandiflora). Computers and Electronics in Agriculture, 123, 264–274. https://doi.org/10.1016/j.compag.2016.02.022

Jones, H. G. (2013). Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology (3rd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9780511845727

Leigh, A., Sevanto, S., Close, J. d., & Nicotra, A. b. (2017). The influence of leaf size and shape on leaf thermal dynamics: Does theory hold up under natural conditions? Plant, Cell & Environment, 40(2), 237–248. https://doi.org/10.1111/pce.12857

Michaletz, S. T., Weiser, M. D., McDowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2016). The energetic and carbon economic origins of leaf thermoregulation. Nature Plants, 2(9), 16129. https://doi.org/10.1038/nplants.2016.129

Murray, F. W. (1967). On the Computation of Saturation Vapor Pressure. Journal of Applied Meteorology and Climatology, 6(1), 203–204. https://doi.org/10.1175/1520-0450(1967)

Tennekes, H. (1973). The Logarithmic Wind Profile. Journal of the Atmospheric Sciences. https://doi.org/10.1175/1520-0469(1973)

Supporting information S3.

References used to compile data for heat tolerance in woody species across the globe

Bigras, F.J., 2000. Selection of white spruce families in the context of climate change: heat tolerance. Tree Physiology, 20(18), pp.1227-1234.

Björkman, O. and Powles, S.B., 1984. Inhibition of photosynthetic reactions under water stress: interaction with light level. Planta, 161, pp.490-504.

Brennan, A.N., Uscola, M., Joly, R.J. and Jacobs, D.F., 2021. Cold and heat tolerances of hybrids for restoration of the endangered *Juglans cinerea* L. Annals of Forest Science, 78(2), pp.1-11.

Cook, A.M., 2021. Variation in the Photosynthetic Heat Tolerance of Plants from Extreme Environments: The Influence of Heat Exposure and Environmental Context. Doctoral dissertation, University of Technology Sydney, Australia.

Cunningham, S.C., Read, J., 2006. Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. Tree Physiology 26, 1435–1443. https://doi.org/10.1093/treephys/26.11.1435

French, K., Jansens, I.B., Ashcroft, M.B., Ecroyd, H., Robinson, S.A., 2019. High tolerance of repeated heatwaves in Australian native plants. Austral Ecology 44, 597–608. https://doi.org/10.1111/aec.12700

González-Rodríguez, Á.M., 1998. Caracterización fotosintética de árboles de la laurisilva canaria (*Laurus Azorica, Persea indica y Myrica faya*) Doctoral dissertation, Universidad de La Laguna ,Canary Islands, Spain.

González-Rodríguez, Á.M., Pérez-Martín, E.M., Brito, P., Fernández-Marín, B., 2021. Unexpected Vulnerability to High Temperature in the Mediterranean Alpine Shrub *Erysimum scoparium* (Brouss. ex Willd.) Wettst. Plants 10, 379. https://doi.org/10.3390/plants10020379

Guha, A., Vharachumu, T., Khalid, M.F., Keeley, M., Avenson, T.J., Vincent, C., 2022. Short-term warming does not affect intrinsic thermotolerance but induces strong sustaining photoprotection in tropical evergreen citrus genotypes. Plant, Cell & Environment 45, 105–120. https://doi.org/10.1111/pce.14215

Haldimann, P., Feller, U., 2004. Inhibition of photosynthesis by high temperature in oak (Quercus pubescens L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant, Cell & Environment 27, 1169–1183. https://doi.org/10.1111/j.1365-3040.2004.01222.x

Konôpková, A., Kurjak, D., Kmeť, J., Klumpp, R., Longauer, R., Ditmarová, Ľ., Gömöry, D., 2018. Differences in photochemistry and response to heat stress between silver fir (Abies alba Mill.) provenances. Trees 32, 73–86. https://doi.org/10.1007/s00468-017-1612-9

Kurjak, D., Konôpková, A., Kmeť, J., Macková, M., Frýdl, J., Živčák, M., Palmroth, S., Ditmarová, Ľ., Gömöry, D., 2019. Variation in the performance and thermostability of photosystem II in European beech (Fagus sylvatica L.) provenances is influenced more by acclimation than by adaptation. Eur J Forest Res 138, 79–92. https://doi.org/10.1007/s10342-018-1155-7

Larcher, W., J. Wagner, G. Neuner, M. Méndez, M.S. Jiménez and D. Morales. 1991. Thermal limits of photosynthetic function and viability of leaves of *Persea indica* and *Persea americana*. Acta Oecol. 12:529–541.

Martinazzo, E.G., Ramm, A., Bacarin, M.A., 2013. The chlorophyll a fluorescence as an indicator of the temperature stress in the leaves of Prunus persica. Braz. J. Plant Physiol. 24, 237–246. https://doi.org/10.1590/S1677-04202013005000001

Méthy, M., Gillon, D., Houssard, C., 1997. Temperature-induced changes of photosystem II activity in Quercus ilex and Pinus halepensis. Can. J. For. Res. 27, 31–38. https://doi.org/10.1139/x96-127

Münchinger, I.K., Hajek, P., Akdogan, B., Caicoya, A.T. and Kunert, N., 2023. Leaf thermal tolerance and sensitivity of temperate tree species are correlated with leaf physiological and functional drought resistance traits. Journal of Forestry Research, 34(1), pp.63-76.

Neuner, G. and Buchner, O., 2023. The dose makes the poison: the longer the heat lasts, the lower the temperature for functional impairment and damage. Environmental and Experimental Botany, 212, p.105395.

Offord, C.A., 2011. Pushed to the limit: consequences of climate change for the Araucariaceae: a relictual rain forest family. Annals of Botany 108, 347–357. https://doi.org/10.1093/aob/mcr135

Okubo, N., Inoue, S. and Ishii, H.R., 2023. Tolerance and Acclimation of the Leaves of Nine Urban Tree Species to High Temperatures. Forests, 14(8), p.1639.

Peñuelas, J., Llusià, J., 2002. Linking photorespiration, monoterpenes and thermotolerance in Quercus. New Phytologist 155, 227–237. https://doi.org/10.1046/j.1469-8137.2002.00457.x

Perera-Castro, A.V., Brito, P., González-Rodríguez, A.M., 2018. Changes in thermic limits and acclimation assessment for an alpine plant by chlorophyll fluorescence analysis: Fv/Fmvs. Rfd. Photosynthetica 56, 527–536. https://doi.org/10.1007/s11099-017-0691-6

Peters, J., Jiménez, M.S., Morales, D., 1999. Effect of Extreme Temperature on Quantum Yield of Fluorescence and Membrane Leakage of the Canarian Endemic Pine (Pinus canariensis). Zeitschrift für Naturforschung C 54, 681–687. https://doi.org/10.1515/znc-1999-9-1010

Petrik, P., Petek-Petrik, A., Konôpková, A., Fleischer, P., Stojnic, S., Zavadilova, I., Kurjak, D., 2023. Seasonality of PSII thermostability and water use efficiency of in situ mountainous Norway spruce (Picea abies). J. For. Res. 34, 197–208. https://doi.org/10.1007/s11676-022-01476-3

Rubio-Casal, A.E., Leira-Doce, P., Figueroa, M.E., Castillo, J.M., 2010. Contrasted tolerance to low and high temperatures of three tree taxa co-occurring on coastal dune forests under Mediterranean climate. Journal of Arid Environments 74, 429–439. https://doi.org/10.1016/j.jaridenv.2009.10.004

Ruter, J.M., 1993. Foliar Heat Tolerance of Two Hybrid Hollies. HortSci 28, 650–652. https://doi.org/10.21273/HORTSCI.28.6.650

Sastry, A., Barua, D., 2017. Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. Sci Rep 7, 11246. https://doi.org/10.1038/s41598-017-11343-5

Sastry, A., Guha, A., Barua, D., 2018. Leaf thermotolerance in dry tropical forest tree species: relationships with leaf traits and effects of drought. AoB PLANTS 10. https://doi.org/10.1093/aobpla/plx070

Shimada, A., YANG, X., Kozai, N. and Yamamoto, M., 2022. The effects of high temperature on the chlorophyll fluorescence in avocado (Persea americana Mill.) cultivars. Tropical Agriculture and Development, 66(3), pp.110-112.

Valliere, J.M., Nelson, K.C. and Martinez, M.C., 2023. Functional traits and drought strategy predict leaf thermal tolerance. Conservation Physiology, 11(1), p.coad085.

Yamada, M., Hidaka, T., Fukamachi, H., 1996. Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. Scientia Horticulturae 67, 39–48. https://doi.org/10.1016/S0304-4238(96)00931-4

Chapter 3

Early dehydration traits and its relationships to other plant functional traits in Mangroves

Abstract:

Even though the summer patchy dieback and large-scale events occur in the mangroves worldwide and alarming threats from changing climate, mangroves are overlooked mainly for climate change impacts. Globally, only a few mangrove species have been studied for their drought tolerance traits, and hardly any have been studied from Indian mangroves. This chapter explores the physiological mechanisms underlying drought tolerance in mangrove species through pressure-volume curve analyses. We aimed to understand how resistance to early dehydration (using Ψ_{TLP}, RWC_{TLP}) functions in mangroves. We found that both the Ψ_{TLP} and RWC_{TLP} varied significantly between species, suggesting different strategies in turgor regulation to early dehydration. We also studied their relationship to a) stomatal traits, b) the modulus of elasticity of the tissue, and c) leaf capacitance. Only the modulus of elasticity, cell wall rigidity, is positively correlated to RWC_{TLP}, suggesting that a rigid cell wall helps retain higher relative water content even at the turgor loss point. We also examined the relationship of early dehydration traits with other leaf morphological traits such as LMA, LDMC, SSD, and LA. Only Stem Specific Density positively correlated with Ψ_{TLP}; otherwise, none found any correlation with Ψ_{TLP} nor RWC_{TLP}. A dense stem supports the plant in retaining higher water potential at early dehydration and regulates turgor loss. We also studied if the resistance traits to early dehydration and the rate of water loss in these species correlate with their functionality in their natural habitat concerning leaf lifespan. We did not find any relationship between phenology traits and drought tolerance. These studied traits are ecologically important and will help for better understanding of mangrove response to changing climate and its consequences on costal ecosystems and blue carbon storage.

INTRODUCTION

The rising frequency and severity of drought episodes is a critical consequence of climate change, profoundly impacting global ecosystems, especially tropical forests. Research demonstrates that tropical ecosystems exhibit significant susceptibility to drought stress, resulting in alterations in species composition and extensive tree mortality in areas such as the Amazon (Allen et al., 2015; Bauman D et al., 2022; Choat et al., 2018; Hammond, W. M and Adams, H. D. 2019; Ma Q et al., 2023 & Phillips et al., 2009).

Predictive climate models indicate that drought episodes may exacerbate in the forthcoming decades, posing further challenges to plant resilience and adaptation (Dai, 2013; IPCC, 2021). Comprehending plant physiological reactions to drought, especially in ecosystems such as mangroves, is essential for forecasting the enduring effects of climate change on biodiversity.

The Water Potential at Turgor Loss Point (TLP) denotes the water potential at which plant cells experience a loss of turgor pressure and commence wilting (Bartlett et al., 2012). TLP is a critical hydraulic trait for understanding drought tolerance, as plants exhibiting lower TLP values are typically more resilient to drought conditions (Skelton et al., 2017). TLP characterises a plant's capacity to conserve water during stress, rendering it a crucial attribute for assessing plant viability in arid or drought-affected regions (Turner, 1986; Blackman et al., 2016). Understanding TLP in mangroves provides insights into how these species sustain hydration and cellular function under high environmental stressors.

Relative Water Content at Turgor Loss Point (RWC_{TLP}) is another important hydraulic trait used to understand plant drought tolerance. It represents the point at which plant cells can no longer maintain turgor pressure due to dehydration. Species exhibiting lower RWC_{TLP} values are more tolerant to water stress, hence offering RWC_{TLP} a key indicator of drought resistance (Bartlett et al., 2012; Maréchaux et al., 2015). Recent investigations, such as those by Trueba et al. (2019), demonstrated that dehydration precipitates sequential reductions in hydraulic function and stomatal conductance prior to the onset of photochemical damage. Trueba et al. (2019) demonstrate that hydraulic failure precedes more significant cellular and structural damage, indicating that RWC_{TLP} may serve as an early warning indicator of severe water stress prior to the onset of irreparable damage. This underscores the necessity of a comprehensive examination of RWC_{TLP}, since it directly impacts drought adaption techniques across various ecosystems (Sack et al., 2003; Gleason et al., 2018).

RWC_{TLP} is associated with numerous critical functional traits, including leaf hydraulic traits, leaf mass per area (LMA), and stomatal density (SD), which are crucial in managing water use efficiency of a plant (Trueba et al., 2019; John et al., 2013). The reduction in stomatal conductance seen in Trueba et al.'s study is closely associated with water-use efficiency, as evidenced by stomatal characteristics and leaf morphological traits. Mangrove species exhibiting higher Leaf Mass per Area (LMA) and Leaf Dry Matter Content (LDMC) generally demonstrate more conservative water use strategy and are predisposed to lower Relative Water Content at Turgor Loss Point (RWC_{TLP}), and enhanced resilience during prolonged water scarcity (Bartlett et al., 2016; Nguyen et al., 2017). Conversely, species exhibiting elevated stomatal conductance are more susceptible to rapid water loss, potentially resulting in higher RWC_{TLP} values and increased vulnerability to drought (Sack et al., 2016; Maréchaux et al., 2015).

Mangroves can withstand harsh coastal conditions such as high salinity, waterlogging, and intermittent droughts, which impose considerable stress (Ball, 1988; Parida & Jha, 2010). These ecosystems are adept at sustaining water balance, frequently employing osmotic adjustments, stomatal management, and efficient water utilisation techniques (Krauss et al., 2008). TLP is essential in the water management strategies of mangroves, aiding in the regulation of water loss and the maintenance of leaf turgor in saline and arid environments (Santini et al., 2019). Mangroves safeguard coastal areas and offer essential ecosystem services, such as carbon sequestration and shoreline stabilisation (Alongi, 2014). The recent die-back events in mangrove forests, particularly in Australia's Gulf of Carpentaria, highlight the susceptibility of these ecosystems to extended drought conditions (Duke et al., 2017). Such occurrences are frequently associated with hydraulic collapse and a lack of access to soil moisture, resulting in widespread tree mortality (Xu et al., 2020). Investigating TLP in mangroves enables researchers to understand species' responses to drought and pinpoint which are in greater danger. This understanding is crucial for formulating conservation measures to alleviate the effects of climate change on these vital coastal ecosystems (Doughty et al., 2015).

Leaf functional traits, including Leaf Area (LA), Leaf Mass per Area (LMA), and Leaf Dry Matter Content (LDMC), are essential for comprehending plant resilience in challenging conditions (Wright et al., 2004). LMA and LDMC offer insights into leaf resource investment, where elevated values often signify tougher leaves that exhibit greater resistance to water loss and physical damage (Poorter et al., 2009; Onoda et al., 2011). TLP is intricately associated with these characteristics, as plants exhibiting lower TLP values generally possess leaves with elevated LMA and LDMC, enabling prolonged turgor maintenance during drought circumstances (Bartlett et al., 2016; Blackman et al., 2016). Stomatal traits, such as stomatal density, guard cell dimensions, and maximum stomatal conductance (gwmax), are essential for understanding plant water loss and gas exchange (Franks et al., 2009). These characteristics affect a plant's capacity to equilibrate water retention with carbon assimilation, which is especially crucial for mangroves experiencing drought stress (Brodribb & McAdam, 2017). Research indicates that species exhibiting greater stomatal density and reduced guard cell size generally possess more effective water-use strategies, which correlates directly with diminished TLP values and improved drought tolerance (Franks & Farquhar, 2007; Buckley et al., 2012).

Specific Leaf Area (SLA) and leaf lifespan indicate a plant's strategy for resource distribution and durability (Reich et al., 1997). Specific leaf area (SLA), defined as the ratio of leaf area to leaf dry mass, exhibits an inverse relationship with leaf mass per area (LMA) and is frequently correlated with accelerated development and reduced leaf longevity (Poorter et al., 2009). Mangroves characterised by extended leaf lifespans generally have reduced SLA and elevated LMA, signifying a conservative resource-utilization strategy that facilitates diminished TLP values (Wright et al., 2004). The trade-off between rapid growth and drought tolerance is essential for comprehending the ecological strategies of various mangrove species (Reich et al., 1999).

Species-level variation in TLP offers significant insights into the ecological strategies of several mangrove species (Skelton et al., 2017). Certain species demonstrate reduced TLP values, indicating enhanced resilience to drought conditions, whilst others may be more susceptible to hydric stress (Bartlett et al., 2012). This variance in TLP may be associated with species-specific adaptations, including disparities in stomatal regulation, leaf morphology, or hydraulic structure (Choat et al., 2018; Maréchaux et al., 2015). The relationship between TLP and leaf functional attributes, including LMA, LDMC, and stomatal characteristics in mangroves, enhances comprehension of plant water-use strategies (Bartlett et al., 2016). Species exhibiting elevated LMA and LDMC values generally have reduced TLP, indicating that more resilient leaves are more effectively adapted for water conservation during drought conditions (Turner, 1986; Skelton et al., 2017). Traits such as stomatal density and guard cell size contribute to regulating water loss, with more stringent stomatal control linked to reduced TLP values (Franks et al., 2009).

Comprehending TLP and its association with other plant functional traits is significant for mangrove conservation (Xu et al., 2020). With the intensification of climate change, mangrove ecosystems are expected to encounter heightened drought stress, necessitating the identification of species that exhibit greater resilience to these conditions (Doughty et al., 2015). By comprehending the physiological characteristics that enhance drought tolerance, conservationists can formulate strategies to save at-risk species and maintain the ecological integrity of mangrove ecosystems (Alongi, 2014).

We measured leaf resistance to turgor loss to analyse species variations in response to mild dehydration. We quantified this in thirteen coexisting mangrove species from estuarine sedimentary islands in Ashtamudy Lake, located on the southwest coast of India. Alongside these data, we observed leaf phenology in situ and assessed numerous additional plant functional traits across all species. This enabled us to tackle the following questions:

1. How does resistance to early dehydration (using Ψ_{TLP}, RWC_{TLP}) function in mangroves?

2. Are they related to a) stomatal traits, b) the modulus of elasticity of the tissue, and c) leaf capacitance?

3. How are these measures related to other leaf traits such as LMA, LDMC, SSD, and LA?

4. Do the resistance measures to dehydration and the rate of water loss in these species correlate with their functionality in their natural habitat concerning leaf lifespan?

Table 2.1: List of all functional traits examined in the study, category, their abbreviations and their units. RWC expanded as Relative Water Content.

Traits		**Abbreviation**	**Unit**
a) Resistance (early dehydration)	RWC at Turgor Loss	RWC_{TLP}	%
	Water potential at the turgor loss	Ψ_{TLP}	MPa
b) Stomatal traits	Pore size	Pore size	mm
	Guard cell length	Guard size	mm
	Stomatal density	StomDen	mm^{-2}
	Stomatal Pore Index	SPI	
	Max. stomatal conductance – water	Log gwmax	mol m^{-2} s^{-1}
c) Capacitance MPa^{-1}	Capacitance per leaf area at full turgor	Log CFTabs	mol m^{-2}
	Relative capacitance at full turgor	Log CFTrelative	MPa^{-1}
	Relative capacitance - zero turgor	CTLP	MPa^{-1}
d) Leaf water status	Saturated water content	Log SWC	g g^{-1}
	Osmotic potential at full turgor	Ψ_O	MPa
e) Cell wall rigidity	Modulus of elasticity at full turgor	e	MPa
f) Morphological functional traits	Leaf area	Log LA	cm^{2}
	Leaf dry matter content	LDMC	g g^{-1}
	Leaf mass per area	Log LMA	g m^{-2}
	Specific stem density	SSD	g cm^{-3}
	Leaf Vein Density	LogDv	mm^{-2}
g) Phenology trait	Leaf Lifespan	Leaf Life	month

Materials and Methods:

Plant material:

We used thirteen true mangrove species from the estuarine sedimentary islands in the Ashtamudy backwaters in Kerala, Southwest coast of India. The site and species details have been described in detail in the previous chapter, materials and methods section. We used mature individuals for all measurements, and sun-exposed branches were collected for bench-drying assays.

Resistance to drought:

A bench drying assay was conducted to generate leaf pressure-volume curves for the mangroves in December-January 2021 (as in Sack and Pasquet-Kok 2011). Sun-exposed top canopy leaves were taken from five individuals of each species, rehydrated overnight, and subsequently allowed to air dry while measuring their weight (±0.01 mg) and water potential using a PMS pressure chamber (Model 1515D). As the leaf desiccated, we measured water potential at 0.2 to 0.3 MPa intervals until a water potential of -3 MPa was attained. The leaf was subsequently dried in an oven for 72 hours before measuring its dry weight, which was necessary for estimating its relative water content (RWC). These curves illustrate the variation in water potential in relation to changes in relative water content (RWC) and were utilised to estimate the water potential at the turgor loss point (Ψ_{TLP}), the RWC at the turgor loss point (RWC_{TLP}), modulus of elasticity, and capacitance.

