Hydraulic traits in seasonally dry tropical forests

A thesis

submitted in partial fulfilment of the requirements

of the degree of

Doctor of Philosophy

by

Ron Sunny
20132020

Indian Institute of Science Education and Research
Pune

2020

CERTIFICATE

Certified that the work incorporated in the thesis entitled “Hydraulic traits in seasonally dry tropical forests” submitted by Ron Sunny 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.

Date: 10/09/2020

Dr Deepak Barua
Supervisor

DECLARATION

I declare that this written submission represents my ideas in my own words and where others' ideas have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have not been properly cited or from whom proper permission has not been taken when needed.

Date: 10/09/2020

Ron Sunny

ACKNOWLEDGEMENTS

I am grateful to Dr Deepak Barua, for taking me in as a student, for being a very patient teacher, for setting standards that often seemed impossible, but felt right when they were done, for facilitating my growth as a researcher and as a thinker. Thank you for the constant mentorship whenever I was confused, and the brutal honesty that helped me reinvent myself repeatedly.

I thank Dr Sutirth Dey, Dr Ramana Athreya, Dr Richa Rikhy and Dr Mahesh Sankaran for their feedback on my work as research advisory committee members

I am grateful to all past and present fellow lab mates for the many discussions, *chai* breaks, dinners and laughs that made this journey enjoyable. Aniruddh, Shatarupa, and Souparna as seniors in the lab have always set very high standards for me to meet, both at the discussion table and on the field. I am ever grateful to Souparna, whose enormous effort resulted in a wealth of species-level phenology and distribution data in the lab that many students, including myself, are continuing to reap the fruits of. A big thanks to Aboli, my first fieldwork guide – your excitement on the field is infectious! Aparna, Rasmhi and all previous lab members who made this lab fun!

I am grateful to Anirban Guha, who as a post-doc in the lab introduced me to the fascinating world of plant physiology and taught me important lessons of professionalism, consistency and teamwork, apart from all the science.

I thank Asmi, Kavya and Neha for their help in the common garden experiment. Only you girls can make weighing 20 Kg pots fun.

There were several undergraduates and postgraduates with whom I had the pleasure of working with. I especially thank Peddiraju, Malavika and Manish for helping out in standardising a lot of new assays in the lab.

I am ever so grateful to Rishiddh with whom I did the massive hydraulic traits campaign in Sirsi-Karnataka and Nigdale-Maharashtra. You made fieldwork so much fun, and I couldn’t have asked for a better partner in that project. I thank Balachandra Hegde for helping with the campaign in Sirsi, generously allowing us to carry out experiments on his property and challenging my perspectives on conservation. I also thank Balu’s family, especially Kusuma akka, for treating me as family during my stay in Sirsi during the campaigns. I thank

Raghavendra (NCBS- Bangalore) for all his help and constant support during the Sirsi campaign.

I thank Dr Narendra Kadoo (NCL- Pune) who kindly loaned their pressure chamber, necessary for a large part of the work that formed Chapter 3 in this thesis. I am indebted to Amol K Kasodekar for his training and constant help especially in using this instrument.

I thank Dr Mahesh Sankaran for providing species dominance and species abundance data for their field site in Sirsi, Karnataka which was used to select species for the studies based in that region.

Field work would have been impossible without the incredible people who helped me out. I thank Kalu kakka and Gabru kakka from Nigdale and Gappu anna and Vinayaka anna from Sirsi, for not only being pro-actively helpful for all the campaigns but also supporting me when things got tough and tricky during those field works.

A big thaks to Hausa maushi, for providing me with space to set up a work station at her home in Nigdale. I also thank Taku maushi and Saku maushi for their warmth and hospitality during my stay at Nigdale.

I am deeply grateful to Prof Emanuel Gloor and Dr David Galbraith from the University of Leeds, UK who are not just brilliant scientists, but also two very kind and wonderful people. I thank them for taking me on-board in their project to set up a long-term monitoring plot in India. I also thank Prof Emanuel Gloor for hosting me in the University of Leeds under the Newton Bhabha Scholarship program. This exposure and discussions with them has certainly helped me grow as an independent researcher. I also thank Rakesh for all his help during my stay at Leeds, his amazing food and wonderful friendship. I thank Julia Tavares for training me in the usage of the pneumatic method to generate vulnerability curves for the Indian species and for the extremely useful discussions we have had. But most importantly I thank you Julia for being a kind and loving friend (Go Hydraulics team!).

I thank Department of Biotechnology, India and British Council, UK for awarding me the Newton Bhabha scholarship which allowed me to spend 6 months in the University of Leeds, UK for the collaborative work with Prof Emanuel Gloor, Dr David Galbraith and Julia Tavares.

I thank NERC-UK for funding the long term tropical observatory setup in Sirsi and the hydraulic trait campaigns held there.

I thank IISER-Pune for funding and providing facilities including the greenhouse, microscopy facility and microtome facility. I thank the IISER administration staff, especially Kalpesh, Mrinalini and Tushar for their constant support that facilitated smooth running of all the field work and experiments. I especially thank our greenhouse technician Niteesh for the constant help during the experiments.

Life in IISER would not have been this exciting if it weren’t for my wonderful Integrated PhD 2013 batch matches. Apart from having smart consultants readily available across various streams in Biology, Physics and Chemistry, it was wonderful growing up with them (seven years!) and sharing so many memorable experiences. Adarsh, Amar, Anish, Aditi, Akhila, Anshul, Bharat, Charu, Deepak, Dhriti, Divya, Harpreet, Jay, Kashyap, Mehak, Neel, Sandip, Shivani and Swati – I thank you for everything! Thank you Mukul for all the laughs.

A special shout out to the other three *Indian wise men*; Anish, Neel and Sandip; who easily, consistently and fruitfully elevated the level of all discourse and most importantly taught me unconditional friendship. Each of you in a unique way have made me a better person. For all the things we did together, for all the fun you forced me to have, for being there whenever there was a reason and whenever there wasn’t one – I thank you. I am so grateful for being blessed with the three of you guys.

I also thank all those people who constantly did the important job of making me realise that there is more to life than IISER. I thank Naseef for his friendship and support during these years. I thank my friends from *Jesus Youth* especially *Vox Christi* for music, love and laughter.

I thank Acha, Ammi, Don, Samantha and Romy for their constant support, patience and understanding throughout this long process. No, I still cannot explain why this ‘course’ took so long.

Most importantly, I thank God who lovingly made sure that I seek beauty, goodness and truth, above and beyond another degree.

SYNOPSIS

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Chapter 1: Introduction

Water is essential to sustain plant growth and function. The tropical biome has a large variation in temporal and spatial water availability, and hence water is an important limiting factor in these forests. In fact, local, regional and global patterns of species distribution are strongly linked to water availability. However, the underlying mechanisms that bring about these patterns are complex and understanding this has been a long-standing challenge. Further, the rapid pace of climate change and the associated increase in the frequency and severity of drought events pose a significant threat to these forests. Given the disproportionate and essential contribution of the tropics to global ecosystem services, understanding how tropical species will respond to drought is urgent. Hydraulic traits, measured at the individual level, give functional insights into species water use strategies. Over the past few decades, this field of hydraulic trait ecology has been useful in understanding variation in plant-water relations quantitatively. This thesis uses a multi-species, multi-trait approach to examine this variation in water-use strategies in tropical forests.

Chapter 2: Drier tropical forests are more at risk, but may be better at buffering effect of drought than wetter forests

A global synthesis of plant hydraulic safety margins (HSM), a measure of the potential risk of drought-induced hydraulic failure, found that species across biomes operated at very narrow margins but also revealed large within biome variation that needs to be accounted for. We took advantage of the 8-fold increase in HSM data for tropical species. We examined how the minimum water potentials experienced (Ψ_{min}), xylem resistance to embolism (Ψ_{50} and Ψ_{88}), and the resultant HSMs were related to the large variation in precipitation in the tropics. We confirm previous observations with a much larger dataset that these species operate with very low safety margins but report much larger variation across species. Both Ψ_{min} and Ψ_{50} were related to precipitation such that species that experienced more stressful environments were adapted to these conditions with xylem that was more resistant to embolism. However, contrary to previous findings, species in wetter sites were found to be safer than those in drier sites. Moreover, drier sites had higher within-site variation in these hydraulic traits, indicating that these sites with diverse strategies may have greater capability in buffering the effects of drought than wetter tropical forests.

Chapter 3: Hydraulic safety margins in two seasonally dry tropical forests with contrasting rainfall regimes

The hydraulic trait composition of communities can affect ecosystem function and can be used as reliable indicators of community-level mortality risk and resilience to drought. The characterisation of hydraulic trait variation is fundamental in species-rich tropical forests to understand how these systems will respond to future climates with increasing droughts. Despite the rapid increase in information on hydraulic traits, global coverage remains patchy, and studies examining community-level trait assemblages are few. We compared hydraulic traits and hydraulic safety margins (HSM) for dominant species from two seasonally dry tropical forests with contrasting rainfall regimes in the northern and central parts of the Western Ghats range in the Indian subcontinent. We examined the importance of accounting for species dominance, tested if commonly measured functional traits were related to hydraulic traits, and asked how species that were common to both sites differed in these traits. There was a considerable variation in hydraulic traits in species from these forests, comparable to the ranges observed in tropical regions worldwide. The drier northern Western Ghats site had a higher community weighted HSM based on resistance to embolisms that resulted in 88 % loss of hydraulic conductivity, indicating a lower risk of catastrophic hydraulic failure and mortality. Comparison based on species averages and HSM based on 50 % loss of hydraulic conductivity predicted different outcomes. This inconsistency highlights the importance of accounting for differences in species dominance, and the influence of the slope of the xylem vulnerability curves in determining the risk of forest communities to drought. We found no relationship between commonly measured functional traits and hydraulic traits or HSMs, indicating possible independence of their carbon and hydraulic economies. Finally, intraspecific variation in traits was species-specific, implying that the species use diverse strategies beyond resistance to xylem hydraulic failure to occur in these two very different sites.

Chapter 4: Beyond turgor loss point, resistance to loss of cellular function and rate of water loss represent independent ecologically important hydraulic strategies for dry tropical forest trees

The considerable variation in species responses contributes to the complex mortality patterns observed in forests due to recent drought events. In trying to understand variation in species drought tolerance, the focus has been on a few key traits, but the multi-dimensional nature of plant responses to drought is often overlooked. Beyond resistance to loss of leaf turgidity and shrinkage, that are important during the early stages of dehydration, we examined variation in resistance to loss of cellular function and integrity, quantified as the loss of the maximum quantum yield of Photosystem II (F_v/F_m) that represents resistance to severe dehydration. This study was done in eighteen coexisting tree species from a seasonally dry tropical forest. Additionally, we examined rates of water loss after stomatal closure (g_{min}), a trait that indicates how quickly physiological damage thresholds are approached. We observed large variation in the hydraulic traits examined. Interestingly, resistance to early dehydration quantified by loss of turgidity and shrinkage, resistance to severe dehydration quantified by loss of photochemical function (F_v/F_m), and the rate of water loss (g_{min}) were not related to each other in these 18 species. Species that had experienced very low leaf water content in the driest time of the year had high resistance to loss of photochemical function. The rate at which species lost water post stomatal closure was higher for deciduous species with larger stomatal pores and lower leaf mass per area. Thus, resistance to early dehydration, resistance to severe dehydration, and the rates of water loss are ecologically important and may represent independent strategies in dealing with drought in seasonally dry tropical forests. Incorporating these understudied dimensions of response to drought is important to understand drought-induced mortality in these forests.

Chapter 5: Response of whole-plant water use to limiting light and water conditions are independent of each other in seedlings of seasonally dry tropical forests

How co-occurring species vary in the utilisation of the shared and limited supply of water, especially in the context of other limiting resources like light, is essential for understanding processes that facilitate species coexistence and community assembly. For seedlings in a seasonally dry tropical forest that experience large heterogeny in light and water conditions, how water use, leaf physiology, and subsequently plant growth, is affected by limited water and light availability is still not well understood. In a controlled common garden experiment with seedlings of four co-existing and commonly occurring dry tropical forest species, we examined how whole plant water uptake responds to limiting water and light conditions, and whether these responses are reflected in leaf physiology, and translated to growth. Water use varied dramatically in seedlings of the four species with a five-fold difference in well-watered plants grown in full sunlight. Species varied in their response to shade but did not differ in responses to the low water treatment, possibly resulting from the strong selective force imposed by the very low water availability and the long dry period in these seasonally dry forests. Interestingly, species response in water use, physiology, and growth in limiting water conditions was independent of light. Thus, species response to both these limiting conditions may evolve independently. Responses in water use were largely congruent with responses in leaf physiology and growth. However, while magnitude of changes in leaf physiology were largely driven by light conditions, changes in whole-plant water use and growth were influenced to a greater degree by the water treatment. These results highlight the need to measure whole-plant water use to better understand species responses to limiting water and light conditions in these seasonally dry tropical forests.

Chapter 6: Conclusions

This thesis reports large variation in hydraulic trait diversity in seasonally dry tropical forests and highlights important understudied hydraulic strategies in these systems. This is also the first characterisation of key hydraulic traits for species from the Indian peninsula. This is important especially given that this region is rich in biodiversity, is home to a large number of endemic species and yet remains largely understudied. Overall, the large variation in hydraulic traits observed in these tropical forests reflects multiple coexisting hydraulic strategies, which may be an evolutionary outcome of the strong selective pressure that water availability exerts in these systems.

CONTENT

Chapter 2 **Drier tropical forests are more at risk, but may be better at buffering effect of drought than wetter forests**

Chapter 3 **Hydraulic safety margins in two seasonally dry tropical forests with contrasting rainfall regimes**

Chapter 4 **Beyond turgor loss point, resistance to loss of cellular function and rate of water loss represent independent ecologically important hydraulic strategies for dry tropical forest trees**

Chapter 1

Introduction

Water, the tropical biome and climate change

Water is the most abundant molecule on Earth, yet its availability is a crucial factor restricting global plant distribution and productivity. From the wet tropical forests in Amazonia to the seasonally dry grasslands like the Asian steppes to the bare desert systems like the Atacama, rainfall abundance and seasonality drive plant survival, growth and persistence. Water is essential to sustain plant life. It is the medium for all cellular metabolic activity. It maintains the plant in an upright form by exerting a positive pressure (turgor) on the cell wall. It also mediates the transport of carbohydrates, hormones, and nutrients across the plant body. Perhaps most importantly, the unavoidable loss of water while letting CO_2 into the leaf through the stomates (microscopic valves on the leaf surface, that regulate gas exchange), is crucial to sustaining plant function and growth (Lambers et al. 2008).

The tropical biome is home to more than 50 % of global biodiversity, and the tropical countries store two-third of the global plant biomass (Pan et al. 2013). It serves as a significant global carbon sink and strongly influences biogeochemical cycles and global climatic patterns. Apart from providing vital economic, social and ecological services to humans who directly or indirectly rely on them, the tropics sustain a large fraction of the global fauna. These regions are essential to preserving overall global biodiversity. The world's wettest and driest locations are in the tropical biome. Especially in the tropics, dry periods can vary widely from a few weeks in every other year in tropical wet forests, to several months in seasonally dry forests (Markesteijn 2010, Trewin 2014). Thus, water availability through rainfall is an essential factor that shapes the different tropical ecosystems, which broadly include the tropical rainforests, seasonally dry forests, woodland forests and savannas (Dirzo et al. 2011). This is also evident from the various studies that indicate that patterns of species richness, composition and abundance are related to rainfall gradients (Esquivel-Muelbert et al. 2017a, Markesteijn 2010, Lambers et al. 2008). Even at regional and local scales, soil moisture availability is strongly related to species distribution patterns (Engelbrecht et al. 2007). However, the mechanisms that determine these global and local species diversity patterns are complex and have been a long standing challenge in plant ecology.

Climate change has drastically altered timing, magnitude and duration of rainfall resulting in severe drought events (IPCC 2014), especially in the tropics (Feng et al. 2013). There have been several accounts of climate change associated drought induced plant mortality (Allen et al. 2010). This has had huge impact on the flora of tropical forest systems, especially with regard to changes in plant above ground biomass, growth, recruitment and composition (Allen et al. 2010, Fauset et al. 2012, McDowell and Allen 2015, van der Sande et al. 2016, Qie et al. 2017). For instance, in large parts of Amazonia that had experienced more intense dry seasons in the past 30 years, there has been a slow but gradual increase in dry-affiliated species and a decrease in wet-affiliated species (Esquivel-Muelbert et al. 2019). Similarly forests in Ghana that have experienced two decades of long-term drought has seen a shift to deciduous forests (with trees that seasonally shed their leaves) and an increase in above ground plant biomass (Fauset et al. 2012). However, understanding how these trends scale to other tropical systems is complex and challenging, owing partly to the lack of understanding of mechanisms related to drought induced plant mortality. Projections indicate that drought events are likely to increase in intensity and duration at a global scale (Duffy et al. 2015, IPCC 2014) and such extreme climatic events are likely to have drastic impacts globally (Thomas et al. 2004, Cox et al. 2004). Given the high biodiversity of the tropics, the disproportionate and essential contribution of tropics to global ecosystem services and the threat that it is currently experiencing, there is an urgent need to understand how tropical forest will respond to climate change associated drought scenarios.

How do plants respond to drought?

It is the maintenance of an intact ‘water column’ or an intact hydraulic network that helps sustain plant activity. This “thread” of water runs from the wet soil, into the root (via the root hairs), where they enter into plant vascular system, a network of conduits composed of the xylem, through which the water travels up via the stem to the leaf. These xylem vessels branch out to form veins in the leaf, through which the water moves into the cells of the leaf to final exit through the stomata into the drier atmosphere. Cohesion tension theory proposes that this water column is maintained by the cohesive force between the water molecules and the tension generated by the pressure gradient between the wet soil and the dry air (Stephen Hales 1727). This gradient causes a continuous pull of this “thread” of water from the soil into the air. An intact water column from the wet soil to the dry air, ensures the necessary loss of water through the stomates in exchange for CO2. This carbon, forms the building blocks of the growing and thriving life on earth.