Stomatal traits:

Pore size, Guard cell length, Stomatal density, Stomatal Pore Index, Max. Stomatal conductance – water was also measured to analyse the relationship with early dehydration traits in mangroves. Detailed methods for measuring these traits were described in the previous chapter.

Morphological functional traits:

Leaf area, Leaf dry matter content, Leaf mass per area, Leaf Vein Density and Specific stem density were also used in this study. Methods for measuring all traits except Stem Specific Density were described in the previous chapter. Stem specific density (SSD) was estimated from three different branches for six individuals per species. Segments greater than 5 cm in length and around 1-2 cm in diameter were collected from the base of 2nd or 3rd-order branches. The volume of these segments was estimated using the water-displacement method (Pérez et al. 2013). All these segments were subsequently oven-dried to constant weight (three to five days at 70°C), and the dry weight was quantified. SSD was calculated as the dry weight per volume (gm^{-3}).

Phenology:

Leaf phenology was quantified for 15-30 established and mature individuals of each species. The overall canopy of individuals was assessed through direct observation using a semiquantitative scale ranging from 0 to 100%, in increments of 10%. A value of zero signifies complete absence of foliage corresponds to a score of 0, whilst a score of 100 indicates a full canopy. Observations were performed monthly throughout a four-year duration from 2019 to 2021. We labelled and observed three branches for each individual and at least six individuals for each species. We labelled 10-15 leaves each branch, ensuring that a minimum of 180 leaves per species were tagged and monitored to track leaf lifespan. We documented the emergence and abscission of the leaves, and the average duration in months was computed and characterised as the species' leaf lifespan.

Data analysis

All models used for the response curves were fit using the R package ‘drc’ (Ritz and Streibig 2005). All measured and derived traits were tested for normality using the Shapiro-Wilk test and transformed when necessary. We examined variation in traits using nested analysis of variance, with species nested within leaf habit. Bivariate trait relationships were assessed using Pearson’s and Spearman’s correlation analysis. Multivariate trait associations were assessed using a principal component analysis (PCA). As all traits were not measured for all species, we restricted the PCA analysis to the key traits examined. We also excluded three species from the PCA since data for Stomatal traits were unavailable for them. We did ANOVA to find if the difference between species was significant, and whenever it was significant, we did Tukey’s Post-Hoc test to find pairwise differences. All analyses were conducted using R (version 4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) and Python 3 (Van Rossum, G., & Drake, F. L. (2009)).

Results:

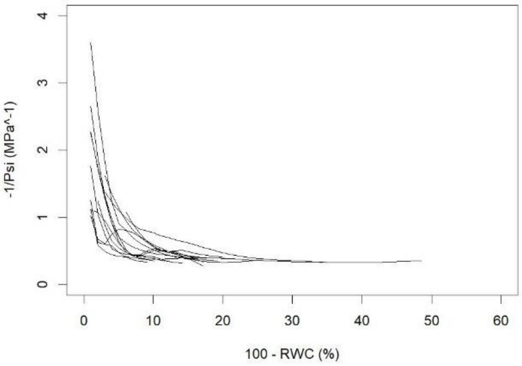


Figure 3.1A shows the Pressure-Volume curves for thirteen species that shows variation in response to early dehydration in mangroves. In Figure 3.1B, RWC at Turgor Loss Point (TLP) shows significant interspecific variability (F= 4.52, P***), with species like KC and BS maintaining higher RWC_{TLP}, indicating better water retention. In contrast, species such as AM show lower values. Similarly, water potential at TLP (Ψ_{TLP}) varies widely (F=3.44, P***), with RA and SC demonstrating lower Ψ_{TLP}, suggesting higher drought tolerance than species such as AO. Ψ_{TLP} ranged from -1.69 Mpa in SA to -2.26 in AO and RWC_{TLP} from 85.81% in CT to 97.79% in RA. Tukey's Post-Hoc test showed CT consistently differs in RWC_{TLP} from various other species (AM, BG, BS, KC, RA, RM), showing CT has a lower or greater value depending on the pair (Table S3). It also shows that SC significantly differed in Ψ_{TLP} from AC, AO and CT (Table S4).

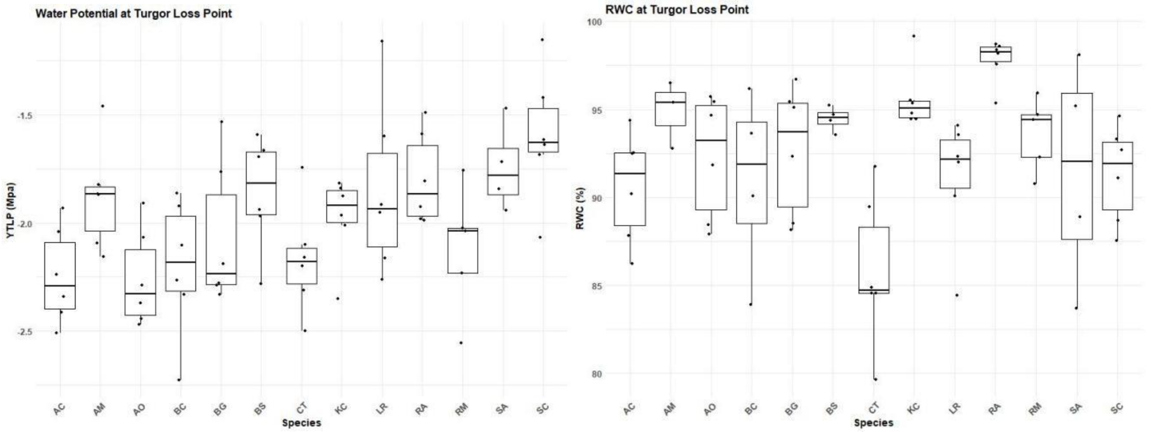


Figure 3.1: (A)Mangrove Pressure-Volume curves for all species. The curve displays the change in leaf water potential (-1/Ψ) with respect to leaf relative water content (RWC). Each line represents a species, which show how leaf water potential declines with relative water content. B) Box plot showing RWC at Turgor Loss Point (TLP) for each species, as medians and outliers. This plot displays 13 mangrove species' TLP RWC variation. The x-axis species abbreviations (AC, AM, AO, etc.) represent mangrove species with high RWC variability at TLP. Water Potential at Turgor Loss Point (ΨTLP) box plot for each species. The x-axis depicts species, while the y-axis displays ΨTLP (in MPa), suggesting drought tolerance techniques vary among species.

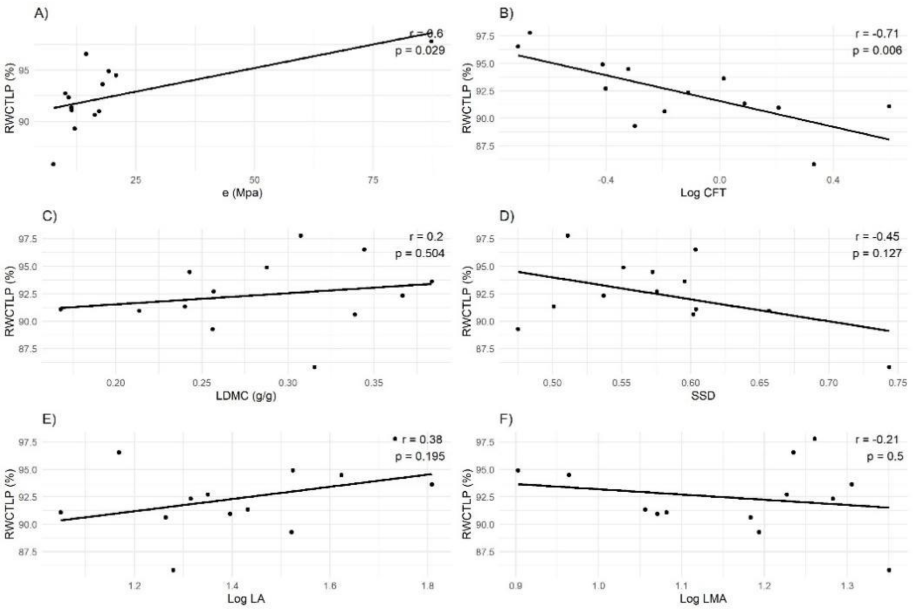


Figure 3.2. Correlation between RWC_{TLP} and key functional traits across mangrove species. Panel A shows the positive correlation between RWCTLP and "e". Panel B illustrates a significant negative correlation between RWCTLP and Log CFT. Panel C presents the non-significant correlation between RWC_{TLP} and LDMC. Panel D shows a moderate, yet non-significant, negative correlation between RWC_{TLP} and SSD. Panel E displays a non-significant positive correlation between RWC_{TLP} and Log LA, and Panel F shows no significant correlation between RWC_{TLP}. These findings highlight the varied relationships between RWC_{TLP} and key traits in mangrove species.

RWC_{TLP} and key functional traits across mangrove species showed mixed correlations. Modulus of elasticity (e) positively correlated with RWC_{TLP} (r = 0.6, p = 0.029), suggesting species with stronger elasticity had higher RWC_{TLP} values (Figure 3.2A). The negative association between $RWC_{TLP}$and Log CFT (Capacitance) showed that species with lower capacitance have greater RWC_{TLP} (r = -0.71, p = 0.006) (Figure 3.2B). RWC_{TLP}had no significant association with LDMC (r = 0.2, p = 0.504) (Figure 3.2C), or SSD (r = -0.45, p = 0.127) (Figure 3.2D), while SSD showed a substantial negative trend. Log LA had a non-significant positive connection with RWC_{TLP} (r = 0.38, p = 0.195) (Figure 3.2E), while Log LMA had no significant relation (r = -0.21, p = 0.5) (3.2F). Only elasticity and capacitance showed significant associations between RWC_{TLP} and key functional traits.

Ψ_{TLP} was negatively correlated with stem specific density (SSD) (r = -0.63, p = 0.022), showing that species with higher SSD have lower Ψ_{TLP} values. Denser wood may be more drought-tolerant due to turgor loss resistance. The "e" (r = 0.31, p = 0.304), LDMC (r = -0.36, p = 0.23), Log CFT (r = -0.34, p = 0.251), Log LA (r = 0.27, p = 0.374), and Log LMA (r = -0.32, p = 0.279) had weak or moderate correlations with Ψ_{TLP}, but none were statistically significant. These findings imply that SSD is critical for mangrove turgor loss point, although other features may indirectly affect Ψ_{TLP}.

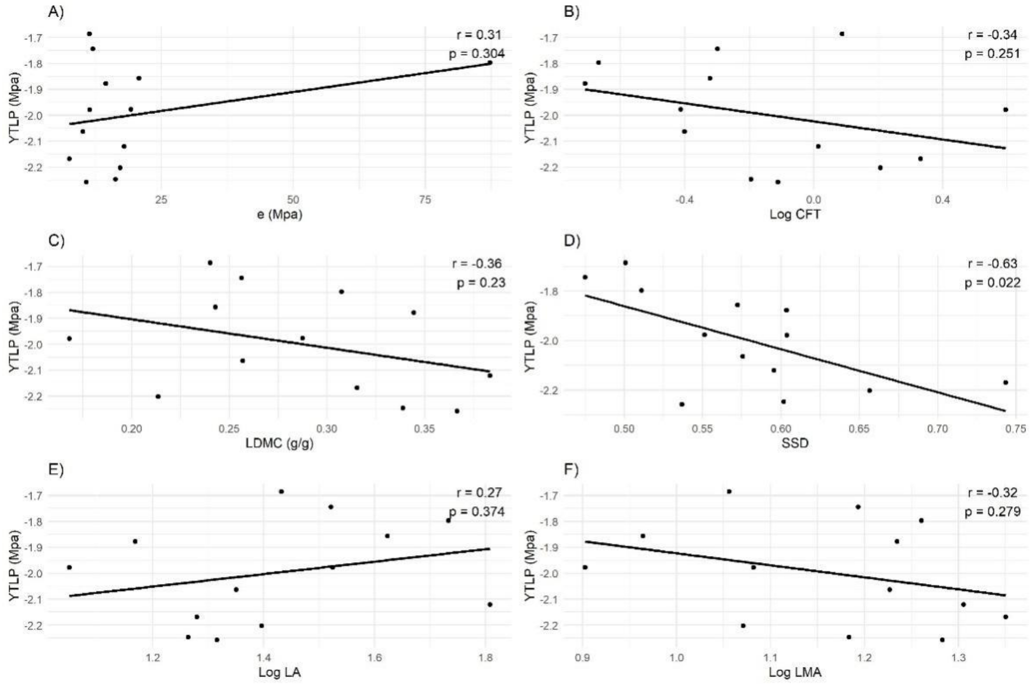


Figure 3.3: Correlation between Ψ_{TLP} key functional traits across mangrove species. Each panel shows the relationship between Ψ_{TLP} and: (A) modulus of elasticity (e, MPa), (B) Log CFT (Capacitance), (C) leaf dry matter content (LDMC, g/g), (D) stem specific density (SSD), (E) leaf area (Log LA), and (F) leaf mass per area (Log LMA). Correlation coefficients (r) and p-values are indicated in the top right corner of each panel.

The relationship between stomatal density and Ψ_{TLP} (Figure 3.4A) shows a weak negative correlation (r = -0.02, p = 0.944), indicating no significant association between stomatal density and water potential at the turgor loss point. Similarly, guard cell size (Figure 3.4B) also demonstrates a weak negative correlation with Ψ_{TLP} (r = -0.2, p = 0.556). Leaf lifespan (Figure 3.4C) shows a slight positive correlation with Ψ_{TLP} (r = 0.1, p = 0.741), but this is not statistically significant. However, maximum stomatal conductance (gw_max) (Figure 3.4D) presents a relatively strong positive correlation with Ψ_{TLP} (r = 0.59, p = 0.056), approaching statistical significance.

In the case of RWC_{TLP}, stomatal density (Figure 3.4E) exhibits a moderately strong positive correlation (r = 0.51, p = 0.113), though not statistically significant. Guard cell size (Figure 3.4F) shows a weak negative correlation with RWC_{TLP} (r = -0.3, p = 0.369), and leaf lifespan (Figure 3.4G) has a very weak negative correlation (r = -0.09, p = 0.767). Lastly, gw_max (Figure 3.4H) displays a moderate positive correlation with RWC_{TLP} (r = 0.36, p = 0.284), but this is not significant. These results suggest that stomatal traits have varying degrees of association with both Ψ_{TLP} and RWC_{TLP}, with gw_max showing the strongest correlations in both cases.

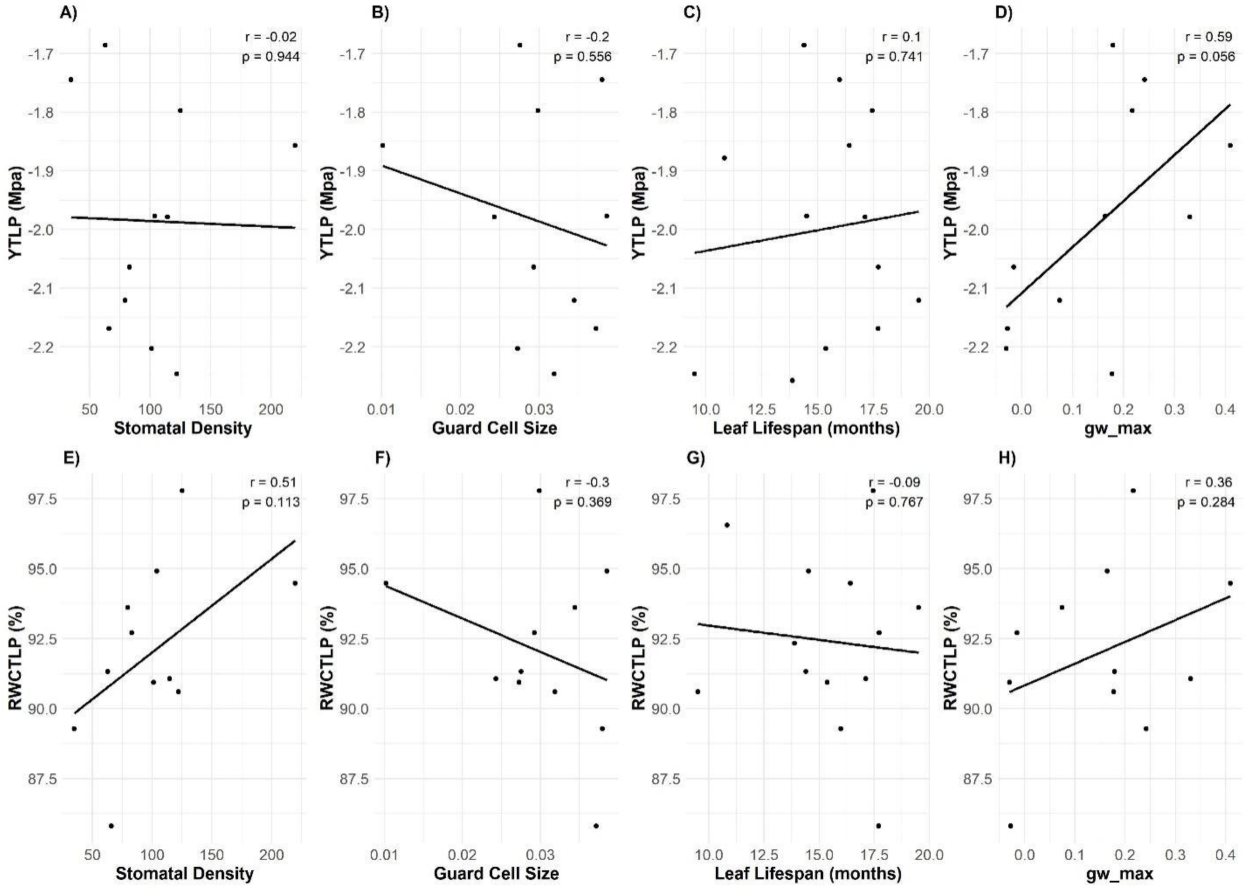


Figure 3.4: Correlations between stomatal traits and turgor loss points in mangrove species. Panels (A-D) display the relationships between Ψ_{TLP} and stomatal density (A), guard cell size (B), leaf lifespan (C), and maximum stomatal conductance (gw_max) (D). Panels (E-H) show relationships between RWC$_{TLP}$ and the same stomatal traits.

PCA showed multivariate correlations between functional traits in mangrove species. The biplots for PC1 vs PC2, PC1 vs PC3, and PC1 vs PC4 (Figure 3.5) show that various features substantially connect with the first two components. PC1, which accounts for most of the variation, is highly related with features including Log LA, Ψ_{TLP} , and RW$_{CTLP}$, indicating their major role in species differences in water-use strategies and drought tolerance. PC2, on the other hand, emphasises Guard Cell Pair Width, LDMC, and SSD, showing that structural leaf features underlie variation in this axis.

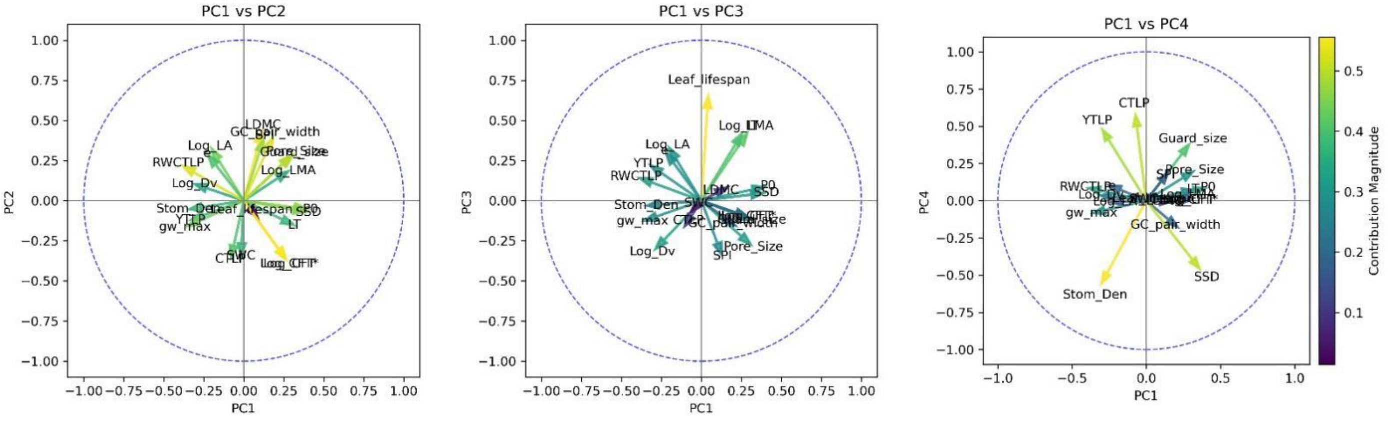


Figure 3.5: Principal Component Analysis (PCA) Biplots showing the relationships between plant functional traits and their contributions to the first four principal component axes of A) PC axes 1 and 2 and B) PC axes 1 and 3 C) PC axes 1 and 4. Solid arrows indicate the weighing and the direction of the traits considered. Trait abbreviations can be found in Table 1. Color intensity represents the magnitude of each trait's contribution to the component axes, with yellow indicating the highest contribution and dark blue the lowest. The dashed blue line represents the correlation circle boundary (unit length).

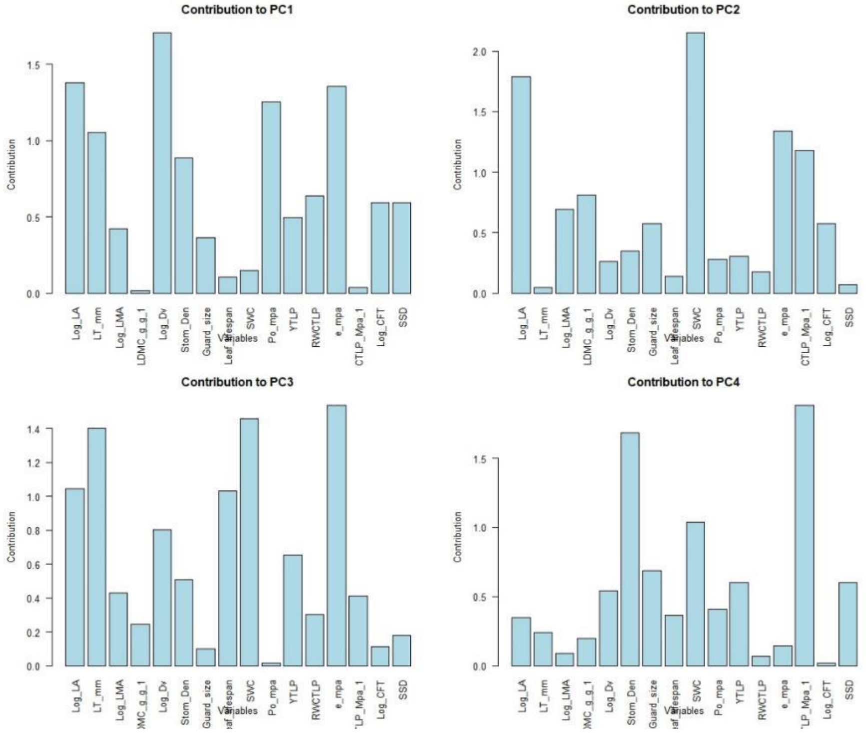


Figure 3.6: Contribution of plant functional traits to the first four principal component axes (PC1, PC2, PC3, and PC4). Bar plots show the relative contribution of each trait to the variation explained by each principal component.

The contribution bar plots (Figure 3.6) show that the first four dimensions explained 81.2 % of the total variations. Log LA and Ψ_{TLP} add the most to PC1, highlighting their importance in explaining variance. Guard Cell Pair Width and SSD dominate PC2, while PC3 is dominated by Ψ_{TLP} , Log gw_max, and Log LMA. PC4 captures variance primarily explained by Pore Size and Log CFT, demonstrating that hydraulic and stomatal features also contribute to species physiological differentiation. These PCA results indicate that structural and hydrodynamic features greatly influence mangrove functioning strategies.

Discussions:

The Ψ_{TLP} and RWC_{TLP} values recorded in this study exhibited considerable species-level variation, aligning with trends identified in prior research on other mangrove species (F. Rada et al., 1989; Herppich, W. B., & Willert, D. J., 1995; Beckett, H., et al., 2023). Notably, Ψ_{TLP} values in this study are mostly higher in comparison to those from mangroves in other regions globally. For example, we documented a Ψ_{TLP} of -1.88 MPa in *Avicennia marina*, the highest recorded to date, while prior investigations consistently indicated values below -4 MPa (Suarez and Subrado 2000, H. T. Nguyen et al. 2017, and Jiang et al. 2022), with one subspecies reaching -5.1 MPa (Nguyen et al. 2017). The RWC_{TLP} values in this study correspond closely with previous research for the majority of the species. Suarez and Sobrado (2000) reported RWC_{TLP} values of 88% in *Avicennia germinans*, which corresponds with our findings for CT (85.81%) and *Rhizophora apiculata* A (97.79%). Herppich and Willert (1995) documented RWC_{TLP} values of 86.7%.

The higher RWC_{TLP} values reported in species such as *Kandelia candel* and *Bruguiera sexangula* indicate superior water retention capabilities; prior research has demonstrated that species exhibiting greater RWC_{TLP} are more resilient to initial dehydration (Zhang et al., 2019; Choat et al., 2018). The notable disparities in Ψ_{TLP}, especially with *Rhizophora apiculata* and *Sonneratia caseolaris* exhibiting reduced Ψ_{TLP} values, underscore their enhanced drought tolerance, aligning with analogous trends observed in Rhizophora species across tropical environments (Trueba et al., 2019). The findings underscore the variety of drought tolerance methods among mangrove species, as noted by Field et al. (2017).

This work shows the correlation between RWC_{TLP} and key functional traits, suggesting the impact of plant water-use strategies and their structural adaptations. The positive correlation between RWC_{TLP} and modulus of elasticity (e) suggests that species exhibiting greater elasticity are likely to sustain higher RWC_{TLP} , indicating that elastic tissues assist in reducing water loss during drought conditions (Sack & Scoffoni, 2013; Zhu et al., 2021). The inverse association between RWC_{TLP} and Log CFT (capacitance) indicates that they undergo turgor loss at higher relative water content (Bartlett et al., 2012).Other morphological functional traits such as LDMC and SSD did not correlate with RWC_{TLP} , suggesting that leaf toughness and structural integrity may not significantly impact mangrove leaf turgor loss (Poorter et al., 2009). RWC_{TLP} and leaf size and mass could not predict drought response in tree species, according to Mencuccini et al. (2015). Species with higher SSD had a negative connection with Ψ_{TLP}, suggesting that denser tissues improve drought tolerance by maintaining cellular turgor (Markesteijn et al., 2011).

This study found mixed relationships between $\Psi_{TLP}$and RWC_{TLP} for stomatal traits such as stomatal density, guard cell size, and maximum stomatal conductance to water (gw_max). In Ψ_{TLP}; stomatal density and guard cell size exhibited weak or non-significant relationships, suggesting that these traits may not directly affect water potential at turgor loss in mangroves. Research by Sack and Buckley (2016) indicates that stomatal traits are frequently decoupled from regulating turgor loss point , particularly in species acclimated to high humidity or wet environments. Nonetheless, gw_max exhibited a more robust, but non-significant, positive connection with Ψ_{TLP}, indicating that increased stomatal conductance may facilitate water transfer, hence resulting in reduced Ψ_{TLP}values during drought stress (Blackman et al., 2016). In RWC_{TLP} , stomatal density exhibited a moderately positive correlation, while not statistically significant. This indicates that species exhibiting elevated stomatal density may maintain higher RWC_{TLP} , perhaps enhancing their regulation of water loss during dehydration. The absence of a significant association between RWC_{TLP} and other stomatal traits (guard cell size, leaf lifespan) suggests that these factors may not considerably influence turgor loss point regulation in the studied mangrove species. Similar findings in related studies suggest that the importance of stomatal traits in water retention may differ among various species and habitats (Martin-StPaul et al., 2017).

Conclusion:

In conclusion, we provided important insights into the turgor loss point and its associations with various functional traits in mangroves. This study was the first one with thirteen mangrove species examined for pressure-volume relations. The significant species-level variation in RWCTLP and ΨTLP demonstrates the diversity of drought response strategies across mangrove species. Key traits such as modulus of elasticity, capacitance, and stem specific density were found to be critical factors influencing turgor loss, while stomatal traits and other morphological traits showed mixed results. An increased stem specific density would cause an increased YTLP and hence lower tolerance to early dehydration. Cell wall rigidity is found to be associated with higher RWC retention at turgor loss point in mangroves. This well explained relatively higher RWC retentions at turgor loss in mangroves compared to other species. We further demonstrated the complex interactions between structural and hydraulic traits that underlie species' drought tolerance. These findings contribute to our understanding of how mangroves cope with water stress, with implications for conservation efforts in the face of rapidly changing climate.

References:

Allen, C. D., et al. (2015). Global change-induced tree mortality: A key driver of landscape evolution. *Bioscience*, 65(7), 680-682. https://doi.org/10.1093/biosci/biv062

Alongi, D. M. (2014). Carbon cycling and storage in mangrove forests. *Annual Review of Marine Science*, 6(1), 195-219. https://doi.org/10.1146/annurev-marine-010213-135020

Ball, M. C. (1988). Ecophysiology of mangroves. *Trees*, 2(3), 129-142. https://doi.org/10.1007/BF00196018

Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species. *Plant Physiology*, 158(2), 531-540. https://doi.org/10.1104/pp.111.18679

Bartlett, M. K., et al. (2016). Drought tolerance as a driver of tropical forest assembly: Resolving spatial signatures for multiple processes. *Ecology Letters*, 19(5), 451-462. https://doi.org/10.1111/ele.12570

Bauman, D., Fortunel, C., Delhaye, G., Murren, C. J., & Rejou-Mechain, M. (2022). Tropical tree mortality has increased with rising atmospheric water stress. *Nature, 608*, 528–533. https://doi.org/10.1038/s41586-022-04737-7

Beckett, H., et al. (2023). Ghosts of dry seasons past: Legacy of severe drought enhances mangrove salinity tolerance. *Plant, Cell and Environment*. https://doi.org/10.1111/pce.14604

Blackman, C. J., et al. (2016). The relationship between leaf turgor loss point and the degree of iso/anisohydry: Insights from a range of woody plant species. *Plant, Cell & Environment*, 39(10), 2012-2024. https://doi.org/10.1111/pce.12758

Brodribb, T. J., & McAdam, S. A. (2017). Evolution of stomatal regulation in non-vascular plants: Implications for early plant life on land. *Plant Physiology*, 174(2), 581-587. https://doi.org/10.1104/pp.17.00299

Buckley, T. N., et al. (2012). The control of stomata by water balance. *New Phytologist*, 198(1), 5-13. https://doi.org/10.1111/nph.12176

Choat, B., et al. (2018). Drought and tree mortality: An integrated perspective. *New Phytologist*, 219(1), 33-50. https://doi.org/10.1111/nph.14984

Dai, A. (2013). Increasing drought under global warming in observations and models. *Nature Climate Change*, 3(1), 52-58. https://doi.org/10.1038/nclimate1633

Doughty, C. E., et al. (2015). Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature*, 519(7541), 78-82. https://doi.org/10.1038/nature14213

Duke, N. C., et al. (2017). Large-scale dieback of mangroves in Australia's Gulf of Carpentaria: A severe ecosystem response, coincidental with an unusually extreme weather event. *Marine and Freshwater Research*, 68(10), 1816-1829. https://doi.org/10.1071/MF16322

F. Rada, et al. (1989). Structural and functional adaptations to water stress in two mangrove species. *Oecologia*, 80, 400-406. https://doi.org/10.1007/BF00379044

Franks, P. J., & Farquhar, G. D. (2007). The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology*, 143(1), 78-87. https://doi.org/10.1104/pp.106.091860

Franks, P. J., et al. (2009). Sensitivity of plants to changing atmospheric CO2 concentration: From the geological past to the next century. *New Phytologist*, 182(1), 12-28. https://doi.org/10.1111/j.1469-8137.2009.02735.x

Gleason, S. M., et al. (2018). Coordination between leaf, stem, and root hydraulics and gas exchange in guayule, a woody desert shrub. *Plant Physiology*, 176(4), 2407-2417. https://doi.org/10.1104/pp.17.01461

Hammond, W. M., & Adams, H. D. (2019). Dying on time: Traits influencing the dynamics of tree mortality risk from drought. *Tree Physiology, 39*(6), 906–909. https://doi.org/10.1093/treephys/tpz050

Herppich, W. B., & Willert, D. J. (1995). Leaf turgor loss point and osmotic adjustment in mangrove species under salinity stress. *Plant Physiology*, 96(1), 151–161. https://doi.org/10.1104/pp.96.1.151

IPCC. (2021). Climate change 2021: The physical science basis. Contribution of Working Group I to the Sixth *Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press*. https://doi.org/10.1017/9781009157896

John, G. P., et al. (2013). The functional coordination of leaf water transport properties is conserved across environmental gradients and tissue types. *Plant, Cell & Environment*, 36(5), 910-920. https://doi.org/10.1111/pce.12024

Jiang, L., et al. (2022). Hydraulic traits and drought response of mangroves: Insights from the water potential at turgor loss point. *Functional Ecology*, 36(2), 418-428. https://doi.org/10.1111/1365-2435.14044

Krauss, K. W., et al. (2008). Environmental drivers in mangrove establishment and early development: A review. *Aquatic Botany*, 89(2), 105-127. https://doi.org/10.1016/j.aquabot.2007.12.014

Ma, Q., Su, Y., Niu, C., Zhang, X., Cheng, L., Wang, X., & Liu, Z. (2023). Tree mortality during long-term droughts is lower in structurally complex forest stands. *Nature Communications, 14*, 7467. https://doi.org/10.1038/s41467-023-43083-8

Maréchaux, I., et al. (2015). Leaf drought tolerance cannot fully explain tree species distributions across the Amazon rainfall gradient. *Ecology Letters*, 18(12), 1290-1299. https://doi.org/10.1111/ele.12512

Markesteijn, L., et al. (2011). Hydraulics and life history of tropical dry forest tree species: Coordination of species' drought and shade tolerance. *New Phytologist*, 191(2), 480-495. https://doi.org/10.1111/j.1469-8137.2011.03708.x

Mencuccini, M., et al. (2015). Physiological basis of drought resilience in conifers: Hydraulic control of stomatal conductance, carbon balance and growth. *Plant, Cell & Environment*, 38(5), 682-695. https://doi.org/10.1111/pce.12450

Nguyen, H. T., et al. (2017). Variation in water potential at turgor loss point and other drought resistance traits among subspecies of *Avicennia marina*. *Tree Physiology*, 37(9), 1182-1191. https://doi.org/10.1093/treephys/tpx036

Onoda, Y., et al. (2011). Global patterns of leaf mechanical properties. *Ecology Letters*, 14(3), 301-312. https://doi.org/10.1111/j.1461-0248.2010.01582.x

Parida, A. K., & Jha, B. (2010). Salt tolerance mechanisms in mangroves: A review. *Trees*, 24(2), 199-217. https://doi.org/10.1007/s00468-010-0417-x

Pérez-Harguindeguy, N., et al. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), 167-234. https://doi.org/10.1071/BT12225

Phillips, O. L., et al. (2009). Drought sensitivity of the Amazon rainforest. *Science*, 323(5919), 1344-1347. https://doi.org/10.1126/science.1164033

Poorter, H., et al. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565-588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Sack, L., & Pasquet-Kok, J. (2011). Leaf pressure-volume curve parameters. In L. Sack, C. Scoffoni (Eds.), *Plant Ecophysiology Techniques Manual* (pp. 1-40). University of California, Los Angeles.

Sack, L., & Scoffoni, C. (2013). Leaf venation and its implications for drought tolerance. *Plant Physiology*, 162(1), 1705-1717. https://doi.org/10.1104/pp.113.221461

Santini, N. S., et al. (2019). Turgor loss point and water potential in mangrove species under contrasting salinity conditions. *Functional Plant Biology*, 46(7), 648-659. https://doi.org/10.1071/FP18363

Skelton, R. P., et al. (2017). Drought strategies and plant hydraulics shape species' drought tolerance across a tropical rainforest gradient. *Plant, Cell & Environment*, 40(2), 267-279. https://doi.org/10.1111/pce.12848

Suarez, N., & Sobrado, M. A. (2000). Adjustments in leaf water relations of *Avicennia germinans* to contrasting salinities. *Trees*, 14(3), 175-180. https://doi.org/10.1007/PL00009754

Trueba, S., et al. (2019). Thresholds for leaf damage due to dehydration: Declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *Plant, Cell & Environment*, 42(7), 1912-1925. https://doi.org/10.1111/pce.13524

38.Zhang, J. L., et al. (2019). The role of hydraulic traits in the drought resistance of mangrove species. *Journal of Plant Ecology*, 12(2), 228-239. https://doi.org/10.1093/jpe/rtz006

Zhu, S. D., et al. (2021). Hydraulic vulnerability and drought-induced plant mortality: Mechanisms, trends and management implications. *New Phytologist*, 229(1), 1125-1137. https://doi.org/10.1111/nph.16918

Supplementary figures and tables:

Supplementary figure S1: Species-level Pressure-Volume curves are shown for each individual sample.

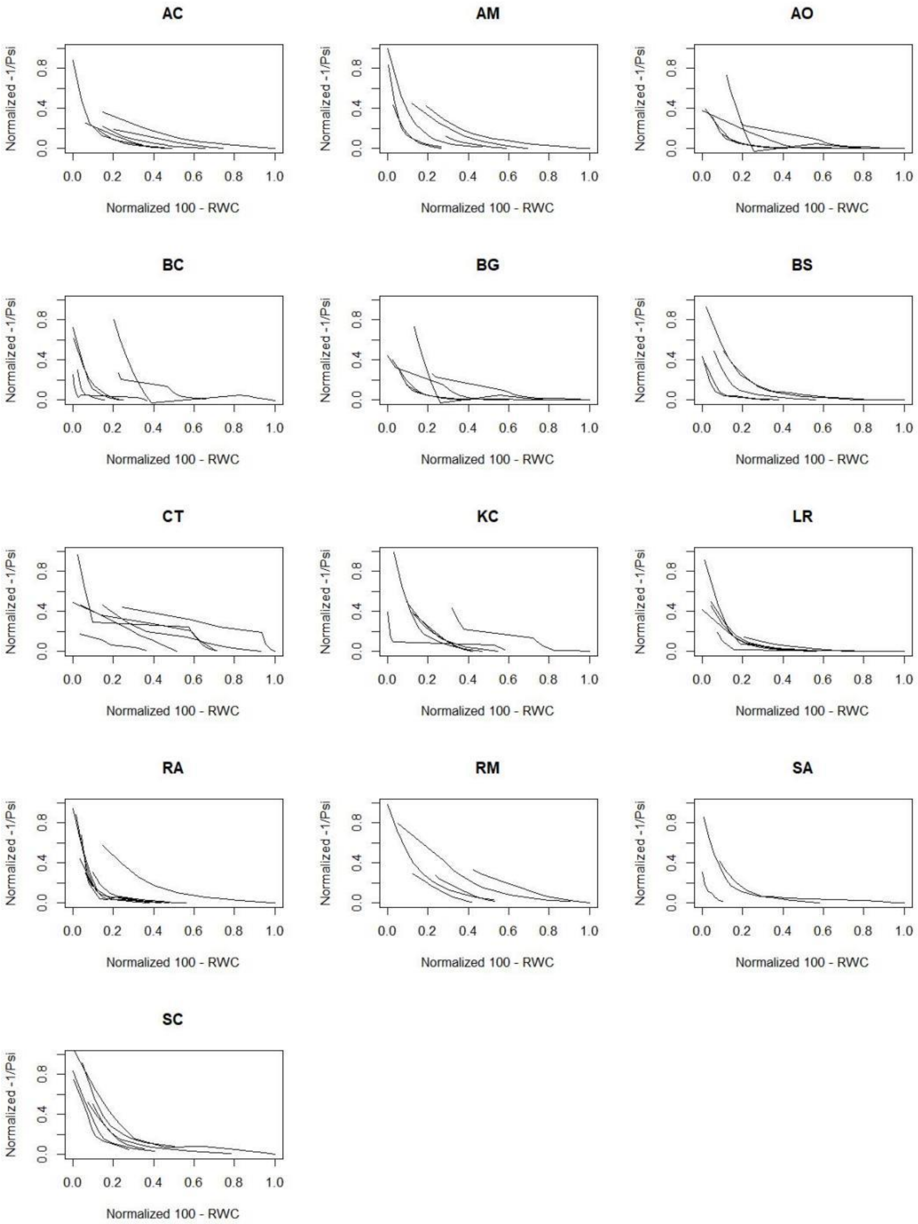
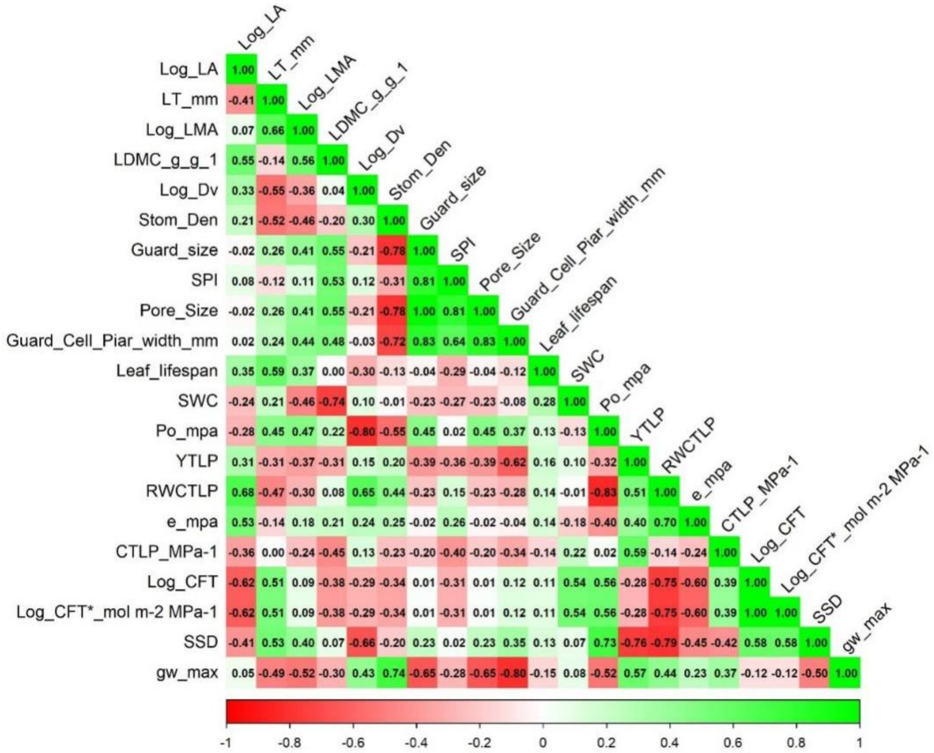


Figure S2: Pearson Correlation Matrix for Key Functional Traits and Turgor Loss Point Measures. The matrix illustrates Pearson correlation coefficients between key functional traits and measures of turgor loss points (YTLP and RWCTLP) in mangroves. Shades of green represent positive correlations, while negative correlations are shown in shades of red. The intensity of the colour corresponds to the strength of the correlation, with stronger relationships appearing in darker shades.