During drought (drying up of the soil), the thread is pulled not only by the dry air upwards, but also downwards by the dry soil, increasing the tension on the thread. This high tension results in breaking of the water column (cavitation) and induces dissolved air and air sucked in from adjacent pores to form an air bubble in the pipe system. This blockage cuts-off the water supply to the leaves. Failure to re-establish the water column breaks hydraulic conductance, disrupts photosynthesis and eventually leads to death. Recent work (Bartlett et al. 2016, Choat et al. 2018, Trueba et al. 2019) summarises our current understanding of the order of events that led up to plant death due to drought. One primary response to early or mild drought is stomatal closure that reduces the tension on the column. However, this stops intake of CO_2 and eventually leads to carbon starvation. While early response to mild drought involves stomatal closure and loss of turgidity (wilting), continued drying leads to tissue shrinkage and loss of hydraulic conductance. Severe dehydration results in the irreversible damage of the cellular machinery. When the use of available energy (ATP) to sustain metabolic activity (respiratory loss) cannot be compensated for (by synthesis of new ATP), it ultimately leads to plant death.

Species, vary widely in their response to drought, some being better than others in dealing with it. For example, some species are able to maintain intact water column and hence hydraulic conductance, even at very low soil water potential. The water potential (Ψ) is the standard unit of water status of a system that corresponds to potential energy of water in the system. By convention pure distilled water with more ‘free’ water molecules, has a water potential of 0 MPa, while water with dissolved solutes such as in the leaf, has a negative water potential. Initial efforts towards the daunting task of understanding species variation in drought tolerance involved categorising species based on habitat preference. Species from drier environments were classified as more tolerant than species that were common to habitats with more abundant water availability. This classification was not useful to compare drought tolerance in species from say very different regions, and could not be used to predict drought tolerance effectively. Using plant traits that are measureable features of the plants that is directly to its performance, has made characterising drought tolerance more quantitative and globally comparable.

One such plant trait that is widely used is the xylem water potential at which xylem hydraulic conductance is lost. This physiological trait can be quantified for species, making comparison across species possible. The water potentials that results in a 50 % (Ψ_{50}) or 88 % (Ψ_{88}) loss of xylem hydraulic conductance is directly linked to physiological function, the ability to maintain hydraulic conductance during drought, and is can be used to predict species mortality in forests that have experienced severe drought (Anderegg et al. 2015). This understanding of the scope of using plant traits that represented a functional aspect of a species led to a large surge in trait measurement and rapid advancement of the field of trait ecology. Ranking species based on vulnerability to drought and prediction of drought induced forest mortality, has thus become more quantitative.

Examining hydraulic trait variation in seasonally dry forests

Earlier ecosystem models that were used to understand response of forests (or plant communities) to extreme climatic scenarios, considered a 'big leaf' approach where the response of one or a few representative species were used to model the response of a forest. Such studies often predicted either large-scale mortality (Cox et al. 2004, Good et al. 2011) or persistence (Friedlingstein et al. 2006, Cox et al. 2013). However, especially over the last three decades, there has been an increased understanding of the fact that not all species are equal. Accounting for the differences in species response to water limitation is crucial to get more realistic and nuanced predictions of how forests will respond to climate change (Fisher et al. 2015, Xu et al. 2016, Manoli et al. 2018). This thesis aims to understand variation in species response to limiting water conditions and associated variation in different strategies that incur tolerance to such conditions. I examine possible underlying mechanisms that bring about this variation and also study their ecological consequences. This thesis also contributes to the much needed understanding of the understudied Indian tropical forests.

The minimum water potential (Ψ_{min}) or relative water content (RWC_{min}) experienced by species in their natural environment in the driest time of the year helps put estimates of hydraulic traits in the context of other traits that influence the likelihood of experiencing low water potentials (Meinzer et al. 2009). For a species, the difference between the water potential that results in 50 % reduction in hydraulic conductance (Ψ_{50}) and the minimum water potential experienced in its natural environment (Ψ_{min}) defines a operational safety margin called the hydraulic safety margin (HSM). Hydraulic safety margins are reliable indicators of the risk of catastrophic hydraulic failure and are related to species mortality during drought (Anderegg et al. 2016). The past three decades has seen a surge in quantification of hydraulic traits globally, and a global analysis that compared hydraulic safety margins across biomes (Choat et al. 2012), revealed large variation in HSMs within biomes. Since species difference in their response to drought maybe related to the rainfall they experience (Trueba et al. 2017, Esquivel-Muelbert et al. 2017b), this biome level variation in HSM may be related to the precipitation these species experience at their sites. Taking advantage of the 8-fold increase in HSM data for tropical species since this global meta-analysis (Choat et al. 2012), we conducted an exhaustive literature survey to put together a dataset for xylem vulnerability to embolism, the minimum water potential experienced by tropical species, their HSMs. This allowed me, in my first chapter, to examine relationships between safety margins and climate for tropical species.

In order to understand how a forest community responds to drought, it is important to account for the relative contribution of each of the component species to the community response. Interaction between species is now increasingly studied using traits (McGill et al. 2006, Miller et al. 2018), and this is useful because it accounts for the variation in species pool available for facilitating dynamics in community assembly, in a quantitative manner. Incorporating performance based traits has more recently been appreciated to be a powerful tool in understanding community level function, and also as a response to varying environmental conditions (Miller et al. 2018, Barros et al. 2019). In my second chapter, I quantified hydraulic traits and HSMs in dominant species from two seasonally dry tropical forests communities that differ in the rainfall they experience, to understand how these forests will respond to changing rainfall patterns. This is also the first study that examines xylem hydraulic vulnerability for forests from the Indian peninsula.

Physiological function based tolerance indices like the resistance to loss of hydraulic conductance (Ψ_{50} or Ψ_{88}) and resistance to loss of turgidity (Ψ_{TLP}) have helped give us invaluable insight into species variation to drought tolerance and have proved useful in ranking species vulnerability to drought induced mortality. However, the rate at which plants lose water to reach critical water potential threshold that result in physiological dysfunction, has been largely overlooked. It is often assumed that the rate of water loss through a leaf after stomatal closure, called the minimal cuticular conductance (g_{min}) is similar across species, or at least that differences in rates of drying across species is small. Thus, the rate at which species approach critical water potential thresholds should be similar, and differences in drought tolerance will largely be determined by the species-specific physiological breakdown thresholds. Recent studies have shown that variation in g_{min} across species can be substantial (Duursma et al. 2019), and when integrated over time these differences in water loss become large enough to be of ecological consequence (Blackman et al. 2016). This has led to an increased attention in species variation in ‘time to death’ as an important factor that determines species ranking in plant mortality. Another important aspect that has been overlooked is species response to severe water stress. While loss of turgidity and loss of hydraulic conductance occurs at early stages of drought, perhaps when damage is mostly reversible (Brodersen and McElrone 2013), response to severe dehydration which can lead to irreversible damage has been largely overlooked. There is a need to identify traits that correspond to resistance to irreversible lethal damage during severe drought that may be more directly linked to plant mortality. Plant water status at which Photosystem II, an important component of the photosynthetic machinery, breakdown occurs (RWC_{flbrk}) is a candidate trait that has been used in crops to understand variation in resistance to severe drought. In my third chapter, I incorporated these two understudied aspects of plant drought resistance, minimising the rate of water loss and resistance to severe drought, with the widely used early response measure of resistance to turgor loss, to examine how these different strategies are related to each other in coexisting species in a seasonally dry tropical forest in India.

Light is one of the most important limiting factor in the tropics (Nemani 2003). Especially for seedlings growing under the canopy in seasonally dry tropical forests, apart from dealing with limitation in water they have to necessarily deal with limitation in light availability. Thus seedlings of these systems are expected to have evolved strategies to simultaneous deal with these limiting light and water (Sterck et al. 2011, Schönbeck et al. 2015). How limitation in light influences species drought response has been a topic of interest for over half a century, but primarily for temperate species (Niinemets and Valladares 2006, Holmgren et al. 2012, Kupers et al. 2019). How these multiple limiting factors affect use of shared resources (such as water) in co-occurring seedlings, is crucial to understand species interactions, possible niche partitioning, and community assembly. Moving from understanding organ level responses in the previous chapters, in the fourth chapter I examined whole plant water use in seedlings of four dominant species from a seasonally dry tropical forest. I examined the responses to limiting light and water conditions mimicking those levels that these species experience on field. Unlike the previous chapters, this experiment was carried out using seedlings in a controlled common garden experiment, focussing on examining how responses quantified at the leaf level scales to whole plant level water use and growth.

Indian forest and climate change

India with its large topographic and climatic gradients, is home to 11% of the world’s flora. It is one of twelve mega biodiversity countries (Myers et al. 2000) with four of the 34 biodiversity hotspots falling partly within its political boundaries. The Indian forests which span almost 700,000 km^2 is directly or indirectly linked to the livelihoods of 275-400 million people (MOEF 2006). Recent studies indicate that the spatial extent and co-occurrence of drought events and heat-waves have increased across India. The occurrence of drought has increased in the central-north east India, and west central India during the last 60 years (Sharma and Mujumdar 2017, Mondal et al. 2015). Increased droughts have had massive impact on livelihood, economy as well as ecology. Very few studies have documented the effects of these droughts on the Indian forest vegetation (Khan et al. 1994, Suresh et al. 2010). Few studies have examined the effect of drought, and these are limited to examining affects on the spatial distribution of a handful of species (Remya et al. 2015, Priti et al. 2016, Deb et al. 2017a, 2017b, Chitale et al. 2012). These studies have shown that large changes in spatial distribution of these species were primarily driven by changes in water availability. Using vegetation dynamics models these studies have made predictions for how the spatial distribution of species are likely to change based on various projected future climate scenarios. The few studies that examined regional vegetation change patterns (Chaturvedi et al. 2011, Gopalkrishnan et al. 2011) predicted large and varied response of the Indian forests to projected climate scenarios. Gopalkrishnan et al. (2011) predicted that 45 % of the forested area are likely to undergo large changes, and the Northern Western Ghats, the Eastern Ghats, parts of central India, and the vegetation in the upper Himalayan stretches may be particularly vulnerable. These studies that model the effect of future climate change scenarios on vegetation dynamics do not account for the large species differences in their physiological thresholds (Behera et al. 2019). Similar to our understanding of variation in temperature tolerance (Sastry and Barua 2017), there are only a handful of studies that have examined species variation in water use (Chitra-Tarak et al. 2018) and variation in response to limiting water conditions through controlled garden experiments (Bhadouria et al., Tripathi et al., Khurana and Singh 2004, Sastry et al. 2018). Given that climate predictions suggest more frequent droughts in 2020-2049 (Joseph et al. 2016), there is an urgent need to understand species variation in response to climate change associated drought in Indian forests. There are however several recent commendable concerted efforts towards addressing this lack of understanding as can be seen in (Behera et al. 2019), and other ongoing projects including 'LEMoN' (long term ecosystem monitoring network) and 'Indian observatory for tropical forest response to climate change' (funded by NERC, UK). However these efforts will still remain short of gaining a holistic understanding of how Indian forests will respond to climate change scenarios (Chitale and Behara, 2014), given the immense and unique diversity of the forests of the Indian peninsula. This thesis is a much needed characterisation of the functional variation in Indian dry forest tropical tree species in response to limiting water conditions.

The thesis primarily focuses on the Western Ghats, which is a global biodiversity hotspot, with 56 % of the tree species found here being endemic to this region (Pascal et al., Myers et al. 2000). This mountain range, which runs parallel to the western coast of the Indian peninsula, varies widely in spatial and temporal water availability; from moderate rainfall and high seasonality in the north to high rainfall and low seasonality in the south (Davidar et al. 2005). This makes the Western Ghats of India an ideal system to examine variation in species response to water limitation, which I take advantage of in this thesis.

Thesis outline

This thesis uses a multi-species, multi-trait approach to examine functional variation in species response to limiting water conditions. To summarise, the following questions are addressed in the four subsequent chapters:

1. *Examining pan-tropical patterns of risk of hydraulic failure*; how do species that differ in the rainfall they experience across the tropical biome, differ in their resistance to xylem hydraulic failure and in the minimum water potential they experience in the driest time of the year? How does this translate to differences in their hydraulic safety margin?
2. *Towards examining community level differences*; for two Indian forest communities that differ in the rainfall they experience, how do the dominant species differ in their hydraulic safety margin? How do these sites compare with other tropical forests? Further, do species that are common in both these sites differ in their hydraulic traits?
3. *Towards examining other dimensions of resistance to drought*; for coexisting species of a seasonally dry tropical forest, how is early response to mild dehydration related to response to severe dehydration? Are these strategies related to the ability to minimise water loss during dehydration post stomatal closure?
4. *Towards examining whole plant level response to limiting light and water conditions*; how do seedlings of species that differ in their water use, differ in how they respond to limiting light and water conditions, especially with respect to growth and water use? Do responses at the level of the leaf translate to whole plant level responses?

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Chapter 2

Drier tropical forests are more at risk, but may be better at buffering effect of drought than wetter forests

Abstract

A global synthesis of plant hydraulic safety margin (HSM), a measure of the potential risk of drought induced hydraulic failure, found that species across biomes operated at very narrow margins but also revealed large within biome variation that needs to be accounted for. We took advantage of the 8-fold increase in HSM data for tropical species, and examined how Ψ_{min}, Ψ_{50} or Ψ_{88} and the resultant HSM were related to the large variation in precipitation in the tropics. With a much larger dataset we confirm previous observations that these species operate with a very low safety margin but have a much larger variation. Both Ψ_{min} and Ψ_{50} were related to precipitation such that species that experienced more stressful environment were adapted to these conditions with xylem that was more resistant to embolism. However, contrary to previous findings, species in wetter sites were found to be safer than those in drier sites. Moreover, drier sites had higher within site variation in these hydraulic traits, indicating that these sites with diverse strategies may have greater capability in buffering the effects of drought than wetter tropical forests.

Introduction

The predicted increase in the frequency and intensity of droughts associated with climate change is expected to decrease productivity and increase mortality in many tropical forests (Gatti et al. 2014, Duffy et al. 2015). Advances in our understanding of the underlying mechanisms suggest that hydraulic failure resulting from embolisms is the primary cause of drought induced tree mortality (Rowland et al. 2015, Adams et al. 2017, Choat et al. 2018). Hydraulic traits provide mechanistic insights that allow us to assess how species will respond to changing water availability and increased vapor pressure deficit, and help predict species vulnerabilities to drought (McDowell et al. 2015, Anderegg et al. 2015, Benito Garzón et al. 2018).

The xylem water potentials that result in a 50 % and 88 % loss of hydraulic conductance (Ψ_{50} and Ψ_{88}) are commonly used measures of embolism resistance (Maherali et al. 2004, Choat et al. 2012). Hydraulic safety margins (HSM) for species represent a measure of the potential risk of hydraulic failure, and are quantified as the difference between Ψ_{50} or Ψ_{88}, and the minimum water potential experienced by species in their natural environments (Ψ_{min}) (Meinzer et al. 2009, Choat et al. 2012). Species that operate with low HSMs are more at risk of hydraulic failure and mortality during drought (Anderegg et al. 2016, Benito Garzón et al. 2018, Powers et al. 2020).

In a global analysis of hydraulic traits, Choat et al. (2012) found that most tree species operated with low HSMs and are therefore at high risk of hydraulic failure. Average HSMs for biomes were largely independent of annual precipitation indicating a global convergence of vulnerability to drought (Choat et al. 2012, Engelbrecht 2012). However, there was large variation within biomes for species HSMs. For example, in the tropics, HSM-Ψ_{50} ranged from -1 MPa, indicative of risky strategies and high vulnerability, to relatively safer margins of greater than 2 MPa. While the reason for this wide range of HSMs within species in biomes is not well understood, these differences likely represent different hydraulic strategies, and importantly, imply different vulnerabilities and consequences for species to drought.

Water availability varies widely in the tropics (Feng et al. 2013), and this variation in water availability shows strong association with species distributions (Steege et al. 2003, Engelbrecht et al. 2007, Kursar et al. 2009, Esquivel-Muelbert et al. 2017). Tropical species from drier sites typically have hydraulic traits that are better adapted to low water availability, including stem xylem with more negative Ψ_{50} and Ψ_{88} that are more resistant to embolism (Choat et al. 2012, Zhu et al. 2017). However, species from drier sites typically also experience more negative minimum water potentials (Bhaskar and Ackerly 2006), and it is unclear how resultant HSMs change in tropical species across gradients of water availability (Adams et al. 2017, Allen et al. 2017).

Since Choat et al. (2012), which included 81 sites from across the globe, and 226 forest tree species, there has been a large number of studies that have quantified hydraulic traits. Tropical sites were underrepresented in Choat et al. (2012), with data for HSM from only 12 sites and 53 species, and this is particularly poor given the high diversity in the tropics. We take advantage of the rapid increase in information on hydraulic traits to examine variation in hydraulic traits and safety margins of adult angiosperm tree species from tropical regions. We compiled a dataset that included published and unpublished data to ask whether hydraulic traits, particularly HSM are related to water availability in tropical regions.

Methods

Literature search, selection criteria and data extraction: We carried out an extensive literature search using Google Scholar for published and unpublished studies that reported either Ψ_{50} and/or Ψ_{88}, and Ψ_{min}. We used 'hydraulic safety margin', 'p50', 'p88', 'minimum water potential', 'xylem vulnerability' and 'plc' as keywords for this search. Additional searches were conducted for references found in the bibliography of, and for studies that had cited, the initial sources identified. We included studies that reported data for HSM-Ψ_{50} and/or HSM-Ψ_{88}, even when they did not report Ψ_{50} or Ψ_{88}. As midday water potential is often much lesser than the predawn water potential during the dry season, in order to capture the maximum stress the xylem experiences in the driest time of the year, only data for the minimum leaf water potentials at midday (Ψ_{min}) was considered. Information for Ψ_{min}, Ψ_{50} and Ψ_{88} were extracted from the text or tables when provided or extracted from figures using Plot Digitizer (Joseph A. Huwaldt, http://plotdigitizer.sourceforge.net/).

We restricted all analysis to adult angiosperm tree species from tropical regions. We used the Whittaker biome classification to select tropical sites (Whittaker 1975). We excluded shrubs and lianas, and studies that examined seedlings to avoid life-form and developmental effects that are known to influence xylem vulnerability and could confound results. We limited this analysis to angiosperms trees and did not include gymnosperms that are less abundant in the tropics and have characteristically different Ψ_{50} and Ψ_{88}. Species from mangrove forests and montane forests (altitude greater than 1500m) were excluded as these often have unique strategies adapted for these unique habitats, and may not be comparable with other tropical species. As the focus of this study was on natural forest systems, we also excluded data from plantations.