	Log_LA	LT_mm	Log_LMA	LDMC_g_g_1	Log_Dv	Stom_Den	Guard_size	SPI	Pore_Size	Guard_Cell_Piar_width_mm	Leaf_lifespan	SWC	Po_mpa	YTLP	RWCTLP	e_mpa	CTLP_MPa-1	Log_CFT	Log_CFT*_mol m-2 MPa-1	SSD	gw_max
Log_LA	1.00																				
LT_mm	-0.41	1.00																			
Log_LMA	0.07	0.66	1.00																		
LDMC_g_g_1	0.55	-0.14	0.56	1.00																	
Log_Dv	0.33	-0.55	-0.36	0.04	1.00																
Stom_Den	0.21	-0.52	-0.46	-0.20	0.30	1.00															
Guard_size	-0.02	0.26	0.41	0.55	-0.21	-0.78	1.00														
SPI	0.08	-0.12	0.11	0.53	0.12	-0.31	0.81	1.00													
Pore_Size	-0.02	0.26	0.41	0.55	-0.21	-0.78	1.00	0.81	1.00												
Guard_Cell_Piar_width_mm	0.02	0.24	0.44	0.48	-0.03	-0.72	0.83	0.64	0.83	1.00											
Leaf_lifespan	0.35	0.59	0.37	0.00	-0.30	-0.13	-0.04	-0.29	-0.04	-0.12	1.00										
SWC	-0.24	0.21	-0.46	-0.74	0.10	-0.01	-0.23	-0.27	-0.23	-0.08	0.28	1.00									
Po_mpa	-0.28	0.45	0.47	0.22	-0.80	-0.55	0.45	0.02	0.45	0.37	0.13	-0.13	1.00								
YTLP	0.31	-0.31	-0.37	-0.31	0.15	0.20	-0.39	-0.36	-0.39	-0.62	0.16	0.10	-0.32	1.00							
RWCTLP	0.68	-0.47	-0.30	0.08	0.65	0.44	-0.23	0.15	-0.23	-0.28	0.14	-0.01	-0.83	0.51	1.00						
e_mpa	0.53	-0.14	0.18	0.21	0.24	0.25	-0.02	0.26	-0.02	-0.04	0.14	-0.18	-0.40	0.40	0.70	1.00					
CTLP_MPa-1	-0.36	0.00	-0.24	-0.45	0.13	-0.23	-0.20	-0.40	-0.20	-0.34	-0.14	0.22	0.02	0.59	-0.14	-0.24	1.00				
Log_CFT	-0.62	0.51	0.09	-0.38	-0.29	-0.34	0.01	-0.31	0.01	0.12	0.11	0.54	0.56	-0.28	-0.75	-0.60	0.39	1.00			
Log_CFT*_mol m-2 MPa-1	-0.62	0.51	0.09	-0.38	-0.29	-0.34	0.01	-0.31	0.01	0.12	0.11	0.54	0.56	-0.28	-0.75	-0.60	0.39	1.00	1.00		
SSD	-0.41	0.53	0.40	0.07	-0.66	-0.20	0.23	0.02	0.23	0.35	0.13	0.07	0.73	-0.76	-0.79	-0.45	-0.42	0.58	0.58	1.00	
gw_max	0.05	-0.49	-0.52	-0.30	0.43	0.74	-0.65	-0.28	-0.65	-0.80	-0.15	0.08	-0.52	0.57	0.44	0.23	0.37	-0.12	-0.12	-0.50	1.00

Table S2. ANOVA results for Ψ_{TLP} (A) and RWC_{TLP} (B)across species.

A)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	12	3.110759	0.25923	3.44415	0.000658
Residuals	62	4.666538	0.075267	NA	NA

B)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	12	589.2785	49.10654	4.295581	8.94E-05
Residuals	55	628.7531	11.43187	NA	NA

Table S3 Tukey's Post-hoc test results for $\mathrm{RWC}_{\mathrm{TLP}}$

	diff	lwr	upr	p adj		diff	lwr	upr	p adj
AM-AC	4.295142	-3.98198	12.57227	0.842888	BG-BC	1.761141	-5.7948	9.317087	0.999802
AO-AC	1.718985	-5.03926	8.477228	0.99952	BS-BC	3.521425	-4.7557	11.79855	0.956124
BC-AC	0.339007	-7.21694	7.894953	1	CT-BC	-5.14026	-12.6962	2.415689	0.489179
BG-AC	2.100148	-4.6581	8.858392	0.996711	KC-BC	4.665297	-2.89065	12.22124	0.637985
BS-AC	3.860432	-3.69551	11.41638	0.856114	LR-BC	0.124886	-7.43106	7.680832	1
CT-AC	-4.80125	-11.5595	1.956994	0.420469	RA-BC	6.839354	-0.71659	14.3953	0.113056
KC-AC	5.004304	-1.75394	11.76255	0.356211	RM-BC	2.667337	-5.18503	10.51971	0.992721
LR-AC	0.463893	-6.29435	7.222137	1	SA-BC	0.532564	-7.74456	8.809688	1
RA-AC*	7.178361	0.420117	13.9366	0.027869*	SC-BC	0.38074	-7.17521	7.936686	1
RM-AC	3.006344	-4.08176	10.09445	0.957084	BS-BG	1.760284	-5.79566	9.31623	0.999803
SA-AC	0.871571	-6.68438	8.427517	1	CT-BG	-6.9014	-13.6596	-0.14315	0.041138
SC-AC	0.719747	-6.0385	7.477991	1	KC-BG	2.904155	-3.85409	9.662399	0.952858
AO-AM	-2.57616	-10.8533	5.700967	0.996664	LR-BG	-1.63626	-8.3945	5.121988	0.999708
BC-AM	-3.95613	-12.8965	4.984181	0.942078	RA-BG	5.078212	-1.68003	11.83646	0.334139
BG-AM	-2.19499	-10.4721	6.08213	0.999273	RM-BG	0.906195	-6.18191	7.994301	1
BS-AM	-0.43471	-9.37503	8.505606	1	SA-BG	-1.22858	-8.78452	6.327368	0.999996
CT-AM*	-9.09639	-17.3735	-0.81927	0.019397*	SC-BG	-1.3804	-8.13865	5.377842	0.999951
KC-AM	0.709162	-7.56796	8.986286	1	CT-BS*	-8.66168	-16.2176	-1.10574	0.011975*
LR-AM	-3.83125	-12.1084	4.445875	0.921574	KC-BS	1.143872	-6.41207	8.699818	0.999998
RA-AM	2.883219	-5.39391	11.16034	0.990962	LR-BS	-3.39654	-10.9525	4.159407	0.93549
RM-AM	-1.2888	-9.83738	7.259779	0.999998	RA-BS	3.317929	-4.23802	10.87387	0.945089
SA-AM	-3.42357	-12.3639	5.516745	0.980258	RM-BS	-0.85409	-8.70646	6.998281	1
SC-AM	-3.5754	-11.8525	4.701729	0.951081	SA-BS	-2.98886	-11.266	5.288263	0.987758
BC-AO	-1.37998	-8.93592	6.175969	0.999985	SC-BS	-3.14068	-10.6966	4.415261	0.962974
BG-AO	0.381164	-6.37708	7.139407	1	KC-CT*	9.805554	3.04731	16.5638	0.000387*
BS-AO	2.141447	-5.4145	9.697393	0.998613	LR-CT	5.265143	-1.4931	12.02339	0.281877
CT-AO	-6.52023	-13.2785	0.238009	0.06847	RA-CT*	11.97961	5.221367	18.73785	7.0E-06*
KC-AO	3.285319	-3.47292	10.04356	0.89292	RM-CT	7.807594	0.719488	14.8957	0.018912
LR-AO	-1.25509	-8.01333	5.503152	0.999982	SA-CT	5.672821	-1.88313	13.22877	0.335398
RA-AO	5.459376	-1.29887	12.21762	0.233353	SC-CT	5.520997	-1.23725	12.27924	0.219228
RM-AO	1.287359	-5.80075	8.375465	0.999986	LR-KC	-4.54041	-11.2987	2.217833	0.509005
SA-AO	-0.84741	-8.40336	6.708532	1	RA-KC	2.174057	-4.58419	8.932301	0.9955
SC-AO	-0.99924	-7.75748	5.759006	0.999999	RM-KC	-1.99796	-9.08607	5.090146	0.998683
SC-KC	-4.28456	-11.0428	2.473687	0.598904	RM-RA	-4.17202	-11.2601	2.916089	0.704203
RA-LR	6.714468	-0.04378	13.47271	0.053027	SA-RA	-6.30679	-13.8627	1.249156	0.193483
RM-LR	2.54245	-4.54566	9.630556	0.988422	SC-RA	-6.45861	-13.2169	0.29963	0.074119
SA-LR	0.407677	-7.14827	7.963624	1	SA-RM	-2.13477	-9.98714	5.717596	0.999072
SC-LR	0.255854	-6.50239	7.014097	1	SC-RM	-2.2866	-9.3747	4.801509	0.995384
					SC-SA	-0.15182	-7.70777	7.404122	1

Table S4 Tukey's Post-hoc test results for Ψ_{TLP}

	diff	lwr	upr	p adj		diff	lwr	upr	p adj
AM-AC	0.368684	-0.17698	0.914352	0.506669	LR-AO	0.415557	-0.13011	0.961225	0.31893
AO-AC	-0.01111	-0.55678	0.534558	1	RA-AO	0.460658	-0.08501	1.006326	0.182912
BC-AC	0.043979	-0.50169	0.589647	1	RM-AO	0.136923	-0.43538	0.709225	0.999763
BG-AC	0.182786	-0.36288	0.728454	0.993988	SA-AO	0.51355	-0.09653	1.123625	0.18619
BS-AC	0.389935	-0.15573	0.935603	0.417101	SC-AO*	0.660665	0.114997	1.206333	0.005827*
CT-AC	0.077736	-0.46793	0.623404	0.999999	BG-BC	0.138808	-0.40686	0.684475	0.999559
KC-AC	0.269937	-0.27573	0.815605	0.885374	BS-BC	0.345956	-0.19971	0.891624	0.605901
LR-AC	0.404447	-0.14122	0.950115	0.359877	CT-BC	0.033757	-0.51191	0.579425	1
RA-AC	0.449548	-0.09612	0.995216	0.211735	KC-BC	0.225958	-0.31971	0.771626	0.96586
RM-AC	0.125813	-0.44649	0.698114	0.999902	LR-BC	0.360468	-0.1852	0.906136	0.542419
SA-AC	0.50244	-0.10764	1.112515	0.212152	RA-BC	0.405569	-0.1401	0.951237	0.355621
SC-AC*	0.649555	0.103887	1.195223	0.007286*	RM-BC	0.081834	-0.49047	0.654136	0.999999
AO-AM	-0.37979	-0.92546	0.165873	0.459158	SA-BC	0.458461	-0.15161	1.068536	0.338855
BC-AM	-0.32471	-0.87037	0.220962	0.696586	SC-BC*	0.605576	0.059908	1.151244	0.01711*
BG-AM	-0.1859	-0.73157	0.35977	0.993028	BS-BG	0.207149	-0.33852	0.752816	0.982658
BS-AM	0.02125	-0.52442	0.566918	1	CT-BG	-0.10505	-0.65072	0.440617	0.999976
CT-AM	-0.29095	-0.83662	0.254719	0.823423	KC-BG	0.087151	-0.45852	0.632819	0.999997
KC-AM	-0.09875	-0.64442	0.44692	0.999988	LR-BG	0.221661	-0.32401	0.767329	0.970473
LR-AM	0.035762	-0.50991	0.58143	1	RA-BG	0.266762	-0.27891	0.81243	0.893407
RA-AM	0.080864	-0.4648	0.626532	0.999999	RM-BG	-0.05697	-0.62927	0.515328	1
RM-AM	-0.24287	-0.81517	0.32943	0.959064	SA-BG	0.319654	-0.29042	0.929729	0.839641
SA-AM	0.133755	-0.47632	0.743831	0.999905	SC-BG	0.466769	-0.0789	1.012437	0.168351
SC-AM	0.280871	-0.2648	0.826538	0.855007	CT-BS	-0.3122	-0.85787	0.233469	0.746802
BC-AO	0.055089	-0.49058	0.600757	1	KC-BS	-0.12	-0.66567	0.42567	0.999901
BG-AO	0.193897	-0.35177	0.739565	0.989977	LR-BS	0.014512	-0.53116	0.56018	1
BS-AO	0.401045	-0.14462	0.946713	0.372935	RA-BS	0.059613	-0.48605	0.605281	1
CT-AO	0.088846	-0.45682	0.634514	0.999996	RM-BS	-0.26412	-0.83642	0.30818	0.9264
KC-AO	0.281047	-0.26462	0.826715	0.854482	SA-BS	0.112505	-0.49757	0.72258	0.999985
SC-BS	0.25962	-0.28605	0.805288	0.910168	SA-KC	0.232503	-0.37757	0.842578	0.982104
KC-CT	0.192201	-0.35347	0.737869	0.990699	SC-KC	0.379618	-0.16605	0.925286	0.459904
LR-CT	0.326711	-0.21896	0.872379	0.688265	RA-LR	0.045101	-0.50057	0.590769	1
RA-CT	0.371812	-0.17386	0.91748	0.493175	RM-LR	-0.27863	-0.85094	0.293667	0.896094
RM-CT	0.048077	-0.52422	0.620379	1	SA-LR	0.097993	-0.51208	0.708068	0.999997
SA-CT	0.424704	-0.18537	1.034779	0.458854	SC-LR	0.245108	-0.30056	0.790776	0.938725
SC-CT*	0.571819	0.026151	1.117487	0.031753*	RM-RA	-0.32374	-0.89604	0.248566	0.760364
LR-KC	0.13451	-0.41116	0.680178	0.999679	SA-RA	0.052892	-0.55718	0.662967	1
RA-KC	0.179611	-0.36606	0.725279	0.994855	SC-RA	0.200007	-0.34566	0.745675	0.986992
RM-KC	-0.14412	-0.71643	0.428177	0.999601	SA-RM	0.376627	-0.25738	1.010636	0.698885
SC-RM	0.523742	-0.04856	1.096043	0.105131	SC-SA	0.147115	-0.46296	0.75719	0.999743

Table S5. Species mean values for all functional traits used in this study, refer to Table 1 for units and expansions.

Species	**Log_LA**	**LT_mm**	**Log_LMA**	**LDMC**	**Vein_Density**	**Stom_Den**	**Guard_size**
Aegiceras corniculatum	1.26	0.42	1.18	0.34	7.351183	122.08	0.03
Avicennia marina	1.17	0.47	1.23	0.34	5.415075	NA	NA
Avicennia officinalis	1.32	0.50	1.28	0.37	5.894208	NA	NA
Bruguiera cyindrica	1.40	0.58	1.07	0.21	6.390092	101.08	0.03
Bruguiera gymnorhiza	1.35	0.72	1.23	0.26	5.68845	82.92	0.03
Bruguiera sexangula	1.62	0.37	0.96	0.24	6.351542	219.86	0.01
Ceriops tagal	1.28	0.76	1.35	0.32	3.715388	65.96	0.04
Kandelia candel	1.52	0.39	0.90	0.29	6.50863	103.82	0.04
Luminitzera racemosa	1.05	0.73	1.08	0.17	6.517492	114.46	0.02
Rhizophora apiculata	1.73	0.56	1.26	0.31	6.629497	125.02	0.03
Rhizophora mucronata	1.81	0.60	1.31	0.38	7.401894	79.42	0.03
Sonnerata caseolaris	1.52	0.60	1.19	0.26	7.854275	34.65	0.04
Sonneratia alba	1.43	0.47	1.06	0.24	6.369953	62.94	0.03
	SPI	Pore_Size	GC_Piar_widt	Leaf_lifespan	SWC	Po	Log_gwmax
Aegiceras corniculatum	0.12	0.03	0.02	9.50	0.16	-1.29	0.18
Avicennia marina	NA	NA	NA	10.83	0.35	-1.15	NA
Avicennia officinalis	NA	NA	NA	13.88	0.37	-1.57	NA
Bruguiera cyindrica	0.08	0.02	0.02	15.37	0.79	-1.25	-0.03
Bruguiera gymnorhiza	0.07	0.02	0.01	17.72	0.37	-1.36	-0.02
Bruguiera sexangula	0.02	0.01	0.01	16.42	0.45	-1.41	0.41
Ceriops tagal	0.09	0.03	0.02	17.70	0.35	-0.73	-0.03
Kandelia candel	0.15	0.03	0.02	14.52	0.54	-1.33	0.16
Luminitzera racemosa	0.07	0.02	0.01	17.12	0.79	-1.32	0.33
Rhizophora apiculata	0.11	0.02	0.02	17.45	0.36	-1.42	0.22
Rhizophora mucronata	0.09	0.03	0.02	19.52	0.44	-1.23	0.07
Sonnerata caseolaris	0.05	NA	NA	15.98	0.46	-1.24	0.24
Sonneratia alba	0.05	0.02	0.01	14.40	0.49	-1.16	0.18
	YTLP	RWCTLP	e	CTLP	Log_CFT	Log_CFT*	SSD
Aegiceras corniculatum	-2.25	90.61	16.32	0.04	-0.19	-0.19	0.60
Avicennia marina	-1.88	96.55	14.50	0.02	-0.71	-0.71	0.60
Avicennia officinalis	-2.26	92.33	10.81	0.04	-0.11	-0.11	0.54
Bruguiera cyindrica	-2.20	90.95	17.21	0.02	0.21	0.21	0.66
Bruguiera gymnorhiza	-2.06	92.71	10.11	0.04	-0.40	-0.40	0.58
Bruguiera sexangula	-1.86	94.47	20.79	0.04	-0.32	-0.32	0.57
Ceriops tagal	-2.17	85.81	7.57	0.03	0.33	0.33	0.74
Kandelia candel	-1.98	94.91	19.24	0.03	-0.41	-0.41	0.55
Luminitzera racemosa	-1.98	91.07	11.43	0.10	0.60	0.60	0.60
Species	**YTLP**	**RWCTLP**	**e**	**CTLP**	**Log_CFT**	**Log_CFT***	**SSD**
Rhizophora apiculata	-1.80	97.79	87.28	0.03	-0.67	-0.67	0.51
Rhizophora mucronata	-2.12	93.61	17.97	0.03	0.01	0.01	0.60
Sonnerata caseolaris	-1.74	89.27	12.06	0.18	-0.30	-0.30	0.47
Sonneratia alba	-1.69	91.33	11.38	0.14	0.09	0.09	0.50

Chapter 4

Mangrove leaf photosynthetic response to elevated Carbon dioxide (CO_2) and its relationship with other functional traits.

Abstract:

Mangroves are essential to coastal ecosystems, offering ecosystem services including carbon sequestration, coastal protection, livelihood, recreation and habitat for wildlife. Despite their ecological importance, their response to environmental factors is largely overlooked in mangrove species, especially in the context of climate change. This study aimed to examine the response of twelve true mangrove species to elevated CO_2 concentrations (A/Ci curves) from the southwestern Indian coast. The study also investigated the correlations between photosynthetic parameters (Amax, Vcmax, Jmax, Rd) and some key functional traits (LMA, LDMC, LA, stomatal attributes, SSD). Measurements of CO_2 response were performed on mature plants from mangrove islands in the Ashtamudy backwaters estuary using an Infra-Red Gas Analyser. Our experiments revealed considerable species-level variation in Amax, Vcmax, and Jmax, with *Avicennia marina* and *Sonneratia caseolaris* were displaying the highest photosynthetic capacity, whereas *Sonneratia alba* exhibited the lowest. Nevertheless, no substantial associations were found between photosynthetic parameters and leaf morphological or stomatal traits across species. The results offer novel insights into the potential responses of mangroves to environmental alterations, including increased atmospheric CO_2, and underscore the necessity for more targeted research on mangrove ecophysiology. This research enhances the comprehension of mangrove carbon dynamics and its function in rapidly changing climate scenario-.

Introduction:

The mangrove ecosystems are one of the most important habitats on earth, providing many ecosystem services, including carbon sequestration, coastal protection, peoples' livelihood, aesthetic services and support for biodiversity. They are found in tropical to subtropical intertidal zones. They are uniquely adapted to withstand the threatening conditions of high salinity, high irradiance, heavy atmospheric humidity, water logging and low oxygen availability in the soil (Osland M.J. et al., 2022, Afonso F et al., 2021 & Alongi 2002). Mangroves play a crucial role in global carbon cycling, as they store enormous amounts of carbon both above and below ground, making them unavoidable in the fight against climate change (Donato et al., 2011). Due to their strong ability to capture and store atmospheric carbon even under harsh conditions, mangrove ecosystems act as critical blue carbon sinks, with their carbon sequestration rates surpassing those of terrestrial forests (Kauffman et al., 2017).

The photosynthetic process in mangroves is often can be constrained by factors such as salinity, which can limit the availability of water for transpiration and reduce the overall photosynthetic rate (Ball, 1988). However, research has shown that mangroves exhibit particular adaptations and physiological strategies to cope with the numerous environmental stresses they are under (Gilman.E.L. et al., 2007, Odum & Johannes, 1979 & Ball, 1988.) which includes osmotic adjustment and alterations in stomatal conductance to maintain photosynthetic efficiency (Medina & Francisco, 1997). These multiple adaptations that mangroves have evolved allow them to sustain photosynthesis even under prolonged exposure to saline and waterlogged conditions, although at the cost of reduced growth and productivity compared to non-saline environments (Gilman. E.L. et al., 2007 & Ball, 1988).

Assimilation to intercellular CO_2 concentration (A/Ci) curve is a widely used and potent tool in plant ecophysiology, which helps in understanding the photosynthetic performance of plants to increasing CO_2 levels (Sharkey et al., 2007; Bernacchi et al., 2003 &Wullschleger, 1993). Researchers have been using this method to estimate key photosynthetic parameters, such as the maximum rate of carboxylation by the enzyme Rubisco (Vcmax), the maximum rate of electron transport (Jmax), and the light-saturated rate of net CO_2 assimilation (Amax) (Farquhar et al., 1980; Sharkey et al., 2007; Long & Bernacchi, 2003& Flexas et al., 2006).

These parameters, Amax, Vcmax and Jmax are crucial in understanding how efficiently plants assimilate carbon and balance water use efficiency under different environmental conditions (Farquhar et al., 1980; Bernacchi et al., 2003 & Sharkey et al., 2007). Studies on mangroves and other coastal plants have highlighted the importance of these photosynthetic traits in determining their ability to thrive in fluctuating environments with high salinity, nutrient limitations, and tidal fluctuations (Lovelock et al., 2006; Reef et al., 2016 & Ball, 1988).

The Amax; the maximum photosynthetic assimilation rate, is an indicator of a species' capacity for carbon gain under optimal conditions (Farquhar et al., 1980; Sharkey et al., 2007; Long & Bernacchi, 2003; Ball & Critchley, 1982). Like many other physiological trait values in mangrove species, Amax values are often influenced by environmental factors such as salinity, temperature, and nutrient availability (Ball, 1988; Naidoo, 2006; Reef et al., 2016). Similarly, Vcmax and Jmax reflect the biochemical limitations to photosynthesis, with Vcmax indicating the capacity for carbon fixation by Rubisco and Jmax representing the electron transport capacity, two rate-controlling processes in photosynthesis (Farquhar et al., 1980; Sharkey et al., 2007; Flexas et al., 2006). In mangroves, these parameters vary between species depending on their level and differences in adaptations to waterlogged and saline conditions (Lovelock et al., 2006; Reef et al., 2016 & Naidoo, 2006). The variability in these photosynthetic parameters provides valuable insights into the functional and ecological strategies of mangroves and their potential responses to future climate change, including elevated atmospheric CO_2 and atmospheric temperature (Reef et al., 2016; Lovelock et al., 2006).

Leaf functional traits, like Leaf Mass per Area (LMA), Leaf Dry Matter Content (LDMC), and Leaf Area (LA), are frequently regarded as critical markers of a plant's photosynthetic potential and ecological strategy. The correlation between these features and photosynthetic measures such as Amax, Vcmax, and Jmax differs among various plant species and habitats (Poorter et al., 2009; Wright et al., 2004; Shipley et al., 2006). Generally, species exhibiting greater leaf mass per area (LMA) are associated with lower photosynthetic rates, since thicker and denser foliage often correlates with reduced carbon assimilation yet extended leaf longevity (Wright et al., 2004; Poorter & de Jong, 1999; Reich et al., 1997). This trade-off is most apparent under stressful conditions, where increased LMA facilitates enhanced resource conservation, but at the expense of diminished photosynthetic capacity (Niinemets, 2001 & Shipley et al., 2006).

There were reports on negative correlation between leaf mass per area (LMA) and photosynthetic capability spans various ecosystems, including tropical forests, temperate forests, and grasslands (Wright et al., 2004; Niinemets, 2001; Reich et al., 1999). Nevertheless, certain research indicate that this relationship is not consistently robust across various habitats, including mangroves and other coastal vegetation (Ball, 1988; Lovelock et al., 2006; Naidoo, 2006). In mangroves, variables such as salinity and waterlogging can independently affect leaf structure and function. This resulting in weaker or no correlations between leaf mass per area (LMA) and photosynthetic attributes (Ball, 1988; Naidoo, 2006). Leaf Area (LA) and Leaf Dry Matter Content (LDMC) frequently exhibit a positive correlation with photosynthetic rates in species that highlight rapid development and carbon acquisition; nevertheless, these correlations can vary significantly over diverse environmental gradients (Wright et al., 2004; Poorter et al., 2009). In mangroves, leaf functional features may be influenced more by environmental stressors than by trade-offs directly associated with photosynthesis (Ball, 1988; Reef et al., 2016).

Stomatal traits such as stomatal density, pore size and stomatal conductance to water (gsw), are essential factors influencing photosynthetic capability, as they control CO_2 absorption and water loss (Franks & Beerling, 2009; Hetherington & Woodward, 2003). Increased stomatal density and enlarged pore diameters enhance CO_2 diffusion into the leaf, increasing Amax, especially when water availability is not restricted (Lawson & Blatt, 2014; Flexas et al., 2012; Wong et al., 1979). In mangroves, stomatal traits are mostly restricted by salt and waterlogging, potentially hindering their ability to regulate gas exchange and water loss (Naidoo, 2006; Lovelock et al., 2006). Research indicates that stomatal conductance is diminished in high-salinity environments, such as mangrove ecosystems, leading to decreased photosynthesis (Ball, 1988; Naidoo, 2006; Lovelock et al., 2006). In today's pressing climate change scenarios, mangroves face severe threats, including rising sea levels, increasing atmospheric temperatures, rising water temperatures in sea and estuaries and more frequent extreme weather events; these situations can severely impact their survival and productivity (Alongi, 2015; Saintilan et al., 2020). Despite their crucial role in coastal protection and carbon sequestration, global attention to the ecophysiology of mangroves still needs to be expanded, with most research concentrated on Australian mangroves (Ball, 1988; Lovelock et al., 2006). Southeast Asia, Africa, and South America, are largely overlooked for studies on mangrove responses to environmental factors (Duke et al., 2007; Reef et al., 2016). As climate change accelerates, there is an urgent need for more comprehensive research to understand how mangroves globally will respond to these changes (Alongi, 2015; Saintilan et al., 2020; Lovelock et al., 2006) and an immediate special need for the Indian coastal regions. Thus, the current study address the following questions as CO_2 response experiment:

1) How will mangroves from the southwest Indian coast respond to elevated CO_2 levels?
2) How do photosynthetic capacity and rate vary in elevated CO_2 conditions?
3) How are photosynthetic traits in mangroves associated with leaf functional traits, including morphological, stomatal and phenology traits?

Materials and Methods:

Plant material/sampling strategy

This study selected the same individuals which were used for other trait measurements and experiments in this study. The in-situ measurement of photosynthetic traits was carried in estuarine mangrove sedimentary islands in the Ashtamudy backwaters; a detailed description of the study site has been given in chapter 2. This study sampled adult healthy mangrove trees between 08:00 to 15:00 h during the day. The campaign is being conducted coinciding with the initial summer period. This study chose fully open, mature, healthy, sun exposed leaves 1-2 meters above water level for all measurements. It was ensured that leaves were not touched, not cleaned or with dirt/dust/insect disturbance. Also ensured that the peripheral branches etc were in intact conditions, as cutting or damaging them could have affect the xylem flow and possibly stomatal response. When trees inaccessible due to dense prop root growth, peripheral trees were sampled (Eg: *Rhizophora apiculata*). For each species, sampled at least three individuals and all of them were under continuous monitoring for phenology observations for last four years, these trees were tagged for future reference and possible repeat measurements.

Leaf Trait Measurements:

This study followed the protocols from Perez H et al., (2013) for the leaf trait measurements as already detailed in the previous chapters; no new traits were used in this chapter.

Photosynthesis measurements:

A reconnaissance measurement exercise using a Li-6800 with a light source chamber (Li-6800-02) conducted during the end of February 2021 revealed that the site experiences ~80% relative humidity throughout the day and a narrow diurnal air temperature range (minimum temperature of 26–27 °C and maximum temperature of 31–33°C). Thus, the ecosystem here is interestingly functions within a narrow threshold of temperature and humidity and the ranges were not changed much throughout the summer.

It must be noted that a) during most parts of the day, the air temperature is often above 30°C and the dewpoint temperature of >30°C. b) The irradiance levels during the peak of the day were about 1600 µ mol m^{-2} s^{-1}. Thus, it is likely that the leaf temperatures of the mangrove species, that are often thick are likely to be warmer than the atmospheric air temperature. Hence to measure photosynthetic traits, the standard portable infrared greenhouse gas analysers (IRGA) are unlikely to achieve lower air temperatures due to high dew point temperature and high humidity. Photosynthetic CO_2 assimilation rate (A_{net}) response to intercellular CO_2 (Ci) are typically measured at 25°C T_{leaf}. Given that during the daytime in summer, the leaf temperatures are unlikely to reach 25°C conditions, measuring A_{net}:Ci at

25°C is not physiologically meaningful.

Although the leaf temperature (T_{leaf}) inferences are based on the IRGA leaf chamber Tleaf snapshot observations, the protocol is invariably constrained by the range of leaf environmental parameters achievable.

Instrument conditioning:

This study have conducted the following measurements using the Li-6800 attached with Li-6800-02 leaf chamber. On a daily basis we changed the CO_2 canister, drierite, replenished water in the humidifier column. Soda lime were changed every three days or as required. We set the saturating radiation for the A/Ci curve measurements at Q_{in}: 1,100 µ mol m^{-2} s^{-1} , T_{leaf} to 37 °C – ensuring the dewpoint temperature is at a safe distance of -5°C from T_{leaf}, Rh chamber to 65% (The maximum attainable at this specific environmental conditions).

The following stability parameters were added to the section and monitored for stability, slope, period of calculation and other details: ΔCO_2, ΔH_2O, T_{leaf}, Rh and A_{net}. Allowed the instrument to reach a stable state for these parameters. After stability is achieved, left the instrument in the same condition atleast for 10 minutes before clipping the leaf or starting a measurement.

Light response measurements:

The Qin values were set to ranging from 1100, 1000, 900, 600, 300, 150, 50, 500, 800, 1200, 1600 and 1100 µ mol m^{-2} s^{-1}. Minimum wait period and maximum wait period were set 180s to 300s respectively. Set to Match IRGAs before every log.

A:Ci measurements:

The CO_2 targets were set to a range of values to 400, 300, 200, 100, 50, 450, 500, 600, 700, 800, 1000, and 1200 µ mol/mol. The minimum wait period and maximum wait period were set to 180s and 300s respectively.

Rd measurements:

While the A:Ci measurement is underway, three to five leaves of the same tree were covered using two layers of aluminium foil on both sides of the leaves, ensuring no light falls on the leaf lamina and kept this dark adaptation condition for a minimum 30 minutes. After the dark adaptation the measurements were taken. We could not complete photosynthesis measurements on *Bruguiera gymnorhiza* as they didn’t respond to the experiment even after trying on 9 individuals in the site. List of the species in this study are added in the supplementary materials (Table S1).

Data Analysis:

This study used Plantecophysiology package in R to analyse A/Ci curve parameters, and fitted the Michaelis Menten model for A/Ci curve and extracted parameters such as Vcmax, Jmax and Rd.

This study used R (Version 4.2.1) for all our analyses and plots. Amax was es taken as the 95th percentile value from the Anet distributions for each species.

Table 1. List of all traits used in this study, trait, abbreviation and unit expressed.

Trait Name	Abbreviation	Unit
Leaf Area	LA	cm^2
Leaf Width	LW	cm
Leaf Thickness	LT	mm
Leaf Mass per Area	LMA	g/m^2
Leaf Dry Matter Content	LDMC	g/g
Vein Density	VD	$veins/mm^2$
Stomatal Density	SD	$stomata/mm^2$
Guard Size	GS	mm
Pore Size	PS	mm
gwmax	gwmax	$mol/m^2/s$
Stem Specific Density	SSD	g/cm^3
Leaf Lifespan	LL	months
Maximum Photosynthetic Rate	Amax	$\mu mol\ CO_2/m^2/s$
Maximum Carboxylation Rate	Vcmax	$\mu mol\ CO_2/m^2/s$
Maximum Electron Transport Rate	Jmax	$\mu mol\ electrons/m^2/s$
Dark Respiration Rate	Rd	$\mu mol\ CO_2/m^2/s$

Results:

This study have performed CO_2 response experiments in twelve true mangrove species (Table S1) from the southwest Indian coast. This study found that A/Ci curves varied significantly (ANOVA, $P<0.05$) in photosynthetic capacity and the transition between electron transport and Rubisco limitation among the studied mangrove species. Species like *Sonneratia caseolaris* and *Avicennia marina* have higher photosynthetic capacities, while others, such as *Ceriops tagal*, exhibit more constrained photosynthesis even under elevated Co_2 conditions (Figure 4.1).

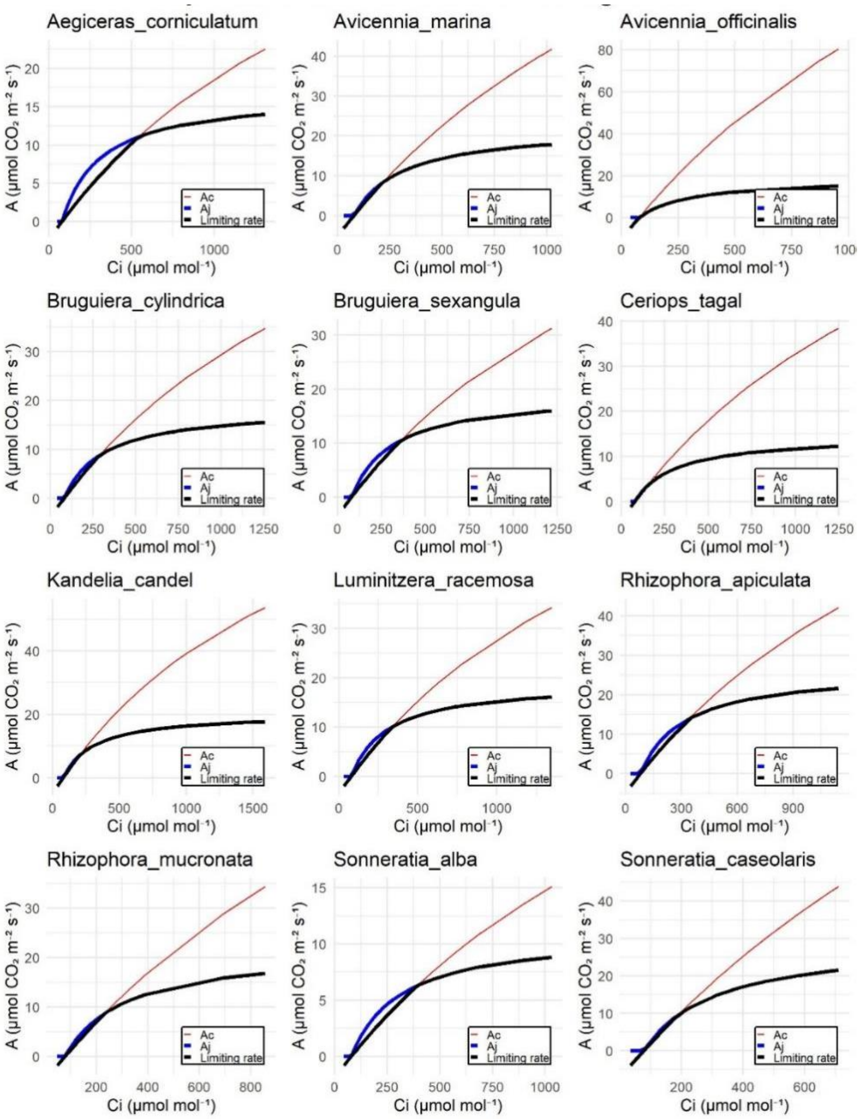


Figure 4.1: *Species-level A/Ci curves for 12 mangrove species showing the relationship between photosynthetic rate (A) and internal* CO_2 *concentration (Ci). The red curve (Ac) represents Rubisco-limited carboxylation, while the blue curve (Aj) represents electron transport-limited photosynthesis. The black curve denotes the limiting rate, which is the lower of Ac or Aj at each Ci value. These A/Ci curves allow comparisons of species' photosynthetic capacity and their limitation by Rubisco or electron transport.*

Photosynthetic assimilation, **Amax;** highest was in *Avicennia marina* (23.18 µmol CO_2 m^{-2} s^{-1}) and *Sonneratia caseolaris* (23.09 µmol CO_2 m^{-2} s^{-1}), indicating greater photosynthetic capacity compared to other species. The lowest Amax was observed in *Sonneratia alba* (8.58 µmol CO_2 m^{-2} s^{-1}) (Table S1).

Avicennia officinalis exhibited the highest **Vcmax** (74.66 µmol CO_2 m^{-2} s^{-1}), followed by *Sonneratia caseolaris* (51.96 µmol CO_2 m^{-2} s^{-1}), while *Sonneratia alba* had the lowest Vcmax (13.24 µmol CO_2 m^{-2} s^{-1})(Table S1). Similarly, **Jmax** was highest in *Sonneratia caseolaris* (114.84 µmol electrons m^{-2} s^{-1}) and *Rhizophora apiculata* (100.80 µmol electrons m^{-2} s^{-1}), with *Sonneratia alba* again showing the lowest value (39.33 µmol electrons m^{-2} s^{-1})(Figure 4.2, Table S2).

Dark respiration rates (**Rd**) varied across species, with *Avicennia officinalis* (-1.63 µmol CO_2 m^{-2} s^{-1}) and *Sonneratia caseolaris* (-0.92 µmol CO_2 m^{-2} s^{-1}) showing the most negative values, indicating higher respiratory losses, while *Aegiceras corniculatum* exhibited the highest positive Rd (0.71 µmol CO_2 m^{-2} s^{-1})(Table S1). This consistent negative values for the Rd in many species was not expected in any case and the values varied a lot within species, as much as "s.e" values of 120% of the mean(Table S2).

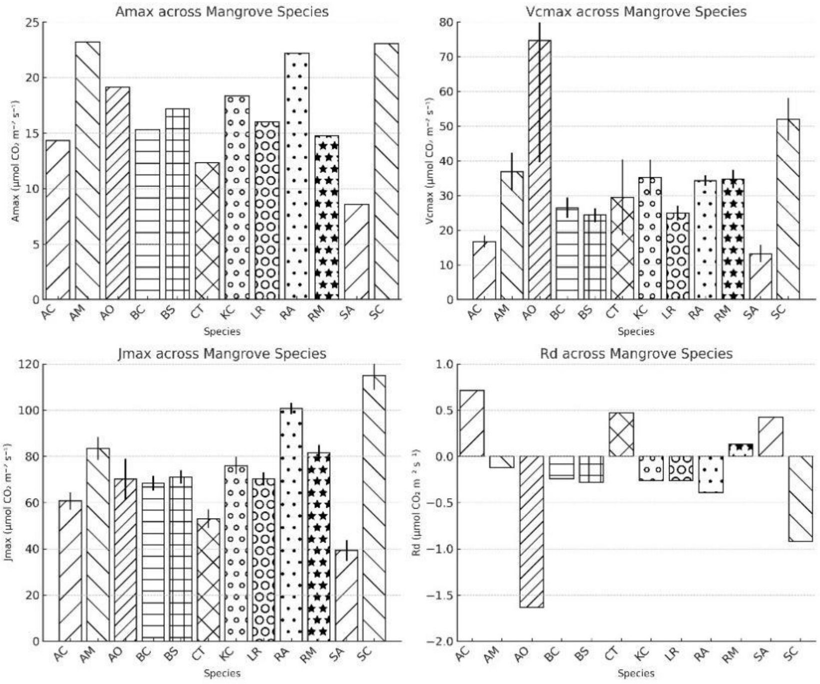


Figure 4.2: Comparative bar plots showing the variation in photosynthetic parameters across 12 mangrove species. **Amax** (top-left) represents the maximum photosynthetic rate, **Vcmax** (top-right) indicates the maximum rate of carboxylation, **Jmax** (bottom-left) reflects the maximum electron transport rate, and **Rd** (bottom-right) represents the dark respiration rate.