From the selected publications, for each species studied, we extracted data for: 1) minimum leaf water potentials (Ψ_{min}) at midday; 2) Ψ_{50}; 3) Ψ_{88}; 4) HSM-Ψ_{50}; 5) HSM-Ψ_{88},; 6) growth habit (tree/shrub/liana); 7) developmental stage (Adult/seedling); 8) method used to generate varying water potentials (bench drying/air injection/centrifugation); 9) method used for determining loss of hydraulic conductivity (pneumatic/ mass flow rate based conductivity); 10) organ used for Ψ_{min} measurement (leaf/stem); 11) Leaf habit (evergreen/deciduous); 12) soil type; 13) site coordinates (latitude and longitude); and, 14) altitude. Additionally, the taxonomic affiliation (family) for the species was obtained from the Plant List (The Plant list 2013, Version 1.1) using the R package 'Taxonstand' (Cayuela et al. 2012). HSM_{50} and/or HSM_{88} were calculated when data were available for Ψ_{50} and/or Ψ_{88}, and Ψ_{min}, but not reported for HSM_{50} and/or HSM_{88}.

In some cases, species level data for a given site was available in more than one publication (i.e. multiple publications reporting data for the same species from the same site). The sites were first checked to be the same by referring to the site descriptions and site coordinates provided in the publications. A difference of greater than 10 minutes in latitude and/or longitude was considered to be significant as the climate data used for this analysis was for 10 minute grids (CRU 2.0, New et al. 2002). If sites with similar coordinates were explicitly described to be different in any aspect such as site topography or soil type, then we considered these as unique sites for our analysis. If the sites were indeed the same in these different publications, then species level data for Ψ_{min}, Ψ_{50} and Ψ_{88}, from these different publications were pooled together. For a given species in a given site, if data was available from more than one publication for Ψ_{min}, Ψ_{50} as well as Ψ_{88}, then the trait values reported were averaged for that species.

Climate data: Monthly rainfall data was extracted from CRU 2.0 (New et al. 2002) for all of the sites identified. Mean Annual Precipitation (MAP) and the average number of dry months (with <100 mm rainfall) was calculated for each site from this monthly data. Data for cumulative water deficit (CWD) for the sites was obtained from Chave et al. (2014). Aridity index was calculated as the ratio of mean annual precipitation and potential evapotranspiration (PET). Since data for PET was not available in CRU 2.0, climate data from CRU 4.0 (Harris et al. 2020) was used to calculate aridity index.

Statistical methods: We analysed the relationships between traits and climate at the level of sites and species using type-I linear regressions. All species at a site were averaged to obtain site level estimates for Ψ_{50}, Ψ_{88}, Ψ_{min}, HSM-Ψ_{50}, and HSM-Ψ_{88}. To understand the relationship between trait variation within sites and climate, we used standard deviation in trait estimates for sites that had six or more species. We used Type II major axis regressions to examine the relationship between Ψ_{50}, Ψ_{88}, and Ψ_{min}. All analysis was done in R (R core team, Version 1.2.5033).

Results and Discussion

An exhausitive search for HSM data for adult tropical angiosperm tree species yielded a dataset that included 419 species from 43 tropical sites from 45 published and unpublished sources (Fig. 1,Table S2, Table S4). These sites represent a wide range of precipitation regimes (Fig. 1C) from 671 mm of annual rainfall and a ten months dry season in Queensland, Australia with (Rice et al. 2004) to 3171 mm of annual rainfall and no dry season in Sucusari, Peru (Tavares et al. unpublished data). Since Choat et al. (2012) which had 11 tropical sites, that included 8 sites in Central and South America, 2 in Australia and 1 in Madagascar, the geographic coverage in the tropics has now vastly increased to 43 sites, which include 9 sites in China, 2 in India, 18 additional sites in Central and South America, and 3 additional sites in Australia. However, there is still no data for HSM from the Congo basin in Africa and a scarcity of information from the Indo-malayan basin (Fig. 1D).

Interestingly, in spite of the large increase in the number of species from a substantially greater number of sites in the current study, the median HSM-Ψ_{50} and HSM-Ψ_{88} for tropical regions (0.43 MPa and 1.76 MPa respectively) was near identical to those reported by Choat et al. (2012) (Fig1A and 1B). However, we observed substantially higher variation in HSMs than previously reported, and these ranged from -3 MPa to 4 MPa for Ψ_{50}, and from -2.5 MPa to 5.5 MPa for Ψ_{88}. Also evident from the current dataset is that a large number of species operate with negative HSMs (greater than 30 % of the species for Ψ_{50}, and nearly 10 % for

Ψ_{88}). This confirms the ealier conclusions that most species in the tropics operate within very low hydraulic safety margins, with almost a third having negative safety margins, and may be at high risk of hydraulic failure in future environments with more frequent and intense droughts.

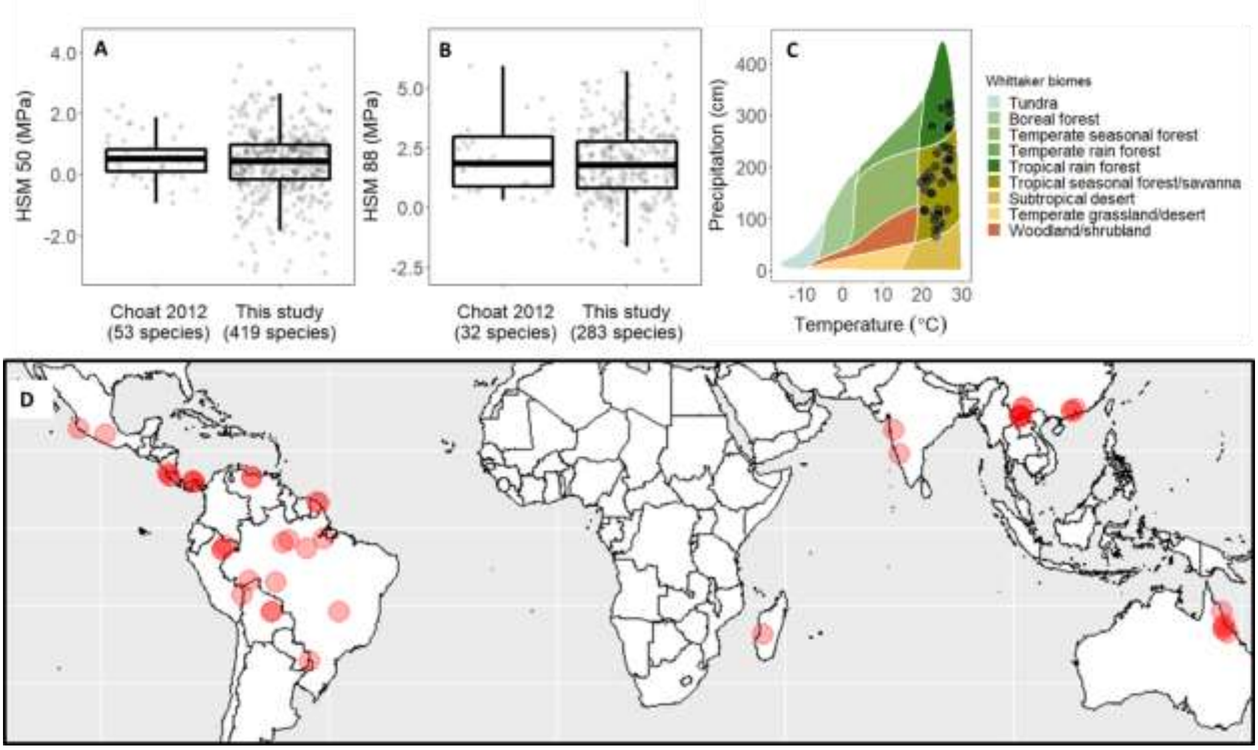

Figure 1: Dataset summary: Hydraulic Safety Margin data for tropical tree species compiled in Choat et al. (2012) and in the current study for: A) HSM-Ψ_{50}; and B) HSM-Ψ_{88}. Each data point corresponds to an adult tree species; C) Whittaker biome plot for the 43 tropical sites included in the current study; and, D) the geographic location of these sites.

Wetter sites are safer

Species from drier sites had more negative Ψ_{min}, likely indicative of the drier conditions that species experience in these environments (Fig. 2A, Table S1). Species at drier sites also had more negative Ψ_{50} and Ψ_{88} (Fig. 2B, Table S1). Thus, tropical species from drier sites are typically more resistant to embolism as compared to species from sites with greater water availability. This is congruent with what has been reported for global analyses (Maherali et al. 2004, Choat et al. 2012), and what has also been observed at smaller geographical scales along gradients of water availability (Choat et al. 2007, Oliveira 2017, Zhu et al. 2017).

In contrast to what was observed by Choat et al. (2012), we found a positive relationship between HSM-Ψ_{50} and MAP (Fig. 2D, Table S1). In examining the relationships between rainfall and Ψ_{50} and Ψ_{min} (Fig. 2A, Fig. 2B), it is evident that the change in Ψ_{min} for a given range of rainfall is higher than the corresponding change in Ψ_{50} for the same rainfall gradient. This difference in how Ψ_{min} and Ψ_{50} change with rainfall likely results in the observed positive relationship between HSM-Ψ_{50} and MAP (Fig. 2D). Thus, our results show that trees at wetter tropical sites had higher hydraulic safety margins compared to drier sites and are therefore likely to be less vulnerable to drought induced hydraulic failure and mortality.

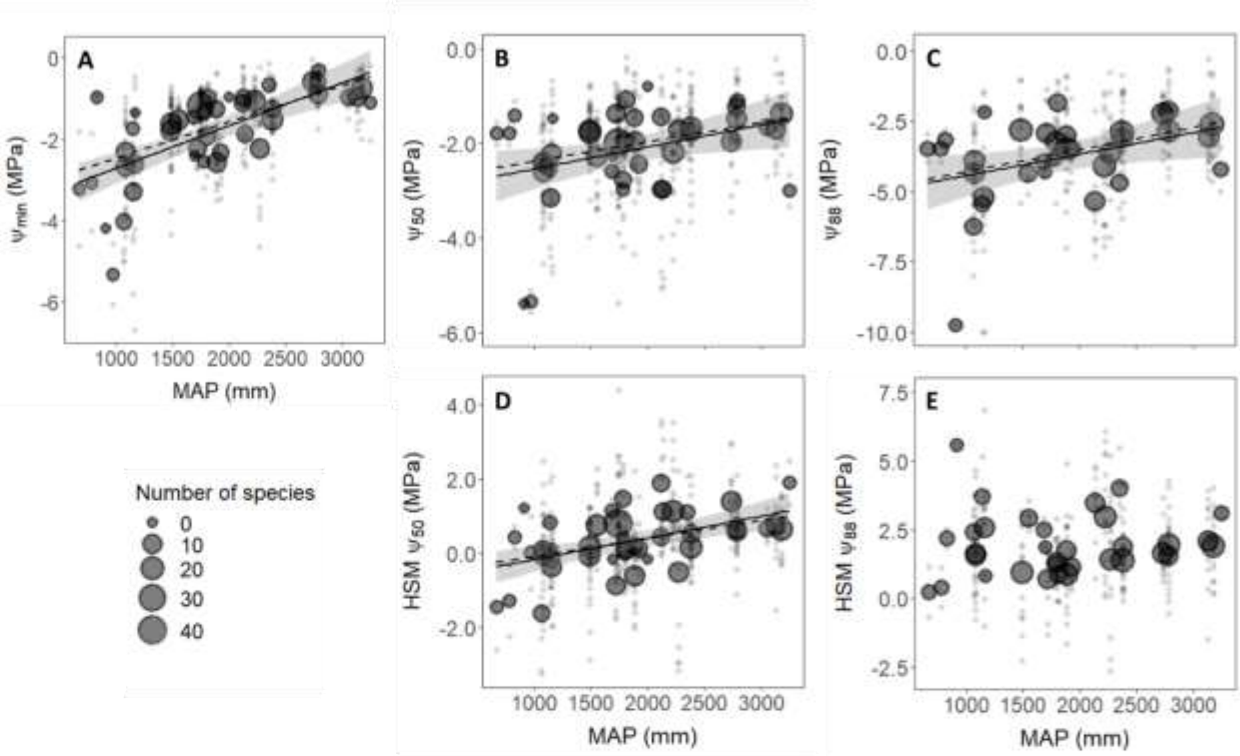

Figure 2: The relationship between mean annual precipitation (MAP), and minimum water potential and hydraulic traits. A) Ψ_{min} - minimum water potential; B) and C) Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50 % and 88 % loss of hydraulic conductance; D) and E) HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and Ψ_{88}. Significant relationships are shown with regression lines for sites (solid line with 95% confidence band in grey), and species (dashed line). The smaller light grey symbols in the background represent individual species and the larger dark grey symbols represent site averages. The size of the larger dark grey symbols are representative of the number of species examined.

The range of Ψ_{min} among species within sites was higher in drier sites where some species were able to maintain water potentials closer to zero (Fig. 3A, Table S1). This indicates that plant water potential in some species in drier sites may be decoupled from the environment, possibly due to stored water in stem (Stratton et al. 2000), access to ground water sources with deep roots (Chitra-Tarak et al. 2018, Brum et al. 2019) and/or strong stomatal regulation (Brodribb and Holbrook 2004, Klein et al. 2014, Bartlett et al. 2016).

Large ranges was also observed for Ψ_{50}, Ψ_{88}, HSM-Ψ_{50} and HSM-Ψ_{88} among species within a site, but with no obvious relationship with MAP. The range of hydraulic safety margins observed within sites, e.g. 5.4 MPa for HSM-Ψ_{50} in the Northern Western Ghats in India, was often substantially higher than the 1.21 MPa difference in mean HSM-Ψ_{50} observed across the 2570 mm gradient of MAP examined. We also examined the relationships of these hydraulic traits with climate variables that accounted for evapotranspiration and seasonality in precipitation, and observed similar relationships to those described for mean annual precipitation (Table S1).

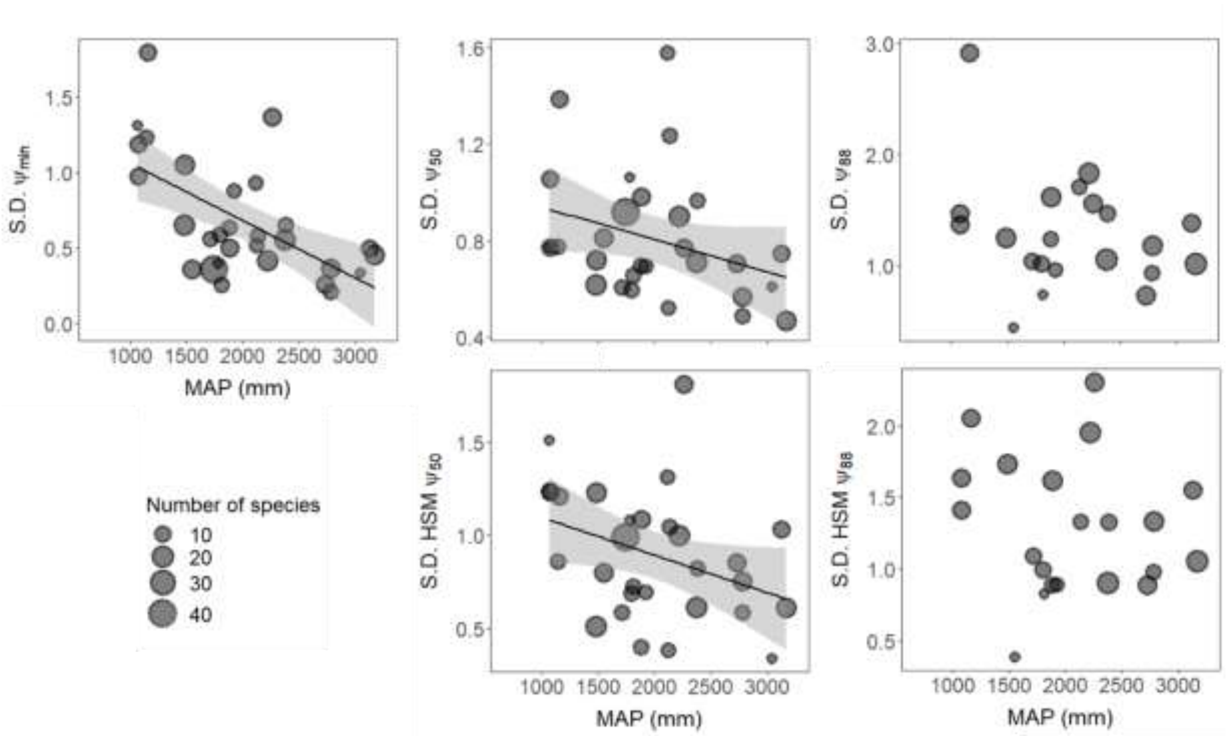

Figure 3: The relationship between mean annual precipitation (MAP) and site standard deviation (S.D.) in minimum water potential and hydraulic traits. A) S.D. of Ψ_{min} - minimum water potential; B) and C) S.D. of Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50 % and 88 % loss of hydraulic conductance; D) and E) S.D. of HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and Ψ_{88}. Significant relationships are shown with regression lines and 95% confidence bands. The size of the larger dark grey symbols are representative of the number of species examined.

Variation in Ψ_{min} and HSM-Ψ_{50} is related to precipitation

Variation in Ψ_{min} and HSM-Ψ_{50} among species within sites was higher in drier sites (Fig. 3A and 3D). Similar relationships were also observed with climate variables that account for evapotranspiration and seasonality in precipitation (Table S1). The observed relationship between variation in HSM-Ψ_{50} and precipitation is driven by variation in both Ψ_{min} and Ψ_{50}.

Thus, differences in species ability to avoid very negative minimum water potentials in drier environments may also be important in the evolution of diverse hydraulic strategies in coexisting species in drier sites.

In a recent study, Anderegg et al. (2018) found that diversity in HSM-Ψ_{50} was the predominant significant predictor of drought responses of temperate and boreal forests. Forests with greater variation in HSM-Ψ_{50} were able to maintain ecosystem function when faced with drought. If also true for tropical systems, given the greater observed variation in Ψ_{min} and HSM-Ψ_{50}, our results suggest that drier tropical sites might be better at buffering effects of drought.