No significant correlations were observed between leaf morphological traits **(Leaf Area, LMA, LDMC)** and A/Ci photosynthetic parameters (**Amax, Vcmax, Jmax**) across the 12 mangrove species (Figure 4.3). The relationship between **Leaf Area** and photosynthetic parameters showed weak and non-significant correlations, with **Amax** (r = 0.01, p = 0.979), **Vcmax** (r = -0.05, p = 0.867), and **Jmax** (r = 0.29, p = 0.369) (Figure 4.3 A-C). Similarly, **LMA** was not significantly correlated with **Amax** (r = -0.17, p = 0.606), **Vcmax** (r = 0.20, p = 0.536), or **Jmax** (r = -0.16, p = 0.608) (Figure 4.3 D-F). Weak correlations were also observed between **LDMC** and the photosynthetic parameters, with **Amax** (r = 0.11, p = 0.733), **Vcmax** (r = 0.36, p = 0.245), and **Jmax** (r = 0.02, p = 0.939) (Figure 4.3 G-I). These results suggest that the studied leaf morphological traits do not have a significant influence on the A/Ci photosynthetic parameters among the mangrove species analysed.

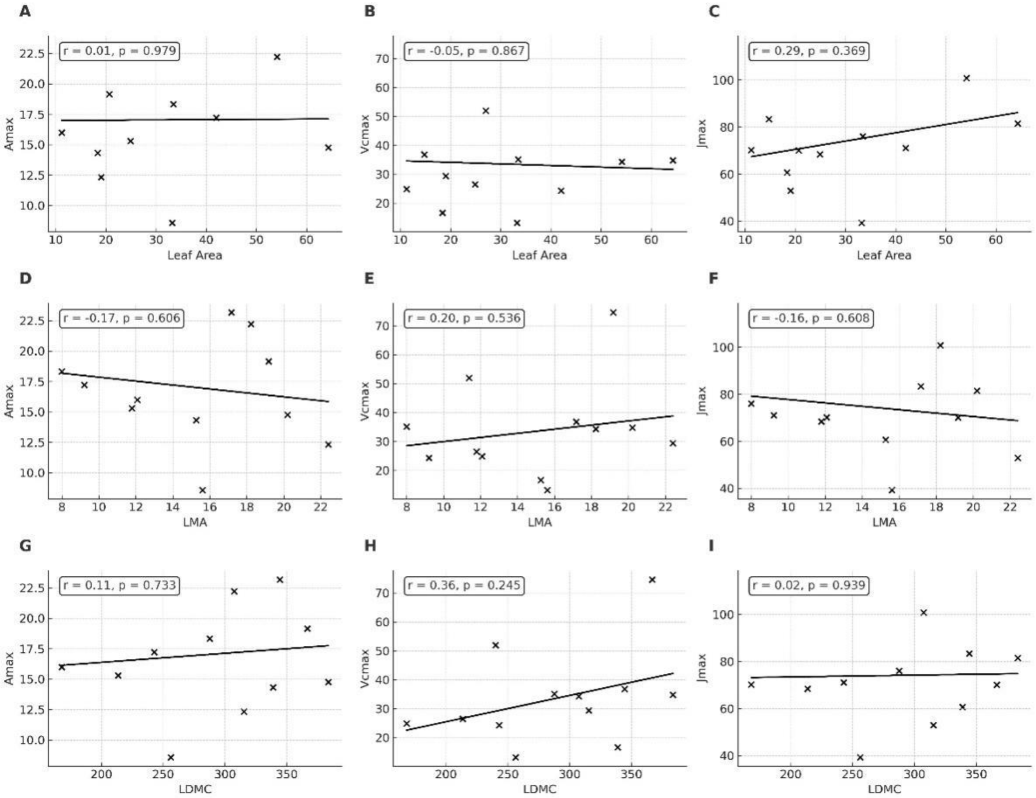


Figure 4.3: Scatter plots showing the relationships between leaf morphological traits (**Leaf Area**, **LMA**, and **LDMC**) and A/Ci photosynthetic parameters (**Amax**, **Vcmax**, and **Jmax**) across 12 mangrove species. Panels A-C depict correlations between **Leaf Area** and **Amax**, **Vcmax**, and **Jmax** respectively. Panels D-F show relationships between **LMA** (Leaf Mass per Area) and **Amax**, **Vcmax**, and **Jmax**. Panels G-I illustrate correlations between **LDMC** (Leaf Dry Matter Content) and the same A/Ci parameters. Pearson correlation coefficients (r) and associated p-values are shown for each relationship.

No significant correlations were found between stomatal traits (**SPI, Stomatal Density**, and **Guard Size**) and the A/Ci parameters (**Amax, Vcmax,** and **Jmax**) across the 12 mangrove species (Figure 4.4). The relationship between **SPI** and photosynthetic parameters was weak and non-significant, with **Amax** (r = 0.10, p = 0.780), **Vcmax** (r = 0.06, p = 0.872), and **Jmax** (r = 0.03, p = 0.926) (Figure 4.4 A, D, G).

Similarly, **Stomatal Density** showed no significant correlations with **Amax** (r = 0.45, p = 0.142), **Vcmax** (r = 0.30, p = 0.348), or **Jmax** (r = 0.13, p = 0.689) (Figure 4.4 B, E, H). Likewise, **Guard Size** exhibited no significant relationships with **Amax** (r = -0.05, p = 0.867), **Vcmax** (r = -0.21, p = 0.522), or **Jmax** (r = 0.23, p = 0.477) (Figure 4.4 C, F, I). This suggests that stomatal traits do not significantly influence the photosynthetic parameters measured in the studied twelve mangrove species from southwest Indian coast.

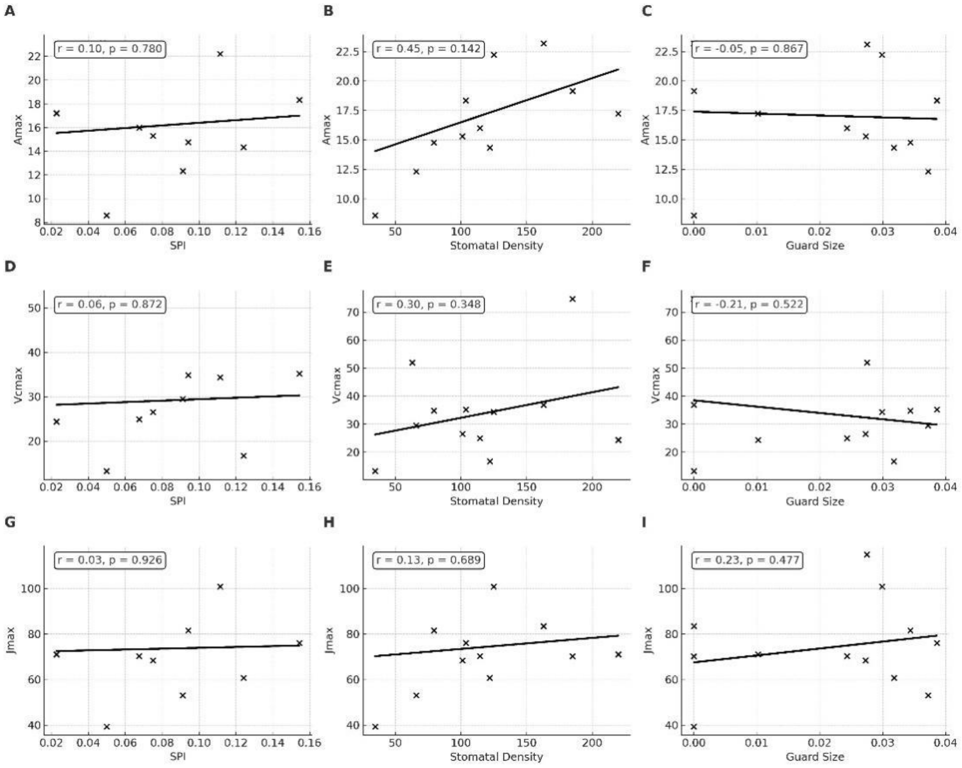


Figure 4.4: Pearson correlation plots illustrating the relationships between stomatal traits (**SPI, Stomatal Density, Guard Size**) and A/Ci parameters (**Amax, Vcmax, Jmax**) across 12 mangrove species. Panels A-C show the correlation between **SPI** (Stomatal Pore Index), **Stomatal Density**, and **Guard Size** with **Amax**. Panels D-F represent the relationships between the same stomatal traits and **Vcmax**, while panels G-I depict the correlations with **Jmax**. Pearson correlation coefficients (r) and associated p-values are provided for each plot. Refer to Table 4.1 for abbreviations and trait units.

This study could not find any significant correlations between the A/Ci parameters (Amax, Vcmax, and Jmax) and the key functional traits (gwmax, SSD, and Leaf Lifespan) across the 12 mangrove species (Figure 4.5). The relationship between **gwmax** and the photosynthetic parameters was weak and non-significant, with **Amax** (r = 0.10, p = 0.780), **Vcmax** (r = 0.06, p = 0.872), and **Jmax** (r = 0.03, p = 0.926) (Figure 4.5 A-C).

Similarly, **SSD** did not show significant correlations with **Amax** (r = 0.11, p = 0.733), **Vcmax** (r = 0.36, p = 0.245), or **Jmax** (r = 0.02, p = 0.939) (Figure 4.5 D-F). The relationship between **Leaf Lifespan** and A/Ci parameters was also non-significant, with **Amax** (r = -0.17, p = 0.606), **Vcmax** (r = 0.20, p = 0.536), and **Jmax** (r = -0.16, p = 0.608) (Figure 4.5 G-I). These results suggest that most of the functional traits examined in this study, including **gwmax**, **SSD**, and **Leaf Lifespan**, do not significantly influence the A/Ci parameters in the studied mangrove species.

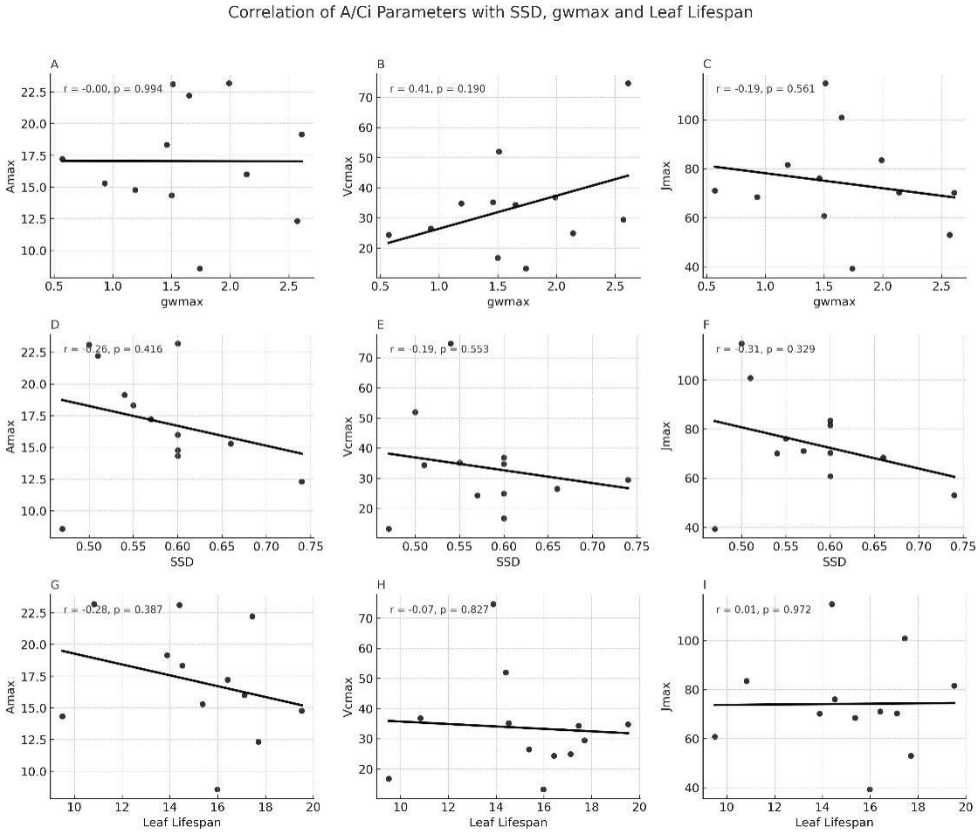


Figure 4.5: Correlation of A/Ci photosynthetic parameters (**Amax**, **Vcmax**, **Jmax**) with key functional traits (**gwmax**, **SSD**, and **Leaf Lifespan**) across 12 mangrove species. Panels A-C depict the relationships between **gwmax** and **Amax**, **Vcmax**, and **Jmax**, respectively. Panels D-F show the correlations between **SSD** (Stem Specific Density) and the same A/Ci parameters, while panels G-I illustrate the relationships between **Leaf Lifespan** and **Amax**, **Vcmax**, and **Jmax**. Pearson correlation coefficients (r) and associated p-values are provided for each relationship.

Contrary to the relationship with other functional traits A/Ci parameters showed significant correlations within themselves (Figure 4.6). Significant positive correlations were found between **Amax** and both **Vcmax** (r = 0.59, p = 0.041) and **Jmax** (r = 0.88, p = 0.000) (Figure 4.6 A, B). Additionally, **Amax** showed a significant negative correlation with **Rd** (r = -0.63, p = 0.029) (Figure 4.6 C). Similarly, **Vcmax** was positively correlated with **Amax** (r = 0.59, p = 0.041), though its correlation with **Jmax** was not statistically significant (r = 0.49, p = 0.107) (Figure 4.6 D, E). A strong negative correlation was observed between **Vcmax** and **Rd** (r = -0.88, p = 0.000) (Figure 4.6 F).

Jmax was highly correlated with **Amax** (r = 0.88, p = 0.000) but did not exhibit a significant relationship with **Rd** (r = -0.52, p = 0.081) (Figure 4.6 G-I). Lastly, **Rd** showed significant negative correlations with both **Amax** (r = -0.63, p = 0.029) and **Vcmax** (r = -0.88, p = 0.000), but its relationship with **Jmax** was not statistically significant (r = -0.52, p = 0.081) (Figure 4.6 J-L). These results suggest that **Amax**, **Vcmax**, and **Jmax** are closely linked, with higher photosynthetic capacities being associated with lower dark respiration rates (**Rd**) in the studied mangrove species.

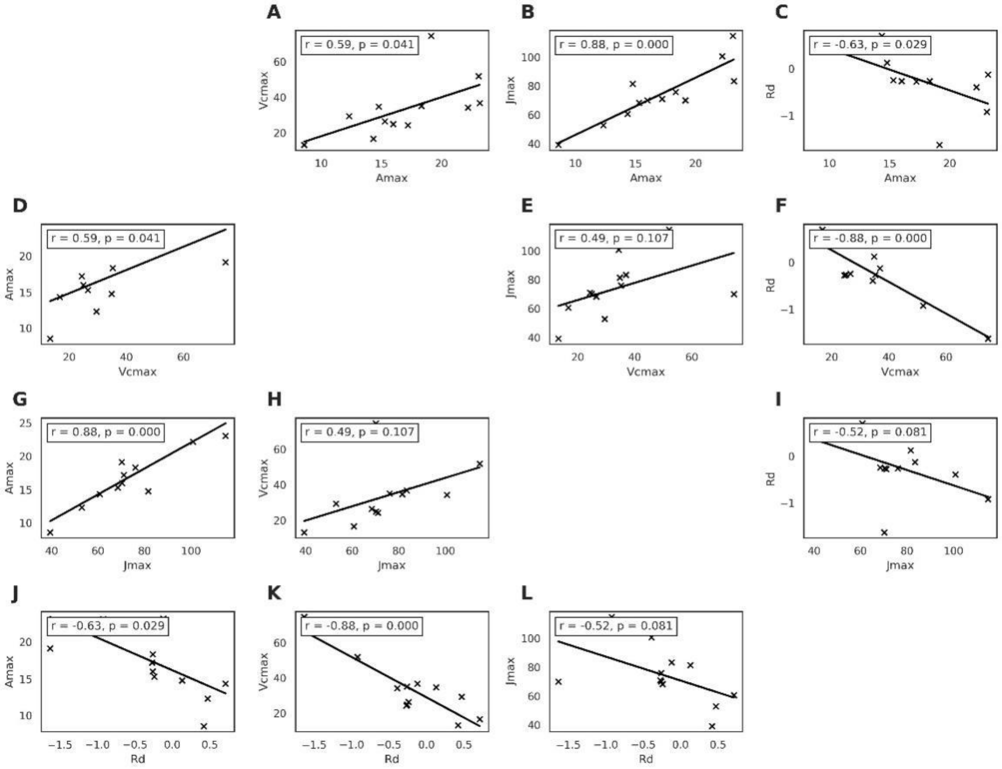


Figure 4.6: Scatter plots with Pearson correlation values showing the relationships between A/Ci parameters (**Amax**, **Vcmax**, **Jmax**, and **Rd**) in 12 mangrove species. Panels A-C display correlations of **Amax** with **Vcmax** , **Jmax** and **Rd,** respectively. Panels D-F depict relationships between **Vcmax** and **Amax** , **Jmax** and **Rd** respectively. Panels G-I show **Jmax** correlations with **Amax** , **Vcmax** , and **Rd,** respectively. Finally, panels J-L illustrate the negative relationships between **Rd** and the other parameters. Significant correlations are indicated with p-values below 0.05.

Discussion:

The considerable variation in A/Ci curves across the twelve mangrove species from the southwest Indian coast underscores the different photosynthetic mechanisms these species utilise to adapt to environmental conditions. The findings, especially the elevated photosynthetic rates in *Avicennia marina* and *Sonneratia caseolaris* (about 23 µmol CO_2 m^{-2} s^{-1}), demonstrate their enhanced carbon assimilation capacity under ambient and elevated CO_2 levels. This discovery corresponds with earlier research indicating that animals exhibiting elevated Amax values generally reside in more open or well-lit habitats, where optimising carbon acquisition is beneficial (Ball & Critchley, 1982; Reef et al., 2016).

Ceriops tagal was previously reported to have lower photosynthetic capacity, Amax values ranging between 8 and 14 µmol CO_2 m^{-2} s^{-1}, demonstrating a conservative approach to photosynthesis, this aligned very well with the lower values found even under elevated CO_2 scenarios in this study (Kao et al., 2004). *Sonneratia alba* also showed notably lower values for Amax in this study was also consistent with previous reports, while *Rhizophora apiculate* and *Rhizophora mucronata* were reported to have a slightly lower value, but varied significantly between different places. This suggests higher plasticity in photosynthetic capacity in mangroves (Clough and Sim 1989, Lovelock. C.E et al., 2006). The results showing higher variation in observed values for Vcmax, Jmax and Rd in this study also resemble reports from mangroves of other parts of the world (Reef et al., 2016, Alongi 2014, Naidoo 2006 and Lovelock et al., 2006). Mangroves like ***Sonneratia alba***, which often inhabit more saline or water-logged areas, typically show lower photosynthetic capacity and thus lower **Vcmax** and **Jmax** values whereas *Avicennia officinalis* and *Sonneratia caseolaris* have higher values (Reef et al., 2016; Lovelock et al., 2006).

The variation in dark respiration rates (Rd) observed in this study, particularly the unexpected negative values, could be possible due to either measurement artefacts or physiological adjustments. However, the reasons still need to be better understood, and the current experimental design is insufficient. Negative Rd values are not typically unexpected but may occur in cases of measurement inconsistencies or variations in environmental conditions during data collection (Lovelock, 2008). The high interspecific variation in Rd values for mangrove species has been previously reported and it reflects the physiological and ecological strategies these species adopt to balance carbon assimilation and respiration in challenging environmental conditions in the mangrove ecosystems (Kao et al., 2004; Naidoo, 2006).

It was surprising that this study did not find any relationship for Amass, Vcmax, Jmax and Rd with LMA,LA and LDMC although they were consistently reported to have a strong relationship and well explained species ecological strategies. Increased LMA leads to greater investment in structural development and resulting in an extended lifespan of the leaves. Amass exhibits a negative correlation with Leaf Mass Area (LMA) and Leaf Logevity, while Leaf Longevity and LMA demonstrate a positive correlation with Leaf Lifespan (Poorter et al., 2009; Wright et al., 2003; Mediavilla & Escudero, 2003). The photosynthetic capacity, specifically Vcmax and Jmax, has a negative correlation with LMA and leaf longevity (Bahar N.H.A et al., 2018; Walker P A et al., 2014 and Kitajima K et al., 1997). A high Amass is typically correlated with short leaf longevity and reduced leaf mass per area due to increased susceptibility to herbivory and physical threats. Additionally, elevated Amass promotes rapid growth, which quickly shades older leaves, causing them to senesce as their resources become more valuable when allocated to better-lit, newer foliage. (Wright et al., 2003). Primary productivity exhibited a positive relationship with specific leaf area; which is the inverse of LMA (Poorter H and de Jong 1999).