Relationship between Ψ_{min} and Ψ_{50} / Ψ_{88}

We observed a significant positive relationship between both Ψ_{50} and Ψ_{88}, and Ψ_{min}. Thus species that experienced more negative water potentials had higher resistance to embolism. The slope of change in Ψ_{50} with Ψ_{min} was not different from unity (Fig. 4, Table S3), suggesting that species are able to compensate for the more negative water potentials experienced with proportional changes in resistance to embolism (Ψ_{50}).

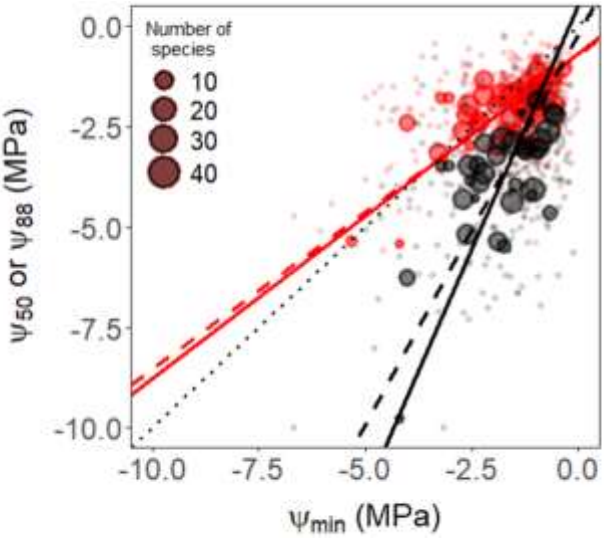

Figure 4: The relationship between minimum water potentials experienced (Ψ_{min}) and resistance to 50 % and 88 % loss of hydraulic conductance (Ψ_{50} - red, and Ψ_{88} - black). The smaller symbols in the background represent individual species and the larger symbols represent site averages. The size of the larger symbols indicate the number of species examined in the site. A type-2 linear regression is plotted for species (solid line) and for sites (dashed line). The dotted line indicates 1:1 relation for reference.

In contrast, the change in Ψ_{88} with Ψ_{min} was greater than unity (Fig. 4, Table S3). Therefore the slopes of the vulnerability curves which represent the rates of embolism spread per unit drop in water potential (Martin StPaul et al. 2017) are shallower for species with more negative Ψ_{min}, and steeper for species that are able to maintain Ψ_{min} closer to zero. Species from wetter sites are more sensitive to changes in water potential and show rapid embolism and a sharp loss of conductivity over a narrow ranges of water potential. In contrast, species adapted to drier conditions which experience very negative Ψ_{min} are less sensitive to changes in water potential and exhibit a gradual loss of conductivity over a wider range of water potentials.

It is evident from Fig.4 that many species experience minimum water potentials that are more negative than their Ψ_{50} (also see Fig 1A). This indicates that these species may have other mechanisms to prevent catastrophic hydraulic failure and mortality. While debated (Delzon and Cochard 2014), refilling and repair of embolized vessels might represent potential mechanisms that allow species to recover from experiencing water potentials below Ψ_{50} (Brodersen and McElrone 2013, Klein et al. 2018). But nevertheless, such species are more at risk and shown to have higher mortality during drought (Benito Garzón et al. 2018).

Conclusion

In agreement with previous studies, but using a much larger dataset, we found that tropical forest trees operate with very low hydraulic safety margins, indicative of high risk of hydraulic failure during drought (Choat et al. 2012). Indeed almost a third of the species examined had negative HSM-Ψ_{50}, implying that they experience water potentials in the driest times of the year that are below those that result in 50% loss of hydraulic conductance. In contrast to earlier studies we observed much larger variation in hydraulic traits. The minimum leaf water potentials (Ψ_{min}) experienced and Ψ_{50} were both positively related to precipitation, likely reflecting the more stressful environments in drier sites and the need for greater resistance to xylem embolism in such sites, respectively. Furthermore, contrary to previous observations we found that HSM-Ψ_{50} were higher in sites with greater mean annual precipitation implying that wetter sites may be safer, and drier sites more vulnerable to risk of hydraulic failure in the face of increasing droughts. However, interestingly, variation in Ψ_{min} and HSM-Ψ_{50} was greater in drier sites and this suggests that drier tropical forests may have greater capability at maintaining ecosystem function during drought than wetter forests.

Contributions

Ron Sunny and Deepak Barua designed the study, compiled the dataset, and performed the analysis. Ron Sunny and Deepak Barua wrote the draft of the manuscript (for submission) with contributions from David Galbraith, Emanuel Gloor, Julia Tavares and Mahesh Sankaran.

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Supplementary figures and tables

List of tables and figures

Table S1: The relationship between hydraulic traits and climate indices. Regression coefficients for relationship between hydraulic traits and: a) mean annual precipitation; b) number of dry months; c) cumulative water deficit; and, d) Aridity index. Analysis were conducted for site averages; all species; and site standard deviations. Significant relationships are denoted by ** for p<0.05, and * for p<0.1.

Climate	**Traits**	**Site**		**Species**		**Site standard deviation**	
a) Mean annual precipitation	Ψ_{min}	**0.47**	**	**0.27**	**	**0.36**	**
	Ψ_{50}	**0.13**	**	**0.07**	**	**0.10**	*
	Ψ_{88}	**0.14**	**	**0.10**	**	**0.07**	
	HSM-Ψ_{50}	**0.27**	**	**0.07**	**	**0.13**	*
	HSM-Ψ_{88}	**0.00**		**0.00**		**0.02**	
b) Dry months	Ψ_{min}	**0.32**	**	**0.17**	**	**0.39**	**
	Ψ_{50}	**0.13**	**	**0.06**	**	**0.12**	*
	Ψ_{88}	**0.13**	**	**0.07**	**	**0.11**	
	HSM-Ψ_{50}	**0.12**	**	**0.03**	**	**0.29**	**
	HSM-Ψ_{88}	**0.00**		**0.00**		**0.15**	*
c) Cumulative water Deficit	Ψ_{min}	**0.36**	**	**0.17**	**	**0.50**	**
	Ψ_{50}	**0.15**	**	**0.04**	**	**0.15**	**
	Ψ_{88}	**0.10**	*	**0.07**	**	**0.17**	*
	HSM-Ψ_{50}	**0.15**	**	**0.05**	**	**0.40**	**
	HSM-Ψ_{88}	**0.00**		**0.00**		**0.21**	**
d) Aridity index	Ψ_{min}	**0.47**	**	**0.30**	**	**0.41**	**
	Ψ_{50}	**0.13**	**	**0.06**	**	**0.03**	
	Ψ_{88}	**0.11**	*	**0.08**	**	**0.01**	
	HSM-Ψ_{50}	**0.29**	**	**0.09**	**	**0.11**	*
	HSM-Ψ_{88}	**0.02**		**0.01**	*	**0.01**	

Table S2 : Number of sites and species (species and site combinations) included in the dataset. Sites with more than 5 species were used for calculating site level standard deviation.

Traits	**Number of sites**	**Number of species & site combinations**	**Number of sites (>5 species per site)**
Ψ_{min}	43	419	29
Ψ_{50}	43	419	29
Ψ_{88}	33	283	21
HSM-Ψ_{50}	43	419	29
HSM-Ψ_{88}	33	283	21

Table S3 : The relationship between minimum water potentials experienced (Ψ_{min}) and resistance to: a) 50 %; and, b) 88 % loss of hydraulic conductance (Ψ_{50} and Ψ_{88} respectively). Results from a type-II major axis linear regression for species and sites. Confidence interval of slope is shown in brackets.

		R^2	***p* value**	**Slope (2.5% CI, 97.5% CI)**	**n**
a) Ψ_{50} vs. Ψ_{min}	Site	0.48	<0.01	0.81 (0.57, 1.12)	43
	Species	0.15	<0.01	0.78 (0.62, 0.97)	419
b) Ψ_{88} vs. Ψ_{min}	Site	0.41	<0.01	1.95 (1.31, 3.26)	33
	Species	0.17	<0.01	2.43 (1.92, 3.24)	283

Table S4: Summary of the 43 sites considered for analysis in the study along with information for country where the site is located, the number of species (No. of spc.) for which data was available for HSM-Ψ_{50} from the site, the sub-biome (SB) to which the site belongs (RF - rainforest, SF - seasonal forest, WS - woodland or shrub land), the latitude (Lat) and longitude (Lon) of the site, the altitude in metres (Alt) of the site, mean annual precipitation in mm (MAP), the number of dry months (DM), the cumulative water deficit (CWD), aridity index (AI) of the site and the references from which the data was extracted. NA = information not available.

	Country	Site	N	SB	Lat	Lon	Alt	MAP	DM	CWD	AI	Ref
1	Argentina	Iguazu National Park	10	RF	-25.62	-54.33	222	1717	0	-1	1.5	[1]
2	Australia	Queensland (Cardwell State Forest)	1	RF	-18.44	146.13	29	2001	5	-563	1.2	[2]
3	Australia	Daintree Rainforest Observatory	3	RF	-16.10	145.45	32	1690	7	NA	2.5	[3]
4	Australia	Queensland (Cardigan Hill)	2	WS	-20.18	146.72	218	672	10	-1053	0.4	[4]
5	Australia	Queensland (Hillgrove Station)	2	WS	-19.67	145.75	330	785	8	-1116	0.4	[4]
6	Australia	Queensland (Many Peaks Range)	2	WS	-19.18	145.75	355	968	8	-1074	0.6	[5]
7	Bolivia	Kenia (1)	12	SF	-16.02	-62.73	392	1079	7	-652	0.7	*
8	Bolivia	Kenia (2)	12	SF	-16.01	-62.74	416	1079	7	-658	0.7	*
9	Brasil	Federal Reserva Biológica Do Jaru	8	RF	-10.19	-61.87	158	2119	4	-450	1.7	[6]
10	Brasil	Fazenda Experimental Catuaba	15	RF	-10.07	-67.62	209	1887	5	-191	1.7	*
11	Brasil	Manaus	21	RF	-2.61	-60.21	114	2221	2	-62	1.8	[7, 8]
12	Brasil	Uatuma Sustainable Development Reserve	8	RF	-2.14	-59.00	135	2123	4	-139	1.7	[8]
13	Brasil	Caxiuana	4	RF	-1.74	-51.46	42	2349	4	-226	2.0	[9]
14	Brasil	Tapajos National Forest	9	SF	-3.89	-54.97	177	2138	3	-291	1.8	[10]
15	Brasil	IBGE ecological reserve	17	WS	-15.93	-47.88	1094	1554	5	-392	1.3	[11 – 14]
16	China	Heshan/Guangdong	6	RF	22.68	112.90	67	1783	6	-199	1.8	[15]

Continued on next page

	Country	Site	N	SB	Lat	Lon	Alt	MAP	DM	CWD	AI	Ref
17	China	Dinghushan/Guangdong	49	RF	23.17	112.52	528	1745	6	-131	1.7	[15]
18	China	Xishuangbanna (1)	1	SF	21.68	101.42	956	1697	6	-285	1.3	[16]
19	China	Xishuangbanna (2)	18	SF	21.87	101.32	853	1488	6	-310	1.3	[17 – 20]
20	China	Xishuangbanna (3)	10	SF	21.90	101.77	1345	1798	6	-271	1.3	[21, 22]
21	China	Menglun/Yuanan	20	SF	21.92	101.27	556	1488	6	-319	1.3	[15]
22	China	Guangxi (South west karst)	9	SF	23.23	113.45	100	1884	5	-142	1.6	[23]
23	China	Xishuangbanna (4)	3	WS	23.45	102.17	747	1148	7	-357	1.2	[24]
24	China	Yuangjiang/Yunnan	10	WS	23.47	102.17	434	1148	7	-363	1.2	[15]
25	Costa Rica	Costa Rican Seasonal Moist Forest	4	RF	10.31	-84.81	1406	2786	3	-212	2.2	[25]
26	Costa Rica	Palo Verde National Park	2	SF	10.35	-85.35	67	1779	5	-625	1.2	[26]
27	Costa Rica	Santa Rosa National Park	8	SF	10.91	-85.60	253	1922	5	-603	1.1	[27]
28	French Guiana	Paracou Research Station (2)	2	RF	5.03	-52.12	0	3242	3	NA	NA	[28]
29	French Guiana	Paracou Research Station (1)	14	RF	5.27	-52.92	36	2732	3	-197	2.5	[29]
30	India	Sirsi, Karnataka	12	SF	14.49	74.75	538	3130	6	-839	2.1	**
31	India	Nigdale, Maharashtra	14	SF	19.09	73.56	962	2266	8	-921	1.5	**
32	Madagascar	Kirindy Forestry Reserve	2	SF	-20.45	44.82	113	827	9	-834	0.6	[30]
33	Mexico	Biosphere Reserve, Chamela-Cuixmala	12	SF	19.48	-105.03	99	1161	7	-891	NA	[31]
34	Mexico	LosLimoncillos	1	SF	18.51	-99.09	965	914	8	-849	0.7	[32]
35	Panama	Barro Colorado Island (BCI)	10	RF	9.15	-79.85	146	2383	4	-321	2.1	[33 – 38]
36	Panama	Parque Nacional San Lorenzo	6	RF	9.28	-79.98	153	3047	3	-232	2.1	[39]
37	Panama	Parque Natural Metropolitano	9	SF	8.98	-79.55	28	1815	4	-414	1.2	[39 – 42]
8	Peru	Allpahuayo (1)	16	RF	-3.95	-73.44	139	2784	0	0	2.6	*

Continued on next page

	Country	Site	N	SB	Lat	Lon	Alt	MAP	DM	CWD	AI	Ref
39	Peru	Allpahuayo (2)	9	RF	-3.95	-73.44	139	2784	0	0	2.6	*
40	Peru	Sucusari	19	RF	-3.25	-72.91	109	3171	0	0	2.6	*
41	Peru	Tambopata	20	SF	-12.83	-69.27	216	2377	3	-134	1.8	*
42	Venezuela	Charallave, Miranda	6	SF	10.25	-67.05	469	1067	6	-504	1.0	[43]
43	Venezuela	Estado Aragua	1	WS	9.92	-66.92	414	1170	6	-558	0.9	[43]

[2]- data from Liu et al. 2019 meta-analysis. Original paper could not be traced from [2]. * Unpublished data collected by Julia Tavares. ** Sunny et al. unpublished data – reported in Chapter 3 of this thesis.

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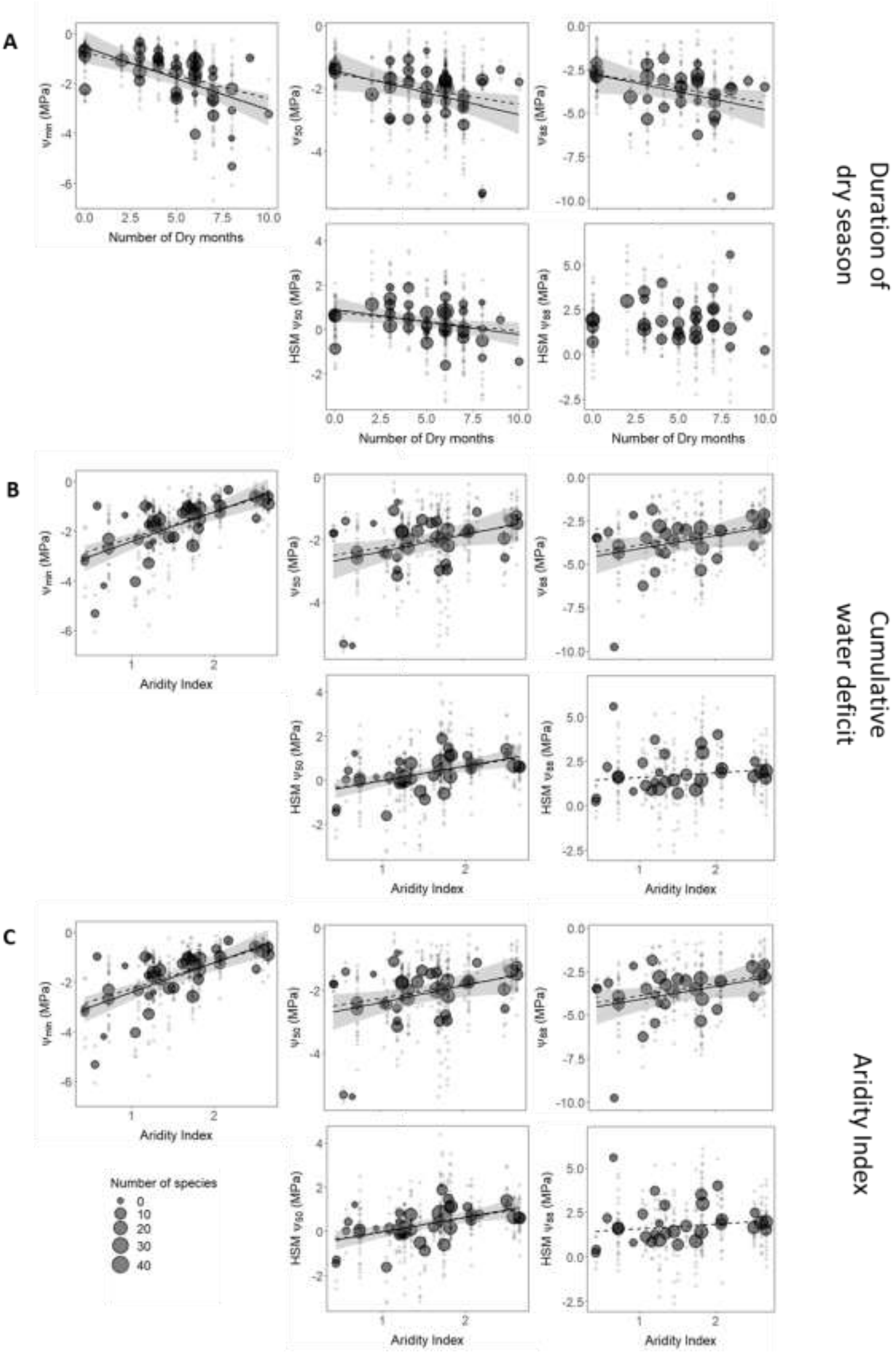
A
B
C
Duration of dry season
Cumulative water deficit
Aridity Index
ψmin (MPa)
ψ50 (MPa)
ψ88 (MPa)
HSM ψ50 (MPa)
HSM ψ88 (MPa)
Number of Dry months
Aridity Index
0.0
2.5
5.0
7.5
10.0
-2.5
-5.0
-7.5
-10.0
0
-2
-4
-6
4
2
1
2
Number of species
0
10
20
30
40

Figure S1: The relationship between minimum water potential (Ψ_{min}) and hydraulic traits (Ψ_{50}, Ψ_{88}, HSM-Ψ_{50} and HSM-Ψ_{88}), and climate indices representing water availability: A) Duration of dry season (in months); B) Cumulative water deficit (CWM); and, C) Aridity index. Significant relationships are shown with regression lines for sites (black line with 95% confidence band in grey), and species (red line). The smaller light grey symbols in the background represent individual species and the larger dark grey symbols represent site averages. The size of the larger dark grey symbols are representative of the number of species examined.

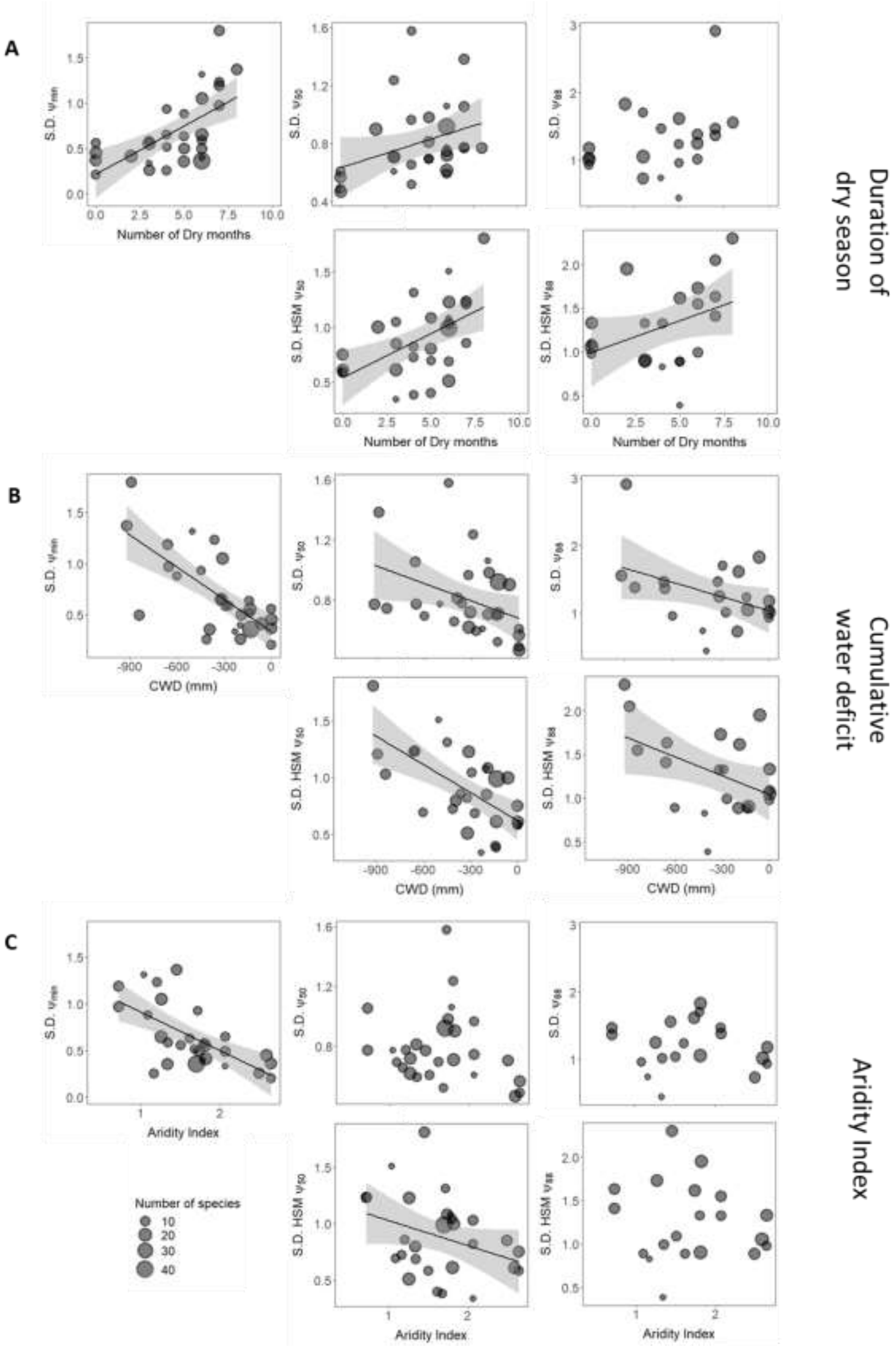
A
S.D. ψmin
Number of Dry months
S.D. ψ50
S.D. ψ88
S.D. HSM ψ50
Number of Dry months
S.D. HSM ψ88
Number of Dry months
Duration of dry season
B
S.D. ψmin
CWD (mm)
S.D. ψ50
S.D. ψ88
S.D. HSM ψ50
CWD (mm)
S.D. HSM ψ88
CWD (mm)
Cumulative water deficit
C
S.D. ψmin
Aridity Index
S.D. ψ50
S.D. ψ88
Number of species
10
20
30
40
S.D. HSM ψ50
Aridity Index
S.D. HSM ψ88
Aridity Index
Aridity Index

Figure S2: The relationship between variation (standard deviation, S.D.) in minimum water potential (Ψ_{min}) and hydraulic traits (Ψ_{50}, Ψ_{88}, HSM-Ψ_{50} and HSM-Ψ_{88}) and climate indices representing water availability: A) Duration of dry season (in months); B) Cumulative water deficit (CWM); and, C) Aridity index. Significant relationships are shown with regression lines with 95% confidence band in grey. The size of the symbols are representative of the number of species examined.

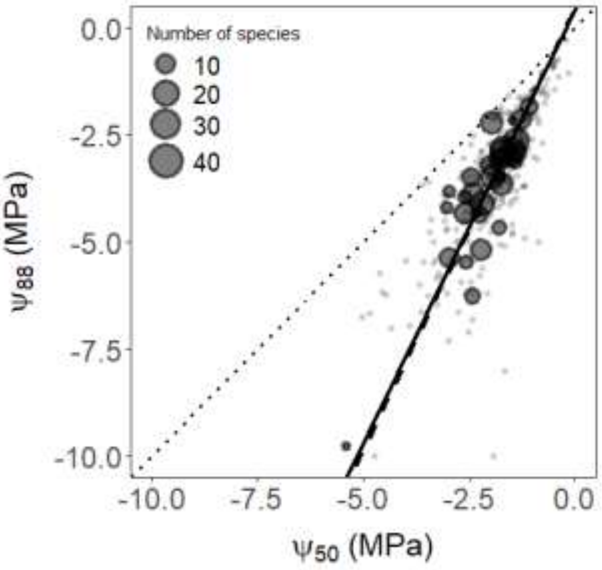

Figure S3: The relationship between Ψ_{50} and Ψ_{88} for sites (larger dark gray symbols), and species (smaller light gray symbols in the background). The size of the larger dark-gray symbols indicate the number of species examined in the site. A type-2 linear regression is plotted for sites (solid line; $R^2 = 0.76$, $p < 0.05$, $n = 33$), and for species (dashed line, $R^2 = 0.62$, $p < 0.05$, $n = 283$). The dotted line indicates 1:1 relation for reference.

Chapter 3

Hydraulic safety margins in two seasonally dry tropical forests with contrasting rainfall regimes

Abstract

The hydraulic trait composition of communities can affect ecosystem function, and can be used as reliable indicators of community level mortality risk and resilience to drought. This is particularly important in species rich tropical forests to understand how these systems will respond to future climates with increasing droughts. Despite the rapid increase in information on hydraulic traits, global coverage remains patchy and studies examining community level trait assemblages are few. We compared hydraulic traits and hydraulic safety margins (HSM) for dominant species from two seasonally dry tropical forests with contrasting rainfall regimes in the northern and central parts of the Western Ghats range in the Indian subcontinent. We examined the importance of accounting for species dominance, tested if commonly measured functional traits were related to hydraulic traits, and asked how species that were common to both sites differed in these traits. There was large variation in hydraulic traits in species from these forests, comparable to the ranges observed in tropical regions worldwide. The drier northern Western Ghats site had a higher community weighted HSM based on measures of resistance to embolisms that resulted in 88 % loss of hydraulic conductivity indicating lower risk of catastrophic hydraulic failure and mortality. However, comparisons based on species averages and HSM based on 50 % loss of hydraulic conductivity gave different results. This highlights the importance of accounting for differences in species dominance, and the influence of the slope of the xylem vulnerability curves in determining the risk of forest communities to drought. We found no relationship between commonly measured functional traits and hydraulic traits or HSMs, indicating possible independence of their carbon and hydraulic economies. Finally, intra-specific variation in traits were species specific, implying that the species use diverse strategies beyond resistance to xylem hydraulic failure to be able to occur in these two very different sites.

Introduction

Predictions for future climate indicate drying trends with more frequent and severe droughts that will reduce productivity, increase mortality, and significantly affect ecosystem structure and function (Choat et al. 2018, Trugman et al. 2020). Indeed, large scale mortality as a result of climate-change type drought events is already evident globally (Allen et al. 2010, Hartmann et al. 2018). Plant hydraulic traits have emerged as key traits that are important in understanding various aspects of plant ecology, and particularly in evaluating species and ecosystem responses to drought (Brodribb et al. 2020). While there has been a large increase in the number of studies examining hydraulic traits in recent years (Cavaleri and Sack 2010, Bartlett et al. 2012, Choat et al. 2012, Nardini and Luglio 2014, He et al. 2020), global coverage is still patchy (Chapter 1, Fig. 1), tropical forests remain highly underrepresented. There are no studies from many ecologically important regions like the Indian subcontinent. Additionally, it is important to understand how hydraulic traits assemblages vary across sites, but studies that examine community weighted estimates are limited. Finally, how different populations of a species differ in the minimum water potential they experience in the driest time of the year and in resistance to embolism remains unclear.

High evaporative demand and low soil water availability result in increased negative pressure in vascular tissue, causing embolisms that disrupt the flow of water to the leaves. Hydraulic failure resulting from embolisms reduces productivity and is recognized as a primary cause of mortality during drought (Rowland et al. 2015, Anderegg et al. 2016, Adams et al. 2017; Choat et al. 2018). Xylem vulnerability to embolism is commonly quantified as the water potential that results in a 50 % and 88 % loss of hydraulic conductance (Ψ_{50} and Ψ_{88}), and these measures provide mechanistic insight into species responses to drought (Urli et al. 2013). In angiosperm tree species, an 88 % loss of hydraulic conductance is a reliable indicator of the lethal water potential that can be tolerated and decreases in plant water potentials below this threshold usually result in mortality (Urli et al. 2013).

Species native to drier environments typically have greater resistance to embolism (Maherali et al. 2004, Choat et al. 2012, Larter et al. 2017, Oliveira 2017, Trueba et al. 2017, Li et al. 2018). However, there is large variation within biomes and sites, and our understanding of the reasons for these differences between co-occurring species remains limited. While Ψ_{50} and Ψ_{88} provide reliable measures of the thresholds that determine catastrophic hydraulic failure (Brodribb & Cochard 2009, Urli et al. 2013), the water potential experienced by species in their natural environments also depend on other traits like stomatal regulation (Klein 2014, Bartlett et al. 2016, Martin StPaul et al. 2017), water storage and capacitance (Stratton et al. 2000), and rooting depth (Chitra-Tarak et al. 2018, Brum et al. 2019). Quantifying the minimum water potential experienced during the driest time of the year (Ψ_{min}) puts estimates of Ψ_{50}/Ψ_{88} in the context of other traits that influence the likelihood of experiencing low water potentials (Meinzer et al. 2009). Thus, the difference between Ψ_{50}/Ψ_{88} and Ψ_{min} represents a hydraulic safety margin that accounts for the diverse strategies among species that may influence the probability of experiencing low water potentials. Additionally, the use of minimum water potentials makes estimates of hydraulic safety margins robust to comparisons across sites that may differ in soil types and vapour pressure deficit (Delzon and Cochard 2014).

Hydraulic trait composition of communities affects ecosystem function and can be used as reliable indicators of community-level mortality risk and resilience to drought (Anderegg et al. 2018, Trugman et al. 2020). Community weighted estimates of hydraulic traits allow us to assess the vulnerability of entire communities to drought. Given the high species diversity and considerable variation in hydraulic traits within co-occurring species, it becomes particularly important to evaluate community weighted estimates in tropical forests (Grime 1998, Garnier et al. 2004, Vitra et al. 2019). However, there is a significant dearth of community-level studies of hydraulic traits, and this is particularly pronounced in the African and Asian tropical regions (Fig. 1, Chapter 1).

While xylem resistance to embolism is a physiological trait mechanistically linked to performance, with the potential to predict growth, drought responses and global distribution of species (Brodribb 2017), measurement of xylem vulnerability is labour intensive, and therefore less commonly quantified. It is important to examine how hydraulic traits are related to more commonly measured stem and leaf functional traits that can serve as potential proxies of drought tolerance (Lens et al. 2011, Maréchaux et al. 2015, Markesteijn et al. 2011, Li et al. 2018). Relationships between xylem resistance and leaf and stem functional traits are also important to understand coordination between hydraulic and carbon economies (Maréchaux et al. 2015, McCulloh 2011, Blackman et al. 2016, Pratt & Jacobsen, 2017, Li et al. 2018). While there is evidence of greater resistance to embolism being related to high stem specific density (Lens et al. 2011, Markesteijn et al. 2011, Li et al. 2018) and greater leaf dry matter content (Markesteijn et al. 2011), these relationships are far from universal

(Lamy et al. 2011, Santiago et al. 2018), and it is not known if the nature of these relationships depend on the environment, and therefore differ across sites with different climate.

Intra-specific variation in traits can potentially impact species responses to drought, species interactions, community assembly and ecosystem function (Albert et al. 2011, Violle et al. 2012, Anderegg et al. 2015). The results from studies that have examined intra-specific variation in hydraulic traits are mixed. Many studies have reported limited variation (Alder et al. 1996, Maherali and DeLucia 2000, Martinez-vilata et al. 2009, Wortemann et al., 2011, Lamy et al. 2014, Anderegg & HilleRisLambers 2016, González-Muñoz et al. 2018, Lobo et al. 2018, Li et al. 2019, Rosas et al. 2019, Skelton et al. 2019, Torres-Ruiz et al. 2019). However, others have found substantial variation in hydraulic traits suggesting that traits conferring resistance to drought may be significantly influenced by the environment (Kavanagh et al. 1999, Choat et al. 2007, Anderegg 2015, Lopez et al. 2016, David-Schwarz et al. 2016, Hajek et al. 2016, Stojnić et al. 2018, Chauvin et al. 2019, Pritzkow et al. 2020). In part, the discrepancy is a result of the different traits examined. Some traits like the ratio of leaf area to the total area of the conducting tissue exhibit large variation between individuals of species while variation in other traits like resistance to embolism is limited (Rosas et al. 2019). Importantly, almost all studies that have examined intra-specific variation with respect to Ψ_{50} have been based on temperate species (primarily from North America and Europe) and very little is known for tropical species.

In this study, we quantified hydraulic safety margins for dominant tree species from two seasonally dry tropical forests in the Indian peninsula that differ in annual rainfall and dry season duration, to understand how these forests will respond to changing rainfall patterns. We asked how differences in the minimum water potential experienced during the dry season (Ψ_{min}), and xylem resistance to embolism (quantified as Ψ_{50} and Ψ_{88}), affect hydraulic safety margins (HSM) for dominant species in these forests. We expected species from the drier site to experience more negative Ψ_{min}, and also have xylem that is more resistant to embolism. How this might affect HSM would depend on the relative changes in Ψ_{min}, Ψ_{50} and Ψ_{88}, and is difficult to predict. To understand how these species estimates of HSM scale up to forests in these regions, we quantified dominance weighted community measures of HSM. Previous studies have suggested that soft traits like stem-specific density and leaf dry matter content are negatively related to minimum water potential and xylem resistance to embolism. We asked whether commonly measured plant functional traits are related to Ψ_{min}, Ψ_{50} or Ψ_{88}, and HSM and whether these relationships differ across the two study sites. For species that were common to both sites, we asked how Ψ_{min} and Ψ_{50} differ across the sites.

Methods

Study sites and species selection: This study was conducted at two sites in the Western Ghats range of the Indian peninsula that differed in annual rainfall and dry season duration (months with precipitation < 100 mm). The drier site with greater seasonality in the Northern Western Ghats (NWG), located near Nigdale, Maharashtra (19.1320 °N, 73.554 °E), had an annual average rainfall of 2266 mm and an eight month long dry season duration. The wetter site with lower seasonality was located in the Central Western Ghats (CWG), near Sirsi, Karnataka (14.610 °N, 74.840 °E) and had a higher annual average rainfall of 2735 mm and a shorter six-month dry season duration. The climate data were obtained from the CRU 2.0 dataset (New et al. 2002). Twelve angiosperm tree species were selected from the CWG site, and fourteen from the NWG site. These represented 81 % and 66 % of the total basal area of the communities in the CWG and NWG sites, respectively (Sankaran M and Barua D, unpublished data). Seven species were common to both these sites.

Xylem vulnerability to embolism: Xylem vulnerability to embolism was quantified for the study species at the end of the rainy season, in October for the NWG site, and in November for the CWG site. Branches greater than 1 m length were collected from at least three individuals of each species from the sun-exposed upper canopy before dawn with the help of climbers. The collected branches were placed in sealed, darkened plastic bags with moistened paper towels to keep the air in the bag water-saturated. The bags were transported back to field stations, and the first measurements were taken within four hours of sampling.

Xylem vulnerability to embolism was assessed by the pneumatic method (Pereira et al. 2016) where the percentage loss of xylem conductivity (PLC) was estimated from percentage air discharge (PAD) for a range of xylem water potentials (Ψ) generated by bench drying (Sperry et al. 1988). The range of water potentials across which PLC was quantified included values > -0.05 MPa representative of a well-hydrated state, to < -10 MPa representative of very dehydrated states. For some species, measurements were terminated before branches reached -10 MPa because all the leaves were shed. Stem water potential was measured as leaf water potential after allowing the branch to equilibrate for 30 min in a sealed darkened plastic bag.