In this investigation, similar to other functional traits examined, this study could not identify any correlation between stomatal characteristics and photosynthetic capacity or Amax. It has been demonstrated that they are closely associated with numerous species across various environments. Stomatal traits were strongly correlated with photosynthetic capacity and rate, encompassing stomatal density, pore size, and conductance. Higher stomatal density and enlarged pore diameters promote increased CO_2 absorption, hence augmenting Amax and total photosynthesis, particularly in high-light conditions (Wong et al., 1979; Lawson & Blatt, 2014). Nonetheless, they also regulate water loss via stomata, as elevated stomatal conductance can enhance transpiration, rendering this relationship intricate and contingent upon environmental factors such as water availability and atmospheric humidity (Hetherington & Woodward, 2003); notably, this study found no correlation between conductance to water vapor (gwmax) and photosynthetic capacity or rate. Stomatal characteristics are essential in balancing the trade-offs between carbon acquisition and water-use efficiency, exhibiting species-specific adaptations to various environmental pressures (Franks & Beerling, 2009; Flexas et al., 2012; Buckley, 2019).

Like LMA, Stem specific density (SSD), is also a measure of mechanical strength and is inversely related to photosynthetic capacity and traits like Amax (Reich 2014 & Freschet T.G. et al., 2010). Species with high SSD often will have to invest more in structural tissue for greater longevity, mechanical strength and resistance to environmental stresses, such as drought or wind, but this comes at the cost of lower photosynthetic rates due to reduced allocation to rapid growth and leaf function (Poorter et al., 2008). Species with a higher SSD are typically slow-growing, with longer leaf lifespans and lower photosynthetic traits, as they prioritise structural integrity over rapid carbon gain (Reich, 2014). Conversely, species with a lower SSD, mostly found in fast-growing environments, invest more in LA and photosynthetic machinery, leading to higher carbon assimilation but shorter-lived leaves and stems (Baraloto et al., 2010 & Chave et al., 2009). While some studies found that specific leaf area (leaf area/leaf mass) and wood density were negatively related (Ishida *et al.* 2008 & Bucci *et al.* 2004), others found no correlation (Wright *et al.* 2007); this suggests SSD has a complex relationship photosynthetic trait in some species.

Conclusion:

This study performed in situ CO_2 response curve experiments on multiple mangrove species and was the pioneer attempt, globally. Twelve mangrove species from the southwest Indian coast were studied for their leaf photosynthetic response to elevated CO_2, and their responses varied significantly. The maximum net photosynthesis rate per unit mass (Amax) varied from 8.58 µmol CO_2 m^{-2} s^{-1} in Sonneratia alba to 23.18 CO_2 m^{-2} s^{-1} in *Avicennia marina*. They also varied significantly in Photosynthetic capacity; Vcmax and Jamx. Species such as ***Avicennia marina*** and ***Sonneratia caseolaris*** exhibited the highest photosynthetic capacities, aligning with their ability to optimize carbon acquisition in well-lit habitats, while others like ***Ceriops tagal*** and ***Sonneratia alba*** showed constrained photosynthetic responses even under elevated CO_2 conditions. Notably, **Vcmax**, **Jmax**, and **Rd** values varied widely among species, consistent with previous findings from global mangrove ecosystems. This reflected the diversity of photosynthetic strategies these species employ to cope with environmental stress. However, we did not observe any significant relationships between photosynthetic traits (Amax, Vcmax, Jmax) and key leaf functional traits (LMA, LDMC, LA), stomatal traits (stomatal density, pore size), or stem-specific density (SSD). This lack of correlation was surprising, as these traits are typically linked to photosynthetic performance in other ecosystems. Nevertheless, strong correlations between **Amax**, **Vcmax**, and **Jmax** themselves suggest that these parameters are closely integrated and contribute collectively to the overall photosynthetic efficiency of mangrove species. Surprisingly, this study got negative Rd values for many species, emphasising the need for a revisit to the field with a more customised experimental design for the mangroves' high-lit and heavily humid conditions. Our findings highlight the complexity of trait-photosynthesis relationships in mangroves suggesting further research is needed to explore the underlying mechanisms and external factors shaping these relationships.

References:

Afonso, F., Félix, P. M., Chainho, P., Heumüller, J. A., de Lima, R. F., Ribeiro, F., & Brito, A. C. (2021). Assessing ecosystem services in mangroves: Insights from São Tomé Island (Central Africa). *Frontiers in Environmental Science*, 9, 501673. https://doi.org/10.3389/fenvs.2021.501673

Alongi, D. M. (2002). Present state and future of the world's mangrove forests. *Environmental Conservation*, 29(3), 331-349. https://doi.org/10.1017/S0376892902000231

Alongi, D. M. (2014). Carbon cycling and storage in mangrove forests. *Annual Review of Marine Science*, 6, 195-219. https://doi.org/10.1146/annurev-marine-010213-135020

Alongi, D. M. (2015). The impact of climate change on mangrove forests. *Current Climate Change Reports*, 1(1), 30-39. https://doi.org/10.1007/s40641-015-0002-x

Ball, M. C. (1988). Ecophysiology of mangroves. *Trees*, 2(3), 129-142. https://doi.org/10.1007/BF00196018

Ball, M. C., & Critchley, C. (1982). Photosynthetic responses to irradiance by the grey mangrove, *Avicennia marina*, grown under different light regimes. *Plant Physiology*, 70(4), 1101-1106. https://doi.org/10.1104/pp.70.4.1101

Bahar, N. H. A., Ishida, F. Y., Weerasinghe, L. K., Guerrieri, R., O'Sullivan, O. S., Bloomfield, K. J., & Atkin, O. K. (2018). Leaf-level photosynthetic capacity in lowland rainforests of Australia. *Functional Plant Biology*, 45(7), 677-691. https://doi.org/10.1071/FP17251

Baraloto, C., Paine, C. E. T., Patino, S., Bonal, D., Hérault, B., & Chave, J. (2010). Functional trait variation and sampling strategies in species-rich plant communities. *Functional Ecology*, 24(1), 208-216. https://doi.org/10.1111/j.1365-2435.2009.01600.x

Bernacchi, C. J., Singsaas, E. L., Pimentel, C., Portis, A. R., & Long, S. P. (2003). Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell & Environment*, 26(9), 1419-1430. https://doi.org/10.1046/j.0016-8025.2003.01060.x

Bucci, S. J., Scholz, F. G., Goldstein, G., Meinzer, F. C., Hinojosa, J. A., Hoffmann, W. A., & Franco, A. C. (2004). Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna trees. *Tree Physiology*, 24(10), 1119-1127. https://doi.org/10.1093/treephys/24.10.1119

Chave, J., Coomes, D., Jansen, S., Lewis, S. L., Swenson, N. G., & Zanne, A. E. (2009). Towards a worldwide wood economics spectrum. *Ecology Letters*, 12(4), 351-366. https://doi.org/10.1111/j.1461-0248.2009.01285.x

Clough, B. F., & Sim, R. G. (1989). Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapour pressure deficit. *Oecologia*, 79(1), 38-44. https://doi.org/10.1007/BF00378238

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011). Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience*, 4(5), 293-297. https://doi.org/10.1038/ngeo1123

Duke, N. C., Ball, M. C., & Ellison, J. C. (2007). Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology and Biogeography*, 7(1), 27-47. https://doi.org/10.2307/2997695

Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, 149(1), 78-90. https://doi.org/10.1007/BF00386231

Flexas, J., Bota, J., Galmés, J., Medrano, H., & Ribas-Carbó, M. (2006). Keeping a positive carbon balance under adverse conditions: Responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, 127(3), 343-352. https://doi.org/10.1111/j.1399-3054.2006.00621.x

Flexas, J., Ribas-Carbó, M., Diaz-Espejo, A., Galmés, J., & Medrano, H. (2012). Mesophyll conductance to CO_2: Current knowledge and future prospects. *Plant, Cell & Environment*, 35(4), 695-707. https://doi.org/10.1111/j.1365-3040.2012.02449.x

Franks, P. J., & Beerling, D. J. (2009). Maximum leaf conductance driven by CO_2 effects on stomatal size and density: A case study using *Ginkgo biloba*. *New Phytologist*, 183(3), 839-847. https://doi.org/10.1111/j.1469-8137.2009.02832.x

Hetherington, A. M., & Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature*, 424(6951), 901-908. https://doi.org/10.1038/nature01843

Ishida, A., Uemura, A., Koike, N., Matsumoto, Y., & Hoe, A. L. (2008). Leaf physiological adaptation of tropical trees to different forest succession stages. *Functional Ecology*, 22(6), 1044-1054. https://doi.org/10.1111/j.1365-2435.2008.01465.x

Kauffman, J. B., Heider, C., Cole, T. G., Dwire, K. A., & Donato, D. C. (2017). Ecosystem carbon stocks of Micronesian mangrove forests. *Wetlands*, 31(2), 343-352. https://doi.org/10.1007/s13157-011-0153-6

Kitajima, K., & Poorter, L. (1997). Functional basis for resource niche partitioning by tropical trees. *Functional Ecology*, 11(6), 694-703. https://doi.org/10.1046/j.1365-2435.1997.00174.x

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*, 164(4), 1556-1570. https://doi.org/10.1104/pp.114.237107

Long, S. P., & Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany*, 54(392), 2393-2401. https://doi.org/10.1093/jxb/erg262

Lovelock, C. E., Ball, M. C., Martin, K. C., & Feller, I. C. (2006). Variation in carbon assimilation and allocation among mangrove species along a nutrient gradient in Panama. *Journal of Ecology*, 94(5), 1132-1144. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Lovelock, C. E. (2008). Soil respiration and belowground carbon allocation in mangrove forests. *Ecosystems*, 11(3), 342-354. https://doi.org/10.1007/s10021-008-9125-4

Mediavilla, S., & Escudero, A. (2003). Photosynthetic capacity, integrated over the lifetime of a leaf, is predicted to be independent of leaf longevity in some tree species. *New Phytologist*, 159(2), 203-211. https://doi.org/10.1046/j.1469-8137.2003.00798.x

Medina, E., & Francisco, A. M. (1997). Osmolality and δ13C of leaf tissues of mangrove species from environments of contrasting rainfall and salinity. *Estuarine, Coastal and Shelf Science*, 45(3), 337-344. https://doi.org/10.1006/ecss.1997.0183

Naidoo, G. (2006). Factors contributing to dwarfing in the mangrove *Avicennia marina*. *Annals of Botany*, 97(6), 1095-1101. https://doi.org/10.1093/aob/mcl066

Odum, W. E., & Johannes, R. E. (1979). The response of mangroves to man-induced environmental stress. In J. H. Steele (Ed.), *Marine ecosystems* (pp. 345-360). Academic Press. https://doi.org/10.1016/B978-0-12-650340-0.50033-1

Osland, M. J., Feher, L. C., Anderson, G. H., & Rogers, K. (2022). The impacts of mangrove range expansion on wetland ecosystem services in the southeastern United States: Current understanding, knowledge gaps, and emerging research needs. *Global Change Biology*, 28(15), 4749-4765. https://doi.org/10.1111/gcb.16111

Poorter, H., & Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, 87(7), 1733-1743. https://doi.org/10.1890/0012-9658(2006)87[1733:LTAGPO]2.0.CO;2

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565-588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275-301. https://doi.org/10.1111/1365-2745.12211

Reef, R., Ball, M. C., & Lovelock, C. E. (2016). The impact of CO_2 and nutrient fertilization on mangrove performance across a latitudinal gradient. *Global Change Biology*, 22(6), 2038-2050. https://doi.org/10.1111/gcb.13294

Saintilan, N., Wilson, N. C., Rogers, K., Rajkaran, A., & Krauss, K. W. (2020). Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global Change Biology*, 26(6), 3054-3065. https://doi.org/10.1111/gcb.15022

Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D., & Singsaas, E. L. (2007). Fitting photosynthetic carbon dioxide response curves for C_3 leaves. *Plant, Cell & Environment*, 30(9), 1035-1040. https://doi.org/10.1111/j.1365-3040.2007.01710.x

Wullschleger, S. D. (1993). Biochemical limitations to carbon assimilation in C_3 plants—A retrospective analysis of the A/Ci curves from 109 species. *Journal of Experimental Botany*, 44(5), 907-920. https://doi.org/10.1093/jxb/44.5.907

Walker, A. P., Beckerman, A. P., Gu, L., Kattge, J., Cernusak, L. A., Domingues, T. F., & Woodward, F. I. (2014). The relationship of leaf photosynthetic traits – Vcmax and Jmax – to leaf nitrogen, leaf phosphorus, and specific leaf area: A meta-analysis and modeling study. *Ecology and Evolution*, 4(16), 3218-3235. https://doi.org/10.1002/ece3.1173

Wright, I. J., Reich, P. B., & Westoby, M. (2003). Least-cost input mixtures of water and nitrogen for photosynthesis. *Plant, Cell & Environment*, 26(8), 1357-1374. https://doi.org/10.1046/j.1365-3040.2003.01055.x

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, F. S., Cornelissen, J. H., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W. S., Lusk, C., ... Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827. https://doi.org/10.1038/nature02403

Supplementary Figures and Tables:

Table 4.S 1. The list of mangrove species used in this study, as well as their family names and species abbreviation codes, is as follows:

	Species	**Family**	**Code**
1.	*Aegiceras corniculatum*	Myrsinaceae	AC
2.	*Avicennia marina*	Avicenniaceae	AM
3.	*Avicennia officinalis*	Avicenniaceae	AO
4.	*Bruguiera cylindrica*	Rhizophoraceae	BC
5.	*Bruguiera sexangula*	Rhizophoraceae	BS
6.	*Ceriops tagal*	Rhizophoraceae	CT
7.	*Kandelia candel*	Rhizophoraceae	KC
8.	*Lumnitzera racemosa*	Combretaceae	LM
9.	*Rhizophora apiculata*	Rhizophoraceae	RA
10.	*Rhizophora mucronata.*	Rhizophoraceae	RM
11.	*Sonneratia alba*	Sonneratiaceae	SA
12.	*Sonneratia caseolaris*	Sonneratiaceae	SC

Table 4.S2: Species-level values for photosynthetic parameters extracted from the A/Ci curve.

Species	Amax_95th	Vcmax	Vcmax_se	Jmax	Jmax_se	Rd	Rd_se
Aegiceras corniculatum	14.32	16.69	1.689	60.718	3.812	0.710	0.529
Avicennia marina	23.18	36.84	5.424	83.424	4.916	-0.115	0.635
Avicennia officinalis	19.14	74.65	35.159	70.151	8.733	-1.632	1.034
Bruguiera cylindrica	15.29	26.49	2.904	68.413	3.119	-0.241	0.450
Bruguiera sexangula	17.21	24.33	2.017	71.056	2.949	-0.275	0.399
Ceriops tagal	12.32	29.45	10.987	53.011	4.122	0.471	0.527
Kandelia candel	18.32	35.15	5.218	76.046	3.772	-0.258	0.548
Luminitzera racemosa	15.6	24.90	2.057	70.261	2.940	-0.261	0.390
Rhizophora apiculata	22.21	34.31	1.517	100.795	2.425	-0.392	0.322
Rhizophora mucronata	14.78	34.77	2.627	81.537	3.368	0.125	0.380
Sonneratia alba	8.58	13.23	2.524	39.327	4.561	0.420	0.609
Sonneratia caseolaris	23.08	51.95	6.182	114.841	6.091	-0.916	0.720

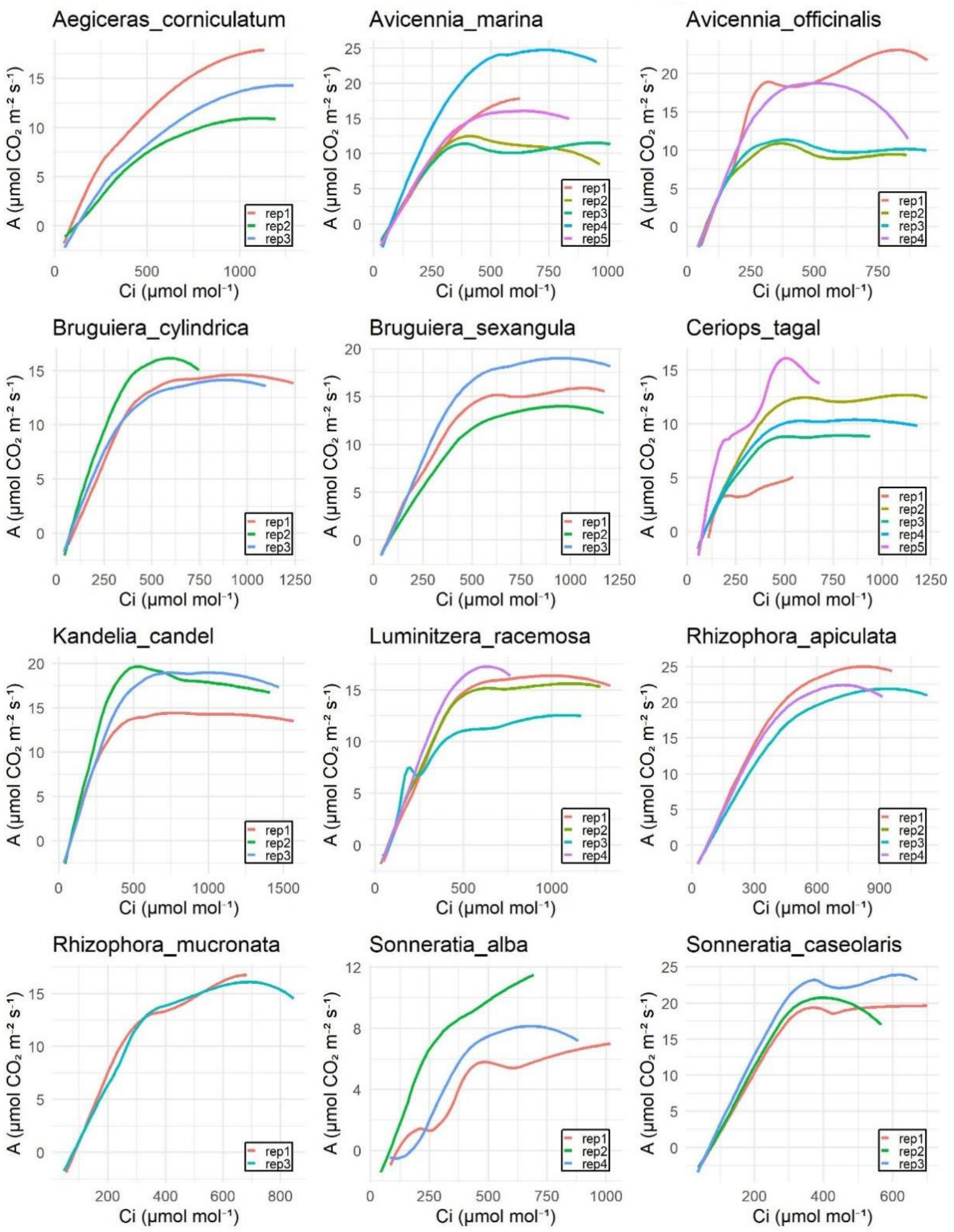
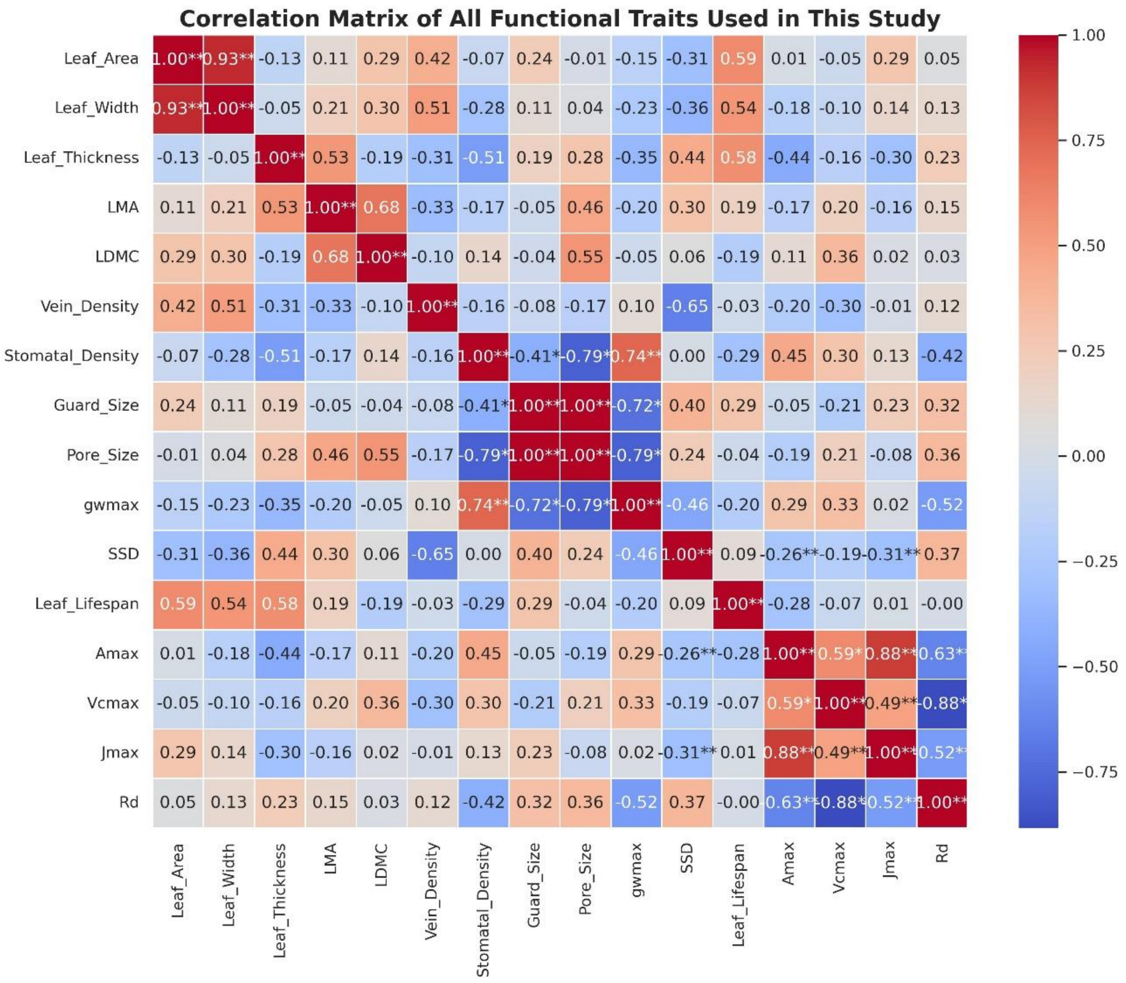


Figure 4.S1: Individual A/Ci response curves for 12 mangrove species showing data from multiple replicates (rep1, rep2, rep3, etc.) per species. The net photosynthesis rate (A, µmol CO_2 m^{-2} s^{-1}) is plotted against the internal leaf CO_2 concentration (Ci, µmol mol^{-1}). Each color represents a different replicate, illustrating the variation within species for their photosynthetic response under changing CO_2 levels. Species exhibit distinct A/Ci responses, reflecting differences in photosynthetic performance and variability among individuals within the same species.