Leaf water potential (MPa) was measured with a pressure chamber (Model 1515D, PMS Instruments Co., Albany, OR, USA). The water potential that resulted in a 50 % (Ψ_{50}) and 88 % (Ψ_{88}) loss of conductivity was calculated by fitting the following sigmoid function to the data (Barros et al. 2019).

$$\text{Percent Air Discharge (PAD)} = \frac{100}{\left(1 + \exp\left(\frac{a}{25} * (\Psi - b)\right)\right)}$$

Here PAD, which represents the percent loss of hydraulic conductivity, and Ψ (xylem water potential, MPa) were quantified, and the parameters '*a*' and '*b*' were estimated. The parameter '*b*' represents the xylem water potential (MPa) when PAD equals 50 %, and the parameter '*a*' represents the slope of the curve. Data from all individuals from a species were pooled to fit a species-level vulnerability curve.

Minimum water potential and estimation of hydraulic safety margins (HSM): To quantify stem xylem water potential at the driest time of the year (Ψ_{min}), midday leaf water potential was measured with a pressure chamber (Model 1515D, PMS Instruments Co., Albany, OR, USA) at the end of the dry season in April for the CWG site, and in May for the NWG site. Mature sun-exposed leaves, collected between 1200-1500 hours, were used for quantification of Ψ_{min}, assuming that stem xylem water potential equals leaf water potential. Estimates of Ψ_{min} were obtained for the same individuals for which we had measured xylem vulnerability to embolism. The difference between Ψ_{min} and Ψ_{50} or Ψ_{88} was used as a measure of hydraulic safety margin (HSM).

Dominance weighted traits: A dominance weighted curve that represents the community level vulnerability curve was generated by calculating the dominance weighted average of the parameters from the species level curves for each site. The relative dominance for the selected species was rescaled to add up to a total of 1. The relative dominance weighted average for the parameters for each site was calculated as:

$$\text{Dominance weighted parameter} = \sum_{n=1}^{N} d(i) * x(i)$$

Here N is the number of species in the site, d is the rescaled relative dominance of the species (i) and x is the estimated parameter (a or b) from the vulnerability curve of the species (i). The parameter b of this dominance weighted curve corresponded to the dominance weighted Ψ_{50} value. This dominance weighted curve was also used to determine dominance weighted Ψ_{88} value (Barros et al. 2019). We estimated the dominance weighted Ψ_{min} to calculate dominance weighted hydraulic safety margins for the sites.

Morphological functional traits: For each individual sampled, a 2nd or 3rd order branch was collected for quantifying leaf and wood traits. The branch was brought back to the field station, cut under water and left with the stem immersed in water for 12 hours in the dark for water saturation. From each branch, five fully expanded mature leaves were collected for quantifying saturated fresh weight and leaf area. Following this, the leaves were oven-dried at 70 °C for at least 72 hours for quantifying dry weight. Leaf mass per area was estimated as the leaf dry weight per leaf area ($g \cdot m^{-2}$), and leaf dry matter content as the leaf dry weight per saturated fresh weight ($g \cdot g^{-1}$) (Pérez-Harguindeguy et al. 2013).

For all individuals sampled, three or more segments greater than 5 cm in length and around 1-2 cm in diameter were collected from the base of these branches. These segments were debarked and the volume estimated by the water-displacement method (Pérez-Harguindeguy et al. 2013). The segments were then oven dried to constant weight (three to five days at 70°C) and the dry weight quantified. Stem specific density was calculated as the dry weight per volume ($g \cdot m^{-3}$).

Analysis: Parameter estimation for fitting the sigmoid model to generate the vulnerability curves were done using the in-built '*nls*' function in R (R core team, Version 1.2.5033). All traits examined in this study were normally distributed (Shipro-Wilk test) with the exception of Ψ_{min} which was log transformed to meet this criteria. A one-way ANOVA (Statistica version 9.1, Statsoft, Tulsa, OK, USA) with site as a fixed effect was used to examine if traits from species in the drier NWG site differed from the wetter CWG site.

For the seven species that occurred in both the sites, we examined intraspecific variation in hydraulic and morphological traits. Data for individual replicates for species were available for Ψ_{min}, LMA, LDMC and SSD, and we used a two-way ANOVA with site and species as fixed effects, and Tukey's post-hoc tests to ask if traits differed for species between sites (Statistica version 9.1, Statsoft, Tulsa, OK, USA). Since Ψ_{50} and Ψ_{88} were quantified using a species level curve, and HSM-Ψ_{50} and HSM-Ψ_{88} derived from these, we could not test for intra-specific differences in these traits in the same manner as above. Ψ_{50} values for species in each site were obtained from sigmoid fits to species-level xylem vulnerability curves. The parameter *b* and the standard error estimates for this parameter from the curve fitting were used to examine difference in Ψ_{50} for species that occurred at both sites, using t-test statistics. Pearson’s correlations were used to examine the relationship between hydraulic traits and leaf mass per area, leaf dry matter content and stem specific density. When relationships between hydraulic traits and leaf and stem functional traits were significant, we asked whether these relationships differed across sites using a type II standard major axis regression analysis using the function *lmodel2* from an R based package with the same name (Pierre 2018).

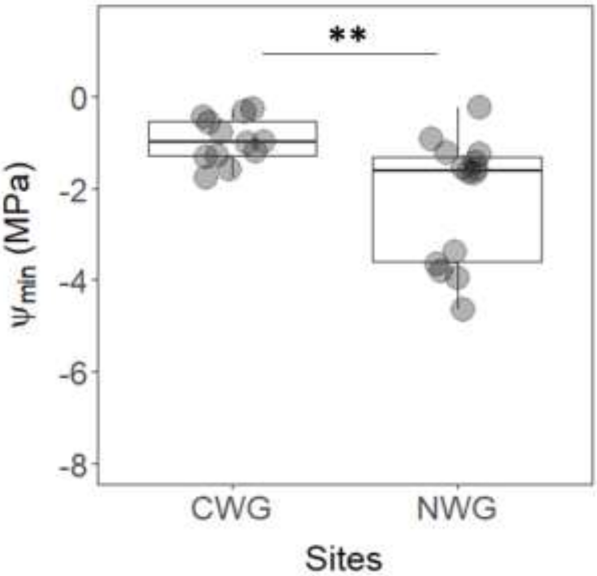

Figure 1: The minimum water potential (Ψ_{min}) experienced by species at the two study sites: Northern Western Ghats (NWG - relatively drier site with a longer dry period) and Central Western Ghats (CWG - wetter site with shorter dry period). Ψ_{min} was quantified as the midday water potential during the driest time of the year at each site. A standard box plot with the lower and upper limits of the box representing the first and the third quartiles are presented, and statistical significance for differences between sites is shown by ** ($p < 0.05$).

Results

Species in drier site experienced more negative minimum water potentials

The minimum water potential (Ψ_{min}) experienced ranged from -4.64 MPa (*Olea dioica*) to -0.24 MPa (*Mangifera indica*) for species in the drier NWG site, and from -1.76 MPa

(*Psydrax dicoccos*) to -0.27 MPa (*Canarium strictum*) for species in the wetter CWG site (Fig. 1). Many species in the NWG site were able to maintain relatively higher water potentials (> -2 MPa) despite the drier conditions at this site, but five species had very low water potentials (< -3 MPa). As expected, the drier NWG site with lower rainfall and a longer dry season had significantly lower minimum water potential than the wetter CWG site.

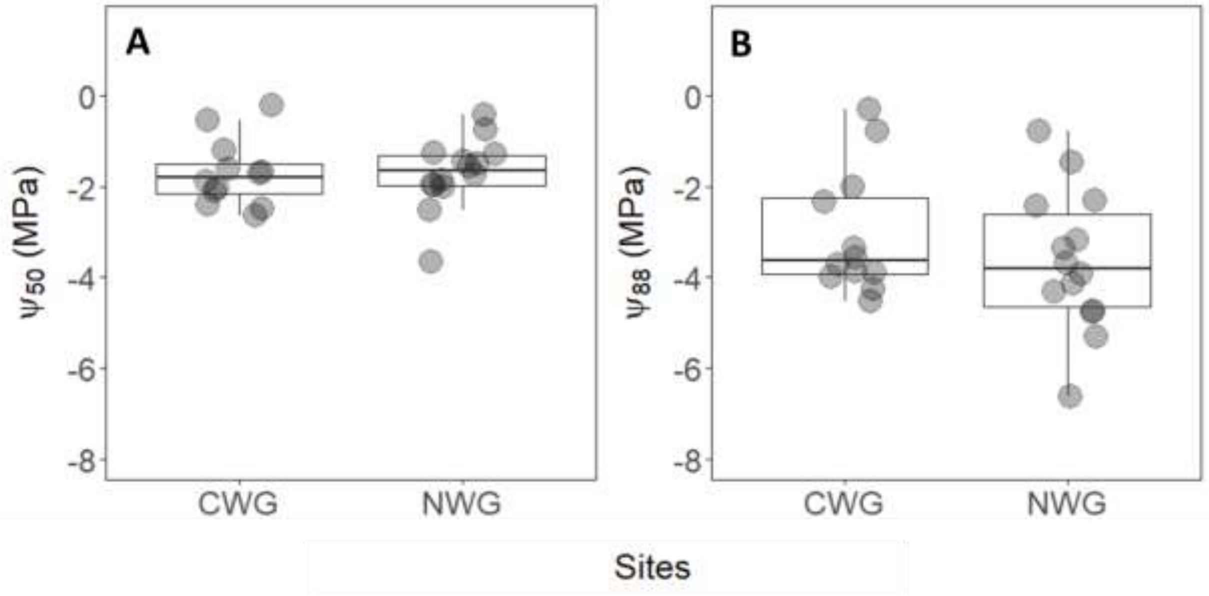

Figure 2: Resistance to xylem embolism for species at the two study sites: Northern Western Ghats (NWG - relatively drier site with a longer dry period) and Central Western Ghats (CWG - wetter site with shorter dry period). Resistance to xylem embolism was quantified as: A) Ψ_{50}; and, B) Ψ_{88} - the stem xylem water potential that results in a 50% and 88% loss of hydraulic conductance, respectively. Standard box plot with the lower and upper limits of the box representing the first and the third quartiles are presented. There were no statistically significant differences between the sites.

No difference in xylem resistance to embolism between the two site

We observed a large range in xylem vulnerability to embolism in species from these seasonally dry tropical forests (Fig. 2). Ψ_{50} ranged from -3.65 MPa to -0.22 MPa, while Ψ_{88} ranged from -6.63 MPa to -0.29 MPa. This represents 63.5 % and 65.3 % of the total range in Ψ_{50} and Ψ_{88} documented in angiosperms from across the tropics (from pan-tropical data summarised in Chapter 1). Despite the drier conditions and the more negative water potentials experienced in the NWG site, we observed no differences between the two sites in xylem resistance to embolism as quantified by Ψ_{50} and Ψ_{88}.

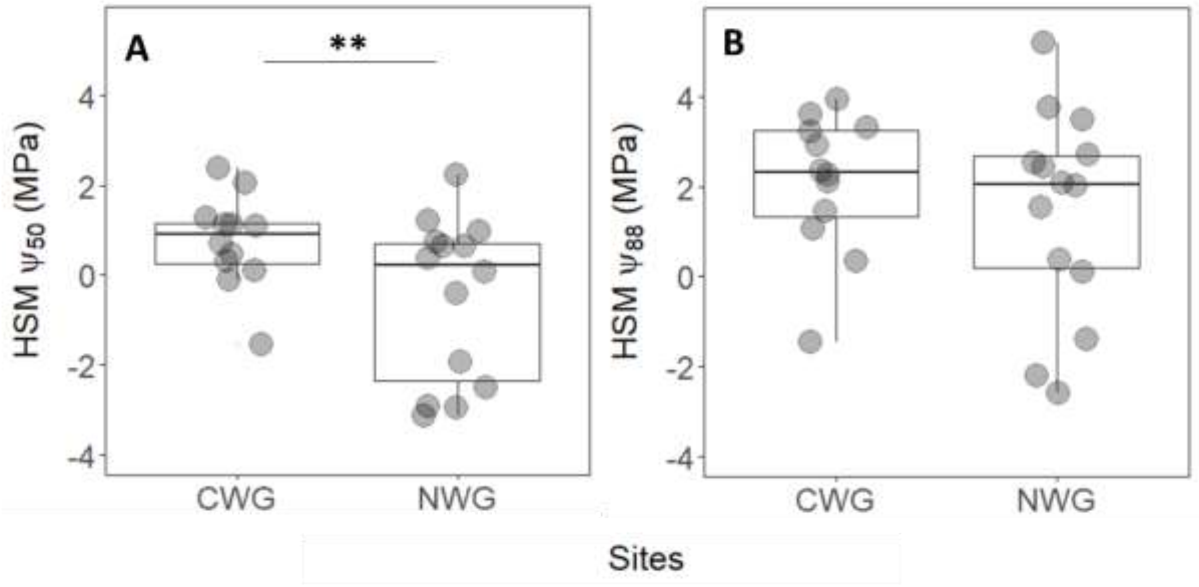

Figure 3: Hydraulic safety margins (HSM) for the two study sites: Northern Western Ghats (NWG - relatively drier site with a longer dry period) and Central Western Ghats (CWG - wetter site with shorter dry period). A) HSM-Ψ_{50}; and, B) HSM-Ψ_{88} were quantified as the difference between Ψ_{50} and Ψ_{88}, and the minimum water potential experienced (Ψ_{min}). Standard box plot with the lower and upper limits of the box representing the first and third quartiles are presented, and statistical significance for differences between sites is shown by ** ($p < 0.05$).

Hydraulic safety margin for Ψ_{50} was higher in the wetter site

HSM-Ψ_{50}, the difference between the minimum water potential and Ψ_{50}, ranged from reasonably safe values of 2.21 MPa (*Syzygium cumini*) to negative water potentials as low as -3.16 MPa (*Olea dioica*), in species from the drier NWG site. HSM-Ψ_{50} was positive but close to zero for most species in the wetter CWG site. HSM-Ψ_{88} ranged from 5.20 MPa (*Syzygium cumini*) to -2.61 MPa (*Atalantia racemosa*) in the drier NWG site with similar values but a smaller range observed in the wetter CWG site, from 3.93 MPa (*Hopea odorata*) to -1.47 MPa (*Psydrax dicoccos*).

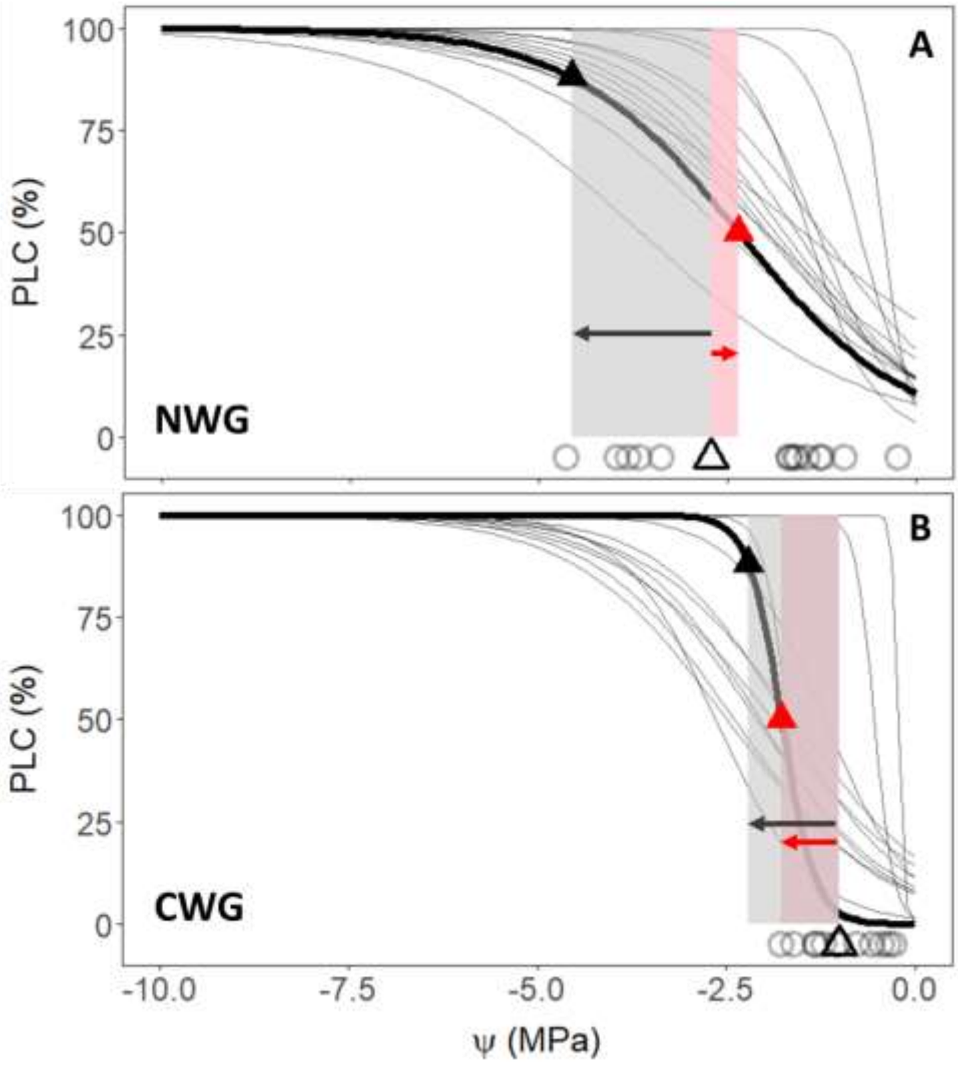

Figure 4: Dominance weighted hydraulic safety margins (HSM) for the two study sites: A) Northern Western Ghats (NWG - relatively drier site with a longer dry period); and, B) Central Western Ghats (CWG - wetter site with shorter dry period). The dominance weighted HSM, the difference between the dominance weighted minimum water potential (Ψ_{min}), and dominance weighted Ψ_{50} or Ψ_{88} are shown as the red (HSM-Ψ_{50}) and black (HSM-Ψ_{88}) arrows. The direction of the arrow indicates whether the dominance weighted HSM was positive (pointed left) or negative (pointed right). The thinner grey vulnerability curves in the background represent individual species, while the bolder black curve represents the dominance weighted curve. The solid red and solid black triangles represent the dominance weighted estimates of Ψ_{50} and Ψ_{88}, respectively. The open black triangle represents the dominance weighted estimate for Ψ_{min} while the open circles represent species Ψ_{min}. PLC - percentage loss of conductivity.