	Leaf_Area	Leaf_Width	Leaf_Thickness	LMA	LDMC	Vein_Density	Stomatal_Density	Guard_Size	Pore_Size	gwmax	SSD	Leaf_Lifespan	Amax	Vcmax	Jmax	Rd
Leaf_Area	1.00**	0.93**	-0.13	0.11	0.29	0.42	-0.07	0.24	-0.01	-0.15	-0.31	0.59	0.01	-0.05	0.29	0.05
Leaf_Width	0.93**	1.00**	-0.05	0.21	0.30	0.51	-0.28	0.11	0.04	-0.23	-0.36	0.54	-0.18	-0.10	0.14	0.13
Leaf_Thickness	-0.13	-0.05	1.00**	0.53	-0.19	-0.31	-0.51	0.19	0.28	-0.35	0.44	0.58	-0.44	-0.16	-0.30	0.23
LMA	0.11	0.21	0.53	1.00**	0.68	-0.33	-0.17	-0.05	0.46	-0.20	0.30	0.19	-0.17	0.20	-0.16	0.15
LDMC	0.29	0.30	-0.19	0.68	1.00**	-0.10	0.14	-0.04	0.55	-0.05	0.06	-0.19	0.11	0.36	0.02	0.03
Vein_Density	0.42	0.51	-0.31	-0.33	-0.10	1.00**	-0.16	-0.08	-0.17	0.10	-0.65	-0.03	-0.20	-0.30	-0.01	0.12
Stomatal_Density	-0.07	-0.28	-0.51	-0.17	0.14	-0.16	1.00**	-0.41*	-0.79*	0.74**	0.00	-0.29	0.45	0.30	0.13	-0.42
Guard_Size	0.24	0.11	0.19	-0.05	-0.04	-0.08	-0.41*	1.00**	1.00**	-0.72*	0.40	0.29	-0.05	-0.21	0.23	0.32
Pore_Size	-0.01	0.04	0.28	0.46	0.55	-0.17	-0.79*	1.00**	1.00**	-0.79*	0.24	-0.04	-0.19	0.21	-0.08	0.36
gwmax	-0.15	-0.23	-0.35	-0.20	-0.05	0.10	0.74**	-0.72*	-0.79*	1.00**	-0.46	-0.20	0.29	0.33	0.02	-0.52
SSD	-0.31	-0.36	0.44	0.30	0.06	-0.65	0.00	0.40	0.24	-0.46	1.00**	0.09	-0.26**	-0.19	-0.31**	0.37
Leaf_Lifespan	0.59	0.54	0.58	0.19	-0.19	-0.03	-0.29	0.29	-0.04	-0.20	0.09	1.00**	-0.28	-0.07	0.01	-0.00
Amax	0.01	-0.18	-0.44	-0.17	0.11	-0.20	0.45	-0.05	-0.19	0.29	-0.26**	-0.28	1.00**	0.59*	0.88**	0.63*
Vcmax	-0.05	-0.10	-0.16	0.20	0.36	-0.30	0.30	-0.21	0.21	0.33	-0.19	-0.07	0.59*	1.00**	0.49**	-0.88*
Jmax	0.29	0.14	-0.30	-0.16	0.02	-0.01	0.13	0.23	-0.08	0.02	-0.31**	0.01	0.88**	0.49**	1.00**	0.52**
Rd	0.05	0.13	0.23	0.15	0.03	0.12	-0.42	0.32	0.36	-0.52	0.37	-0.00	0.63**	0.88*	0.52**	1.00**

Figure 4.S2: Correlation matrix showing relationships between all functional traits used in this study including A/Ci parameters in 12 mangrove species. The colour gradient represents the strength and direction of the correlations, with red indicating positive correlations and blue indicating negative correlations.

Table S3 : Shapiro-Wilk test for normality

Trait	**W-statistic**	**p-value**
Amax	0.957349	0.745419
Vcmax	0.870672	0.066644
Jmax	0.961337	0.802733
Rd	0.920683	0.291619

Chapter 5

Conclusions

Mangroves are facing unprecedented threats from climate change despite their strong resilience to extremely harsh environments in which they live. This thesis explores the physiological responses of mangrove plants to different environmental factors like temperature, water and CO_2. This enquiry will help us understand and predict how they will respond to future rising atmospheric temperatures, including heatwaves, frequent and intense drought events and increasing levels of atmospheric CO_2 concentrations. This work aimed to give insights into mangrove ecological strategies by studying the relationship between plant functional traits and physiological performances. The main findings across the three core studies and their broader implications are summarized below. The expermiment was done on 13 true mangrove species from the Ashtamudy backwaters estuarine sedimentary islands, at Shakthikulangara, near Kollam. Keralam, Southwest coast of India.

Chapter 2 deals with temperature tolerance (T50) and thermal safety margins in 13 true mangrove species. All species had high thermotolerance, with T50 values above 50°C, except Avicennia marina, which had the lowest of 48.9°C. The highest thermotolerance was observed in Bruguiera sexangula (55.32°C). There was significant variation in thermal safety margins and maximum leaf temperatures across species examined. This result is consistent with previous studies, which found Avicennia species to be more vulnerable to heat (Li et al., 2022; Reef et al., 2016; Lovelock et al., 2006). Mangroves from tropical regions, like those in this study, often have higher thermal thresholds due to greater exposure to intense solar radiation (Clough & Sim, 1989; Alongi, 2014).
There was significant variation in thermotolerance between species. Mangroves like Bruguiera sexangula, with larger leaves, had higher T50 values, which may help with heat dissipation (Sastry & Barua, 2017). In contrast, Avicennia marina, with smaller leaves, may be less efficient at regulating leaf temperature than bigger-leaved species, leading to lower thermotolerance (Nguyen et al., 2017). Mangroves from tropical regions tend to have higher thermotolerance than those from subtropical areas, such as China, where average T50 values are lower than what I found in this study (Li et al., 2022; Slot et al., 2021). This higher thermotolerance may become more critical as temperatures continue to rise due to climate change (Duke et al., 2007; Alongi, 2015).

In the second study (Chapter 3), pressure-volume curves were used to understand drought tolerance among mangrove species. Species like Kandelia candel and Bruguiera sexangula retained water better during dehydration, indicating superior drought tolerance levels (Beckett et al., 2023; Choat et al., 2018). These species had higher relative water content at the turgor loss point (RWCTLP). Rhizophora apiculata and Sonneratia caseolaris had lower water potential at the turgor loss point (ΨTLP), showing a quicker loss in turgor during early dehydration (Herppich & Willert, 1995; Markesteijn et al., 2011). Modulus of elasticity (e) was positively correlated with RWCTLP, meaning species with more rigid cell architexture retained water better during drought (Sack & Scoffoni, 2013). In contrast, traits like leaf dry matter content (LDMC) and leaf mass per area (LMA) showed no significant relationship with drought tolerance. This finding suggests that some commonly studied leaf traits may not play a major role in water regulation for mangroves, or mangroves adopt very different ecological strategies to cope with hydraulic stress.

The third study (Chapter 4) examined the leaf photosynthetic responses of mangroves to elevated CO2 in the context of a rapidly changing climate. Species like Avicennia marina and Sonneratia caseolaris had the highest net photosynthesis rates (Amax), while species like Ceriops tagal and Sonneratia alba had lower responses, and this was found to be consistent with previous reports from Australian mangroves (Reef et al., 2016; Lovelock et al., 2006). The mangrove species studied here showed significant variation in photosynthetic responses, suggesting the different strategies that mangrove species use to cope with CO_2 changes; similar results can be found in other mangroves too (Clough & Sim, 1989; Wong et al., 1979). Key photosynthetic parameters, such as maximum carboxylation rate (Vcmax) and maximum electron transport rate (Jmax), were also varied widely. Avicennia officinalis and Sonneratia caseolaris had the highest values for photosynthetic capcity, suggesting they are better adapted to optimise carbon uptake under elevated CO_2 concentrations. In contrast, Sonneratia alba had the lowest values, possibly due to its adaptation to more saline environments (Naidoo, 2006; Alongi, 2014).

Surprisingly, the trend of having no correlations between functional traits and stress tolerance from the previous chapters continued here, too. This study found no significant correlations between photosynthetic traits (Amax, Vcmax, Jmax) and leaf morphological functional traits like LMA, LDMC, or stomatal traits such as density and pore dimensions. This finding contradicts studies in other ecosystems, where these traits are often linked to photosynthesis (Poorter et al., 2009; Wright et al., 2004). This result suggests that mangroves may use unique physiological strategies to optimise photosynthesis in highly stressed environments like high humidity, higher salinity, heavy wind, higher irradiance and water-abundant environments (Lovelock et al., 2006; Lawson & Blatt, 2014). However, in this study also photosynthetic traits showed strong correlations with each other. Amax was positively correlated with Vcmax and Jmax, indicating that higher photosynthetic capacity is closely linked with increased rates of carbon assimilation and electron transport (Reich et al., 2014; Walker et al., 2014). Additionally, the negative relationship between dark respiration (Rd) and both Amax and Vcmax shows that species with higher photosynthesis rates have lower respiratory losses, which may help them conserve energy under high-light conditions (Reich, 2014; Buckley, 2019).
In conclusion, this thesis highlights mangroves' diverse strategies to cope with environmental stressors like heat, drought, and rising CO_2. This study also points towards decoupling established trait-trade-offs in terrestrial plants. None of the leaf economics traits showed any pattern established in the

worldwide leaf economic spectrum, and no significant correlations were found between any studied stress tolerance and functional traits. The multitude of adaptations they had to evolve and the high cost of maintaining them to cope with the unmatched stressful environment they live in might have caused them to compromise on general trait-trade-offs. The findings of this study will help in guiding conservation and restoration policies regarding coastal ecosystem and biodiversity management and the fight against climate change. Further research is needed to explore the physiological mechanisms driving these adaptations and their implications for mangrove ecosystems in the face of climate change.

References:

Alongi, D. M. (2014). Carbon cycling and storage in mangrove forests. *Annual Review of Marine Science, 6*, 195-219. https://doi.org/10.1146/annurev-marine-010213-135020

Alongi, D. M. (2015). The impact of climate change on mangrove forests. *Current Climate Change Reports, 1*(1), 30-39. https://doi.org/10.1007/s40641-015-0002-x

Beckett, H., Choat, B., Delzon, S., & Lovelock, C. E. (2023). Drought tolerance in mangrove species. *Journal of Experimental Botany, 74*(3), 597-606. https://doi.org/10.1093/jxb/erac509

Bernacchi, C. J., Singsaas, E. L., Pimentel, C., Portis, A. R., & Long, S. P. (2003). Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell & Environment, 26*(9), 1419-1430. https://doi.org/10.1046/j.0016-8025.2003.01060.x

Buckley, T. N. (2019). How do stomata respond to water status? *New Phytologist, 224*(1), 21-36. https://doi.org/10.1111/nph.16045

Choat, B., Brodribb, T. J., & Sack, L. (2018). Plant hydraulics and climate change. *New Phytologist, 224*(4), 1206-1212. https://doi.org/10.1111/nph.15974

Clough, B. F., & Sim, R. G. (1989). Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapour pressure deficit. *Oecologia, 79*(1), 38-44. https://doi.org/10.1007/BF00378238

Duke, N. C., Ball, M. C., & Ellison, J. C. (2007). Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology and Biogeography, 7*(1), 27-47. https://doi.org/10.2307/2997695

Herppich, W. B., & Willert, D. J. (1995). Osmotic adjustment and water relations of Mediterranean sclerophyllous species. *Plant, Cell & Environment, 18*(6), 696-705. https://doi.org/10.1111/j.1365-3040.1995.tb00573.x

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology, 164*(4), 1556-1570. https://doi.org/10.1104/pp.114.237107

Li, M., Jiang, Z., Zhang, X., Wang, H., & Lin, G. (2022). High temperature tolerance of mangroves in subtropical China. *Ecology and Evolution, 12*(8), e9224. https://doi.org/10.1002/ece3.9224

Lovelock, C. E., Ball, M. C., Martin, K. C., & Feller, I. C. (2006). Variation in carbon assimilation and allocation among mangrove species along a nutrient gradient in Panama. *Journal of Ecology, 94*(5), 1132-1144. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Markesteijn, L., Poorter, L., Paz, H., Sack, L., & Bongers, F. (2011). Hydraulics and life history of tropical dry forest tree species: Coordination of species' drought and shade tolerance. *New Phytologist, 191*(2), 480-495. https://doi.org/10.1111/j.1469-8137.2011.03708.x

Naidoo, G. (2006). Factors contributing to dwarfing in the mangrove *Avicennia marina*. *Annals of Botany, 97*(6), 1095-1101. https://doi.org/10.1093/aob/mcl066

Nguyen, H. T., Hong, P. N., Phan, N. H., & Armstrong, W. (2017). Salt tolerance in *Avicennia* spp. *Plant Physiology and Biochemistry, 65*(3), 98-106. https://doi.org/10.1016/j.plaphy.2017.04.010

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist, 182*(3), 565-588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Reef, R., Ball, M. C., & Lovelock, C. E. (2016). The impact of CO_2 and nutrient fertilization on mangrove performance across a latitudinal gradient. *Global Change Biology, 22*(6), 2038-2050. https://doi.org/10.1111/gcb.13294

Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology, 102*(2), 275-301. https://doi.org/10.1111/1365-2745.12211

Sack, L., & Scoffoni, C. (2013). Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist, 198*(4), 983-1000. https://doi.org/10.1111/nph.12253

Sastry, A., & Barua, D. (2017). Leaf thermotolerance in tropical trees from a seasonally dry tropical forest. *Plant Physiology, 174*(1), 199-209. https://doi.org/10.1104/pp.17.00105

Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D., & Singsaas, E. L. (2007). Fitting photosynthetic carbon dioxide response curves for C_3 leaves. *Plant, Cell & Environment, 30*(9), 1035-1040. https://doi.org/10.1111/j.1365-3040.2007.01710.x

Slot, M., Winter, K., & Kitajima, K. (2021). Drought tolerance in Panamanian mangroves. *Journal of Tropical Ecology, 37*(2), 120-130. https://doi.org/10.1017/S0266467421000070

Walker, A. P., Beckerman, A. P., Gu, L., Kattge, J., Cernusak, L. A., Domingues, T. F., ... & Wullschleger, S. D. (2014). The relationship of leaf photosynthetic traits and genotype-by-environment interactions to yield in diverse populations. *New Phytologist, 201*(2), 515-531. https://doi.org/10.1111/nph.12512

Wong, S. C., Cowan, I. R., & Farquhar, G. D. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature, 282*(5738), 424-426. https://doi.org/10.1038/282424a0

Wright, I. J., Reich, P. B., Cornelissen, J. H. C., Falster, D. S., Groom, P. K., Hikosaka, K., ... & Westoby, M. (2004). The worldwide leaf economics spectrum. *Nature, 428*(6985), 821-827. https://doi.org/10.1038/nature02403

Chapter 6

Recommendations

This study was one of the most extensive efforts on mangroves to understand a comprehensive idea of their ecophysiology. The study addressed various plant functional traits and the effects of three stress factors: temperature, drought, and elevated CO_2. This was entirely done within a single study and a PhD time frame. Continued attempts should be made to improve our understanding of mangrove physiology and enhance conservation efforts by considering on-field experiments, including on-field in-situ leaf temperature measurements. This would accurately represent the leaf's photosynthetic thermal safety margin, whereas this study estimated leaf temperature based on leaf traits and climatic parameters. The current study used Fv/Fm measurements on heat-treated excised leaf discs, which may not fully capture the in-situ whole plant response to temperature stress.

Additionally, future studies should conduct on-field photosynthetic temperature response curves to enhance our understanding of the photosynthetic temperature response in mangroves. While this study faced technical challenges such as low dew point temperatures and high humidity that prevented successful temperature adjustments on IRGA (Infra-Red Gas Analyser) in the field, developing technical solutions, such as enhanced temperature regulation in chambers or alternative dehumidifying systems, would allow accurate field measurements. This will help researchers understand the optimal and critical temperatures for mangrove photosynthesis in real-world conditions, particularly in humid and hot tropical mangrove ecosystems.

Furthermore, rapid, large-scale screening of key physiological traits like photosynthesis (Amax), dark respiration (Rd), and maximum stomatal conductance (gswmax) is essential. Special attention must be given to the negative Rd values observed in this study, which were not expected for plants during day time. If these negative values represent real physiological processes, identifying their cause could lead to groundbreaking insights into mangrove metabolism, particularly under high-light, high-humidity conditions. A focused study on Rd, coupled with advanced measurement methods, is necessary to resolve this dilemma.

In addition to the current analysis of plant hydraulics using pressure-volume (p-v) curves, we also need to understand the xylem vulnerability to drought to understand the drought tolerance mechanisms in mangroves better. Xylem vulnerability assessments will provide critical insights into how different species respond to water stress and regulate water transport under dry conditions beyond turgor adjustments. A more detailed understanding of mangrove hydraulics also requires continuous, on-field water potential measurements across different seasons. This data would help track species' water use strategies with respect to soil salinity and water availability throughout the year and better explain how mangroves cope with the fluctuating water availability in coastal environments. Long-term monitoring of leaf and stem water potentials will provide critical information on seasonal and species-level variations in water relations.

Phenology, particularly the timing of leaf production, senescence, and flowering, should be systematically monitored for all mangrove species in a long-term study. This will help to link phenological patterns to physiological traits and water status. A detailed understanding of phenology will improve predictions of how mangrove species manage their water resources and respond to environmental stressors such as drought and temperature extremes.

This has yet to attempt to study anatomical and root traits in mangroves. To fully understand the functional relationships between traits, further anatomical studies are required to examine the structural causes and consequences of trait relationships. Exploring how internal leaf and stem structures influence physiological processes will provide a deeper understanding of the ecological strategies employed by mangrove species. Studies should investigate how anatomical features, such as stomatal arrangement, xylem architecture, and leaf tissue composition, influence the observed physiological responses.

While much of the focus has been on aboveground functional traits, the belowground ecophysiology remains critical for understanding the full scope of mangrove response to its environment, hence adaptation and resilience. Root systems in mangroves play a crucial role in nutrient uptake, water absorption, and stability in their often waterlogged, hypoxic and saline environments. The unique adaptations of mangrove roots, such as pneumatophores and prop roots, are vital to their survival, but little is known about how these root traits respond to environmental stressors like drought, salinity, and changing CO_2 levels. There is an immediate need to study the belowground systems to understand mangrove physiology comprehensively. This will help inform conservation and restoration efforts, ensuring holistic approaches are considered in mangrove management strategies.

Finally, these recommendations underscore the need for continued research on mangroves' physiological responses to changing climate. By addressing the gaps in current knowledge regarding leaf temperature, hydraulics, and photosynthetic responses, future studies can better inform conservation strategies to protect these vital coastal ecosystems from the cascading impacts of global warming.