Average HSM-Ψ_{50} was higher for the wetter CWG site as compared to drier NWG site. This difference in HSM-Ψ_{50} appears to be largely driven by the observed differences in the minimum water potential. However, we did not see a significant difference between sites for HSM-Ψ_{88}.

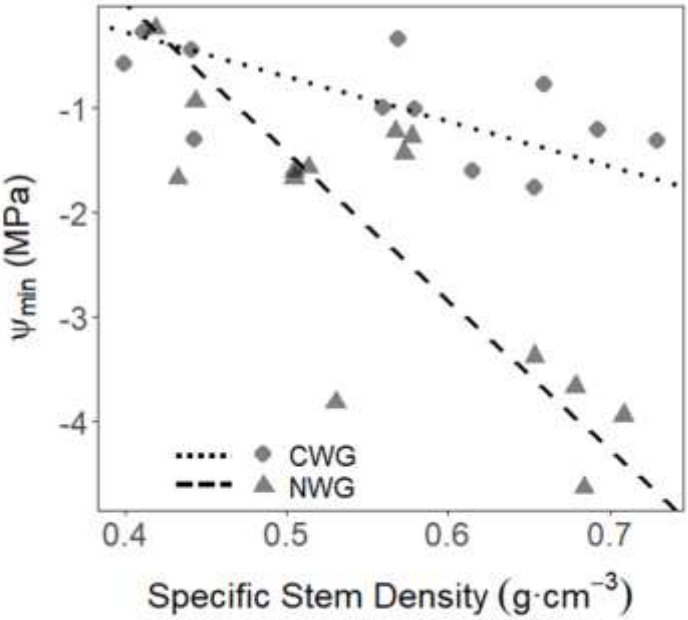

Figure 5: The relationship between specific stem density and minimum water potential (Ψ_{min}) for the two sites: Northern Western Ghats (NWG - relatively drier site with a longer dry period - dashed line, circles), and Central Western Ghats (CWG - wetter site with shorter dry period - dotted lines, triangles). Each point corresponds to a species. The lines correspond to standard major axis type II regression fits for each site.

Dominance weighted estimates for hydraulic saftey margins tells a different story

Dominance weighted Ψ_{min}, Ψ_{50} and Ψ_{88} were more negative than the species averages for the NWG site (Table S3, Fig. 4). In this drier site, the estimates of safety margins, HSM-Ψ_{50} and HSM-Ψ_{88} from community weighted trait values, were higher than margins estimated from species averages. Dominance weighted Ψ_{min} and Ψ_{50} were also more negative than species averages for the wetter CWG site, but Ψ_{88} was higher. Thus, while community weighted HSM-Ψ_{50} was higher than safety margins estimated from species averages for the wetter CWG site, community weighted HSM-Ψ_{88} was lower.

Minimum water potentials experienced were negatively related to stem specific density, but no relationship between functional traits and hydraulic traits

We observed high variation in the commonly measured functional traits examined: specific-stem density (SSD), leaf mass per area (LMA), and leaf dry matter content (LDMA), but no differences between sites in any of these traits. SSD was negatively related to the Ψ_{min}. At both sites, species with higher SSD experienced more negative minimum water potentials during the driest time of the year. The slope of the relationship between SSD and Ψ_{min} differed for the two sites (Fig. 5) with greater decreases in Ψ_{min} per unit change in SSD in the drier NWG site ($r^2 = 0.62$, $p < 0.01$, slope= -0.07, 95% C.I. -0.10, -0.05) than in the wetter CWG site ($r^2 = 0.34$, $p < 0.05$, slope= -0.23, 95% C.I. -0.40, -0.14). Surprisingly, none of the functional traits examined were related to xylem resistance to embolism. SSD was negatively related to HSM-Ψ_{50}, but this relations was likely driven by the relationship between SSD and Ψ_{min}. Contrary to expectations, Ψ_{min} was not related to Ψ_{50} (NWG - $r^2 = -0.37$, $p = 0.18$; CWG - $r^2 = -0.36$, $p = 0.25$) or Ψ_{88} (NWG – $r^2 = -0.23$, $p = 0.42$; CWG - $r^2 = -0.17$, $p = 0.57$) in either of the sites examined.

Intra-specific variation in Ψ_{min}, and resistance to embolism

For the seven species common to both sites the results from the ANOVA indicated that differences in the minimum water potentials (Ψ_{min}) experienced at the sites was dependent on species (significant site x species interaction, Table S5). Ψ_{min} was significantly lower in the drier site for three of the seven species (*Psydrax dicoccos, Memecylon umbellatum, Olea dioica*).

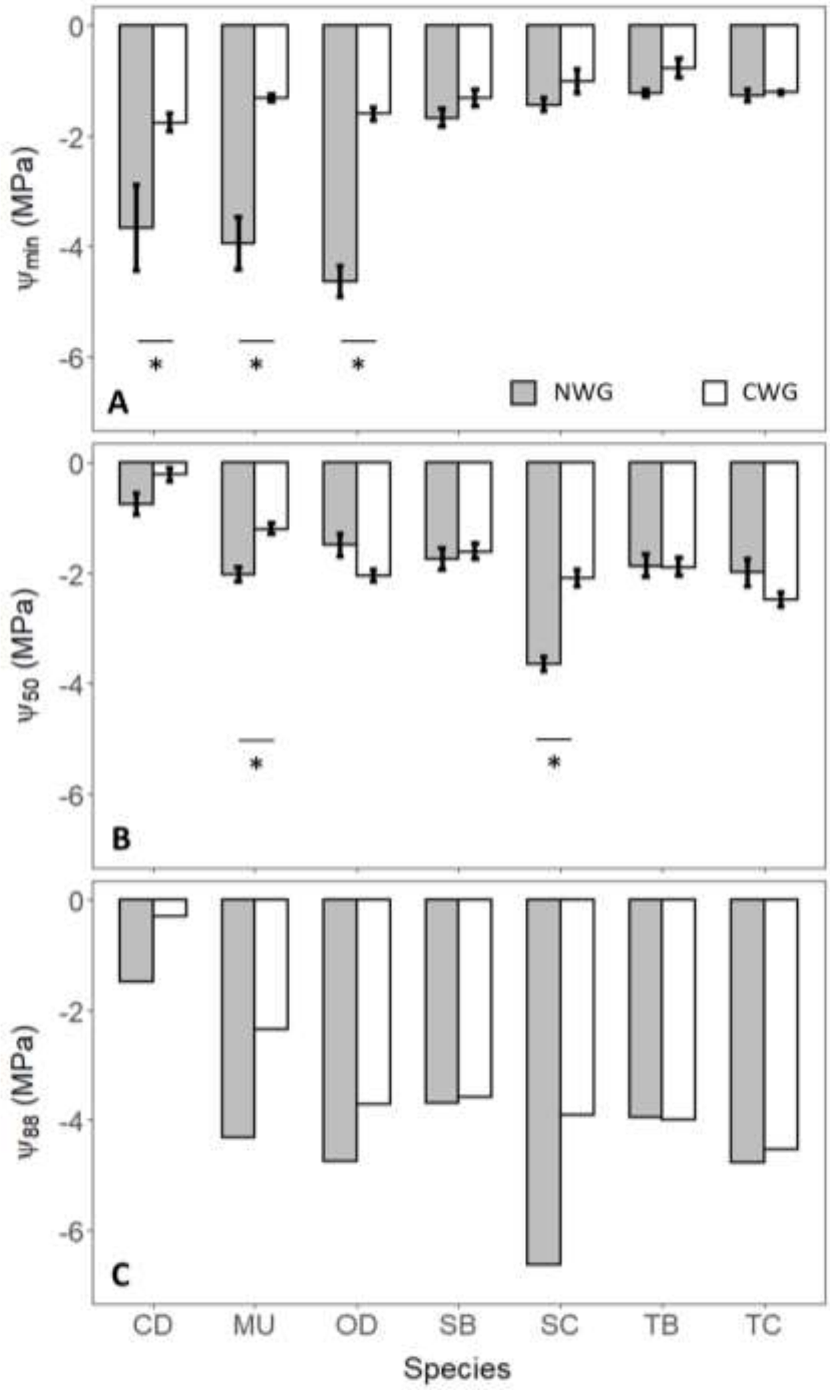

Figure 6: Intraspecific variation in hydraulic traits. Variation betwen sites for the seven species that were common to both sites. A) Ψ_{min} - minimum water potential; B) Ψ_{50}: and, C) Ψ_{88} - the xylem water potential that results in a 50% and 88% loss of hydraulic conductance respectively. The species were *Psydrax dicoccos* (CD), *Memecylon umbellatum* (MU), *Olea dioica* (OD), *Symplocos beddomei* (SB), *Syzygium cumini* (SC), *Terminalia bellirica* (TB), and *Terminalia chebula* (TC). Statistical significance for the difference as indicated * corresponding to $p<0.05$.

Surprisingly, there were no significant differences in Ψ_{min} for the other four species that were found in both sites (*Symplocos beddomei*, *Syzygium cumini*, *Terminalia bellirica and Terminalia chebula*), indicating that these species were able to maintain similar Ψ_{min} at both sites in spite of the drier conditions experienced in the NWG site. Similarly, with respect to $\Psi_{50,}$ two species (*Memecylon umbellatum* and *Syzygium cumini*) had xylem that were more resistant to hydraulic failure in the drier NWG site. Thus intra specific variation in Ψ_{50} was species dependent when compared across these two sites. However, intra-specific variation in Ψ_{50} and in Ψ_{min} did not seem to be coordinated, in these seven species that managed to occur in these two sites.

Discussion

There was large variation in the minimum water potentials experienced, resistance to embolism, and hydraulic safety margins in the dominant tree species from the two seasonally dry tropical forests examined. The minimum water potentials (Ψ_{min}) experienced by species in the wetter CWG site were greater that -2 MPa indicating that species at this site were able to maintain higher levels of hydration. Average Ψ_{min} was lower at the drier NWG site, and for many of the species close to -4 MPa. Interestingly, several species managed to maintain Ψ_{min} close to a well-hydrated state in the drier NWG site. It is possible that these species had access to water via deeper rooting depth (Chitra-Tarak et al. 2018, Brum et al. 2019) or stored stem water (Stratton et al. 2000), or had more efficient stomatal regulation (Klein 2014, Bartlett et al. 2016, Martin StPaul et al. 2017). Surprisingly, despite the drier conditions and the lower Ψ_{min} at the NWG site, there were no significant differences between sites in xylem vulnerability to embolism for both Ψ_{50} and Ψ_{88}. However, the range of xylem vulnerability to embolism observed in these species from seasonally dry tropical forests were very similar to what has been reported from other tropical forests (Fig. 2, Chapter 1).

The drier NWG site had a lower average hydraulic safety margin (HSM-Ψ_{50}) than the wetter CWG site. Given the lack of differences in xylem vulnerability to embolism (Ψ_{50}), this difference in HSM-Ψ_{50} was likely driven by the more negative minimum water potentials experienced by species in the drier site. In comparing the hydraulic safety margins with other tropical sites, average values for HSM-Ψ_{50} indicate that species from the drier NWG site may be at higher risk, while species from the CWG sites may be at lower risk of experiencing water potentials that result in a 50 % loss of hydraulic function than for other sites with similar amounts of rainfall (Fig. 2, Chapter 1). Interestingly, the drier NWG site had the highest range of hydraulic safety margins (5.3 MPa for HSM-Ψ_{50}; 7.8 MPa for HSM-Ψ_{88}) that has been reported from tropical forests. This likely reflects the high diversity in hydraulic strategies that has evolved within this highly seasonal community.

Community weighted estimates for HSM based on Ψ_{50} indicated that the forests in the wetter site were safer, and this is congruent with the site level species averages for HSM-Ψ_{50} for these two sites. However, estimates of safety margins based on Ψ_{88} indicated that the communities in the drier site are safer from drought in contrast to the site level species averages for these two forest communities (which indicated no difference between the two sites). These results highlight two important points: community weighted estimates may differ substantially from average species trait estimates (Barros et al. 2019), and that estimates based on Ψ_{50} and Ψ_{88} can give different answers. The CWM estimates for Ψ_{88} changed in opposite directions from the species level averages for the two sites when relative dominance of species was taken into account. This likely reflects processes regulating community assembly in these two sites. The regional species pools are likely similar in these communities which share a large proportion of species, but the dominance of species with greater resistance to embolism is evident in the drier site.

The results of the comparison of vulnerability between the two sites was dependent on whether Ψ_{50} or Ψ_{88} was used for evaluation. CWM for HSM-Ψ_{50} was greater for the wetter site, but the opposite was true for CWM for HSM-Ψ_{88}. This can result when comparing vulnerability curves that have different slopes, and cross over between the water potentials for Ψ_{50} and Ψ_{88} (Fig. 7). The community weighted slopes were steeper for the wetter CWG site that for the drier NWG site. While variation in slopes for vulnerability curves has been commonly reported, the functional significance of such variation remains unknown. Importantly, we show here that a practical consequence of evaluating species or communities that differ in slopes is that the results may differ based on whether Ψ_{50} or Ψ_{88} is used for the comparison.

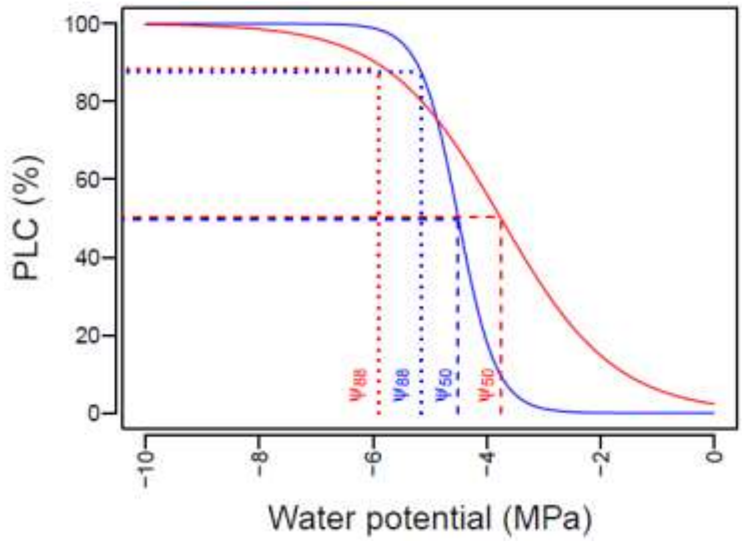

Figure 7: Conceptual xylem vulnerability curves showing the importance of slopes in determining risk for xylem hydraulic failure. The blue curve represent a species or community with a steeper slope while the red curve represent a species or community with a shallower slope. The dotted line corresponds to Ψ_{88} while the dashed line corresponds to Ψ_{50}. Note that the red curve species is more vulnerable to hydraulic failure with respect to Ψ_{88}, while the blue curve species is more vulnerable with respect to Ψ_{50}.

These results imply that plants in the drier NWG site are more likely than those in the CWG site to experience water potentials that are lower than levels that will result in 50 % loss of conductance. However, the opposite is true for the likelihood of experiencing water potential that will result in severe hydraulic stress and an 88 % loss of conductance. Given that Ψ_{88} is a more reliable indicator of mortality in angiosperms (Urli et al. 2013), these results suggest a lower risk of increased mortality in the drier NWG site in the face of increased droughts in future climates.

The commonly quantified leaf and stem functional traits, LMA, LDMC and SSD associated with leaf and wood economic spectrums were not related to xylem vulnerability to embolism or hydraulic safety margins. Thus, carbon-based resource tradeoff axes defined by these commonly quantified functional traits may be independent of xylem resistance to hydraulic failure. Additionally, these functional traits may not represent reliable proxies for xylem vulnerability to embolism (Santiago et al. 2018) and species responses to drought (Maréchaux et al. 2015), in contrast to what has been reported in other studies (Markesteijn et al. 2011, Li et al. 2018). However, we observed consistent relationships between the minimum water potentials experienced by species in the driest time of the year and stem specific density (SSD). Species that experienced had high SSD experienced very low Ψ_{min}.

This relation was stronger in the drier site with a greater decreases in Ψ_{min} per unit change in SSD in the NWG site.

We observed that intra-specific variation with respect to Ψ_{min} and Ψ_{50} was species specific. Of the seven species that occurred at both, two had significant intra-specific variation in Ψ_{50}, having more resistant xylem in the drier site. This is in contrast to suggestions that measures of xylem vulnerability to embolisms may be species-specific and should not differ within species in populations adapted to different environmental conditions (Rosas et al. 2019, Skelton et al. 2019, Torres-Ruiz et al. 2019), and provides further evidence to support recent studies that have shown significant intra-specific variation in these traits (Stojnić et al. 2018, Chauvin et al. 2019, Pritzkow et al. 2020). While Ψ_{min} was more negative in the drier site for three of the seven common species, the other four species were able to maintain similar Ψ_{min} at both sites often at a well-hydrated state even in the drier site. In comparing differences in Ψ_{min} and Ψ_{50} for these species the was no apparent evidence for coordination in these measures.This indicates that diverse strategies beyond those related to xylem resistance to hydraulic failure facilitate the occurrence of these seven species in these two sites that drastically differ in their precipitation and rainfall seasonality.

Conclusion

For the dominant species of two Indian forest communities that differ drastically in their mean annual precipiatation and dry season length, we report large variation in the minimum water potential experienced during the dry season (Ψ_{min}), and xylem resistance to embolism (quantified as Ψ_{50} and Ψ_{88}), and the resultant hydraulic safety margins (HSM). In fact, the ranges in Ψ_{50}, Ψ_{88} and Ψ_{min} were comparable to those observed in the tropics and the drier NWG site had one of the largest ranges in HSM reported in the tropics. This large variation in HSM in these sites, indicate that while some species are very safe, some species are in particularly high risk of catastrophic hydraulic failure, associated loss of function and subsequently mortality. We also highlight that evaluating species (or communities) that differ in the slope of the vulnerability curve (loss of hydraulic conductance per unit decrease in water potential), can result in different predictions. With respect to communtiy weighted HSM-Ψ_{88}, which is shown to be a better predictor of drought induced mortality for angiosperms, the wetter CWG site is more at risk than the drier NWG site. However, the opposite is true for HSM-Ψ_{50}. For these species, popularly measured functional traits, LMA, LDMC, SSD, were not related to Ψ_{50} and Ψ_{88} and hence maynot serve as oppriopriate proxies in this system. We also show that intra specific variation in Ψ_{50} as well as Ψ_{min} was infact species specific indicating that species common to both these sites employ a diverse range of strategies to be able to do so. Overall, we highlight large variation in the hydraulic traits and the resultant saftey margin in these SDTFs, indicating diverse strategies that have evolved in such systems. Further community level studies, especially from the highly diverse and understudied African and Asian basins, are essential to make informed predictions of how global vegetation will respond to extreme climate change scenarios.

Contributions

Ron Sunny, Deepak Barua, Julia Taveres, David Galbraith, and Emanuel Gloor designed the experiment. Ron Sunny, Rishiddh Javeri, Balachandra Hegde quantified xylem vulnerability, leaf and stem morphological traits, and minimum water potential. Ron Sunny performed the analysis. The code used for generating the vulnerability curves were writtin by Paulo Bittencourt with modifications by Julia Taveres and Ron Sunny.

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Supplementary figures and tables

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Table S1: Species list, taxonomic affiliation, leaf habit (LH; E - Evergreen, D - Deciduous), and trait values for: a) Northern Western Ghats; and, b) Central Western Ghats. The traits examined were: Ψ_{min} - minimum water potential; Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50% and 88% loss of hydraulic conductance; the slope of the vulnerability curve fit for each species, HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and $\Psi_{88;}$ LMA - leaf mass per area, LDMC - leaf dry matter content, and SSD - stem-specific density. NA = information not available.

Species	**ID**	**Family**	**LH**	**Ψ_{min}**	**Ψ_{50}**	**Ψ_{88}**	**Slope**	**HSM-Ψ_{50}**	**HSM-Ψ_{88}**	**LMA**	**LDMC**	**SSD**
a) Northern Western Ghats												
1. *Actinodaphne angustifolia*	AC	Lauraceae	E	-1.60	-1.96	-4.13	23.0	0.36	2.53	117.9	447.9	0.50
2. *Atalantia racemosa*	AR	Rutaceae	E	-3.38	-0.43	-0.77	147.4	-2.95	-2.61	123.4	405.1	0.65
3. *Bridelia retusa*	BR	Phyllanthaceae	D	-1.57	-2.52	-5.31	17.9	0.95	3.74	117.6	380.2	0.51
4. *Careya arborea*	CA	Lecythidaceae	D	-0.94	-1.58	-3.37	27.8	0.64	2.43	118.0	NA	0.44
5. *Psydrax diococcos*	CD	Rubiaceae	E	-3.67	-0.75	-1.48	68.6	-2.92	-2.20	198.6	445.8	0.68
6. *Diospyros montana*	DM	Ebenaceae	D	-3.82	-1.31	-2.42	44.6	-2.52	-1.40	101.1	383.6	0.53
7. *Mangifera indica*	MI	Anacardiaceae	E	-0.24	-1.43	-2.31	56.9	1.19	2.07	131.9	437.0	0.42
8. *Mallotus phillipensis*	MP	Euphorbiaceae	E	-1.67	-1.26	-3.19	25.7	-0.41	1.53	88.3	479.5	0.50
9. *Memecylon umbellatum*	MU	Melastomataceae	E	-3.95	-2.02	-4.32	21.7	-1.93	0.37	210.9	439.4	0.71
10. *Olea dioica*	OD	Oleaceae	E	-4.64	-1.49	-4.75	15.2	-3.16	0.11	123.4	442.8	0.68
11. *Symplocos beddomei*	SB	Symplocaceae	E	-1.67	-1.74	-3.70	25.5	0.07	2.03	123.7	NA	0.43
12. *Syzygium cumini*	SC	Myrtaceae	E	-1.43	-3.65	-6.63	16.7	2.21	5.20	103.8	378.3	0.57
13. *Terminalia bellirica*	TB	Combretaceae	D	-1.23	-1.86	-3.95	23.9	0.63	2.72	142.5	330.7	0.57
14. *Terminalia chebula*	TC	Combretaceae	D	-1.27	-1.99	-4.76	18.0	0.72	3.49	127.6	326.6	0.58

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Species	**ID**	**Family**	**LH**	**Ψ_{min}**	**Ψ_{50}**	**Ψ_{88}**	**Slope**	**HSM-Ψ_{50}**	**HSM-Ψ_{88}**	**LMA**	**LDMC**	**SSD**
b) Central Western Ghats												
1. *Psydrax dicoccos*	CD	Rubiaceae	E	-1.76	-0.22	-0.29	646.6	-1.54	-1.47	187.3	438.7	0.65
2. *Canarium strictum*	CN	Burseraceae	E	-0.27	-2.64	-3.87	40.2	2.37	3.61	136.3	358.6	0.41
3. *Holigarna arnottiana*	HA	Anacardiaceae	E	-0.57	-1.69	-2.00	158.5	1.11	1.43	132.5	366.3	0.40
4. *Hopea odorata*	HW	Dipterocarpaceae	E	-0.34	-2.39	-4.27	26.5	2.05	3.93	125.2	429.2	0.57
5. *Knema attenuate*	KA	Myristicaceae	E	-0.44	-0.53	-0.78	201.2	0.09	0.34	102.3	387.1	0.44
6. *Memecylon umbellatum*	MU	Melastomataceae	E	-1.31	-1.19	-2.36	42.9	-0.11	1.05	194.1	454.8	0.73
7. *Olea dioica*	OD	Oleaceae	E	-1.60	-2.05	-3.71	30.0	0.45	2.11	81.7	389.8	0.62
8. *Symplocos beddomei*	SB	Symplocaceae	E	-1.31	-1.61	-3.58	25.3	0.30	2.27	138.3	427.5	0.44
9. *Syzygium cumini*	SC	Myrtaceae	E	-1.01	-2.10	-3.92	27.4	1.08	2.90	133.9	429.6	0.58
10. *Terminalia bellirica*	TB	Combretaceae	D	-0.77	-1.89	-3.99	23.6	1.12	3.22	147.7	355.1	0.66
11. *Terminalia chebula*	TC	Combretaceae	D	-1.21	-2.49	-4.53	24.4	1.27	3.31	166.4	406.4	0.69
12. *Terminalia paniculata*	TP	Combretaceae	D	-1.00	-1.71	-3.36	30.2	0.71	2.36	116.7	325.7	0.56

Table S2: Differences in traits between the two sites. Results for one-way ANOVA with site as a fixed effect for: a) hydraulic traits - Ψ_{min} - minimum water potential; Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50% and 88% loss of hydraulic conductance; and, HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and Ψ_{88}); and, b) plant morphological traits (LMA - leaf mass per area, LDMC - leaf dry matter content, SSD - stem-specific density).

	Traits	***df***	***MS***	***F***	***p***	***R*^{2}**
a) Hydraulic traits	Ψ_{min}	1	17.0	17.89	<0.01	0.36
	Log Ψ_{min}	1	0.7	7.43	0.01	0.24
	Ψ_{50}	1	0.0	0.00	0.98	0.00
	Ψ_{88}	1	2.3	1.04	0.32	0.04
	HSM-Ψ_{50}	1	10.1	4.46	0.05	0.15
	HSM-Ψ_{88}	1	2.8	0.71	0.41	0.02
b) Morphological traits	LMA	1	404.0	0.36	0.55	0.01
	LDMC	1	685.8	0.35	0.56	0.01
	SSD	1	0.0	0.02	0.88	0.00

Table S3: Differences in traits between the two sites. Site average and standard error and community weighted means for : a) hydraulic traits - Ψ_{min} - minimum water potential; Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50% and 88% loss of hydraulic conductance; and, HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and Ψ_{88}); and, b) plant morphological traits (LMA - leaf mass per area, LDMC - leaf dry matter content, SSD - stem-specific density). Statistical significance for differences between sites is shown by ** ($p < 0.05$), * ($p < 0.1$), or NS (not significant).

Traits	**Northern WG**		**Central WG**		
	Species	**CWM**	**Species**	**CWM**	**test**
a) Hydraulic					
Ψ_{min}	-2.22 ± 0.36	-2.7	-0.96 ± 0.14	-1.0	**
Log Ψ_{min}	0.24 ± 0.09		-0.08 ± 0.07		*
Ψ_{50}	-1.71 ± 0.2	-2.4	-1.7 ± 0.21	-1.8	NS
Ψ_{88}	-3.64 ± 0.41	-4.5	-3.05 ± 0.39	-2.4	NS
HSM-Ψ_{50}	-0.5 ± 0.48	-0.2	0.74 ± 0.29	0.9	**
HSM-Ψ_{88}	1.42 ± 0.61	1.9	2.08 ± 0.44	1.3	NS
b) Morphological					
LMA	130.61 ± 9.15	141.23	138.52 ± 9.36	119.22	NS
LDMC	408.08 ± 14.04	410.73	397.38 ± 11.55	394.01	NS
SSD	0.55 ± 0.02	0.61	0.56 ± 0.03	0.57	NS

Table S4: The relationship between hydraulic traits and morphological traits in the two study sites: Northern Western Ghats (NWG - relatively drier site with a longer dry period) and Central Western Ghats (CWG - wetter site with shorter dry period). Pearson's correlation coefficients are presented for relationship between hydraulic traits (Ψ_{min} - minimum water potential; Ψ_{50} and Ψ_{88} - the xylem water potential that results in a 50% and 88% loss of hydraulic conductance; and, HSM-Ψ_{50} and HSM-Ψ_{88} - Hydraulic safety margins for Ψ_{50} and Ψ_{88}), and plant morphological traits (LMA - leaf mass per area, LDMC - leaf dry matter content, SSD - stem-specific density). Significant relationships are depicted by ** ($p < 0.05$).

	Northern WG			**Central WG**		
	LMA	**LDMC**	**SSD**	**LMA**	**LDMC**	**SSD**
Ψ_{min}	-0.38	-0.30	-0.79 **	-0.32	-0.42	-0.59 **
Ψ_{50}	0.18	0.37	0.15	0.25	0.26	0.06
Ψ_{88}	0.19	0.32	0.03	0.20	0.17	-0.13
HSM-Ψ_{50}	-0.37	-0.38	-0.67 **	-0.34	-0.39	-0.32
HSM-Ψ_{88}	-0.36	-0.39	-0.49	-0.29	-0.28	-0.07

Table S5: Summary table with F statistic for two-way ANOVA for effect of species, site and their interaction on the traits examined for 7 species that were present in both the sites. Those traits for which individual level data was available was included; hydraulic traits (Ψ_{min} - minimum water potential) and plant morphological traits (LMA - leaf mass per area, LDMC - leaf dry matter content, SSD - stem-specific density). (** p<0.01, * p<0.05)

	Traits	**Site**		**Species**		**Site x Species**	
a) Hydraulic traits	Ψ_{min}	50.9	**	13.7	**	6.6	**
	Log Ψ_{min}	58.3	**	17.4	**	3.7	**
b) Morphological traits	LMA	0.2		54.1	**	6.6	**
	LDMC	12229.2	**	15.8	**	15.8	**
	SSD	2.4		22.4	**	3.0	*

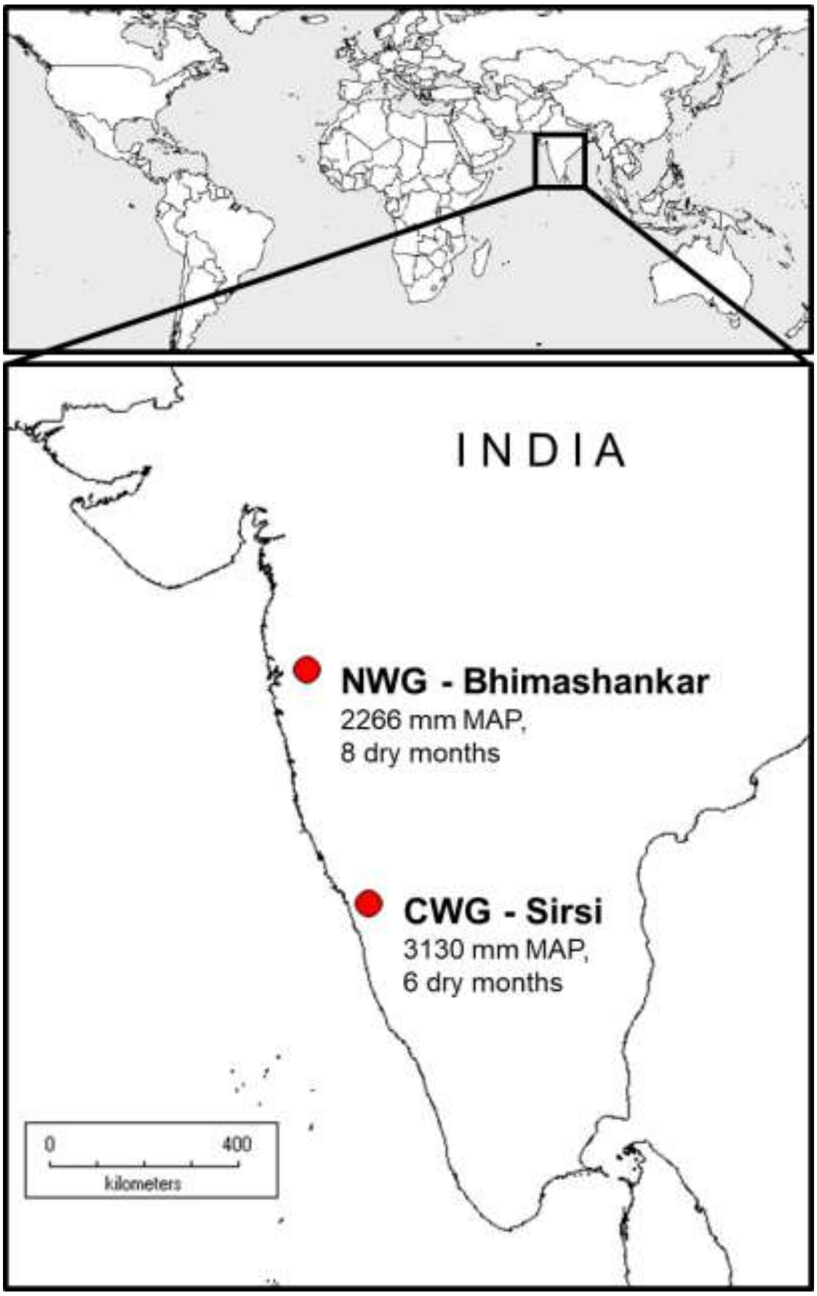

Figure S1: Map showing the location and climate of the study sites. MAP - mean annual precipitation; dry months represent the number of months with rainfall lower than 100 mm. Climate data were obtained from CRU version 2.0 dataset with a climate avergae for the years 1961-1990 (New 2002).

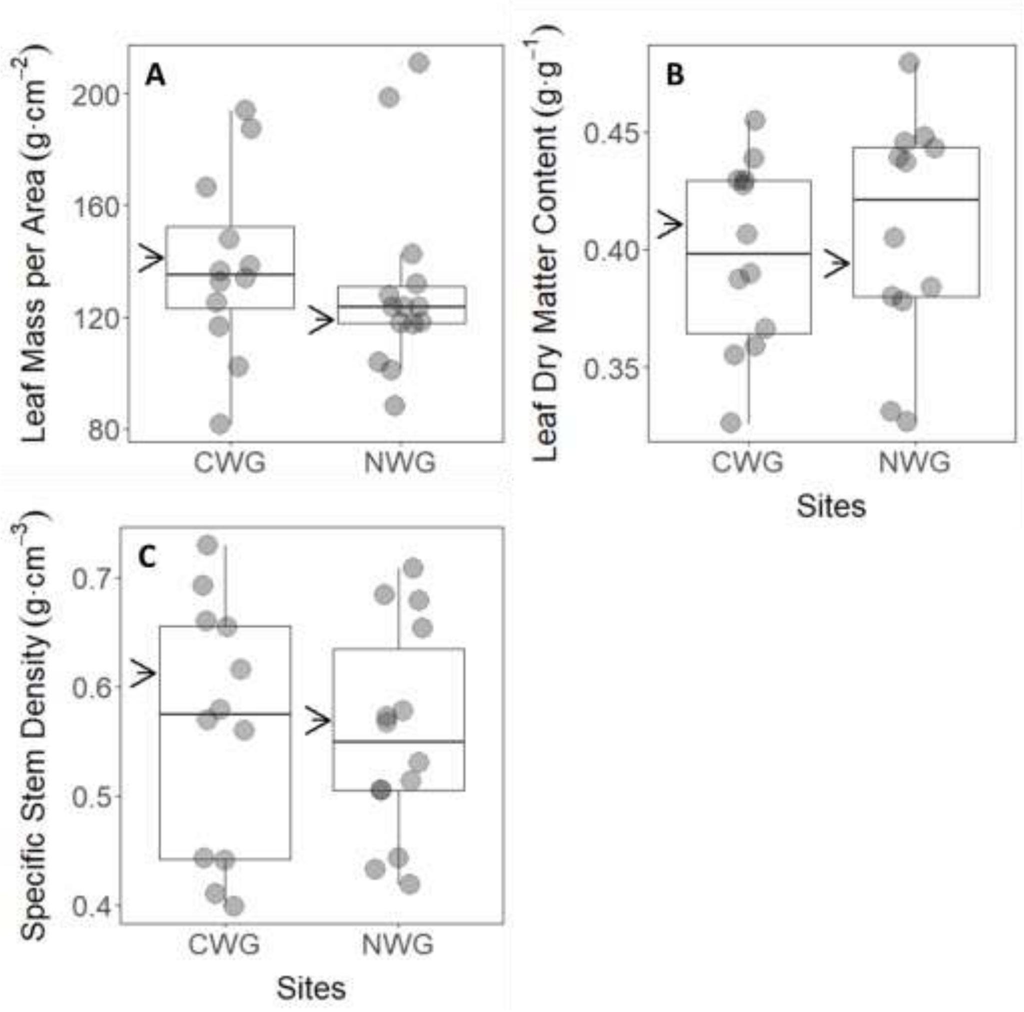

Figure S2: Morphological trait values for the two study sites, Northern Western Ghats (NWG - relatively drier site with a longer dry period) and Central Western Ghats (CWG - wetter site with shorter dry period): a) Leaf mass per area (LMA); b) Leaf dry matter content (LDMC), c) Stem-specific density (SSD). Standard box plots with the lower and upper limits of the boxes representing the first and the third quartiles are presented. Arrows on each box corresponds to dominance weighted trait values.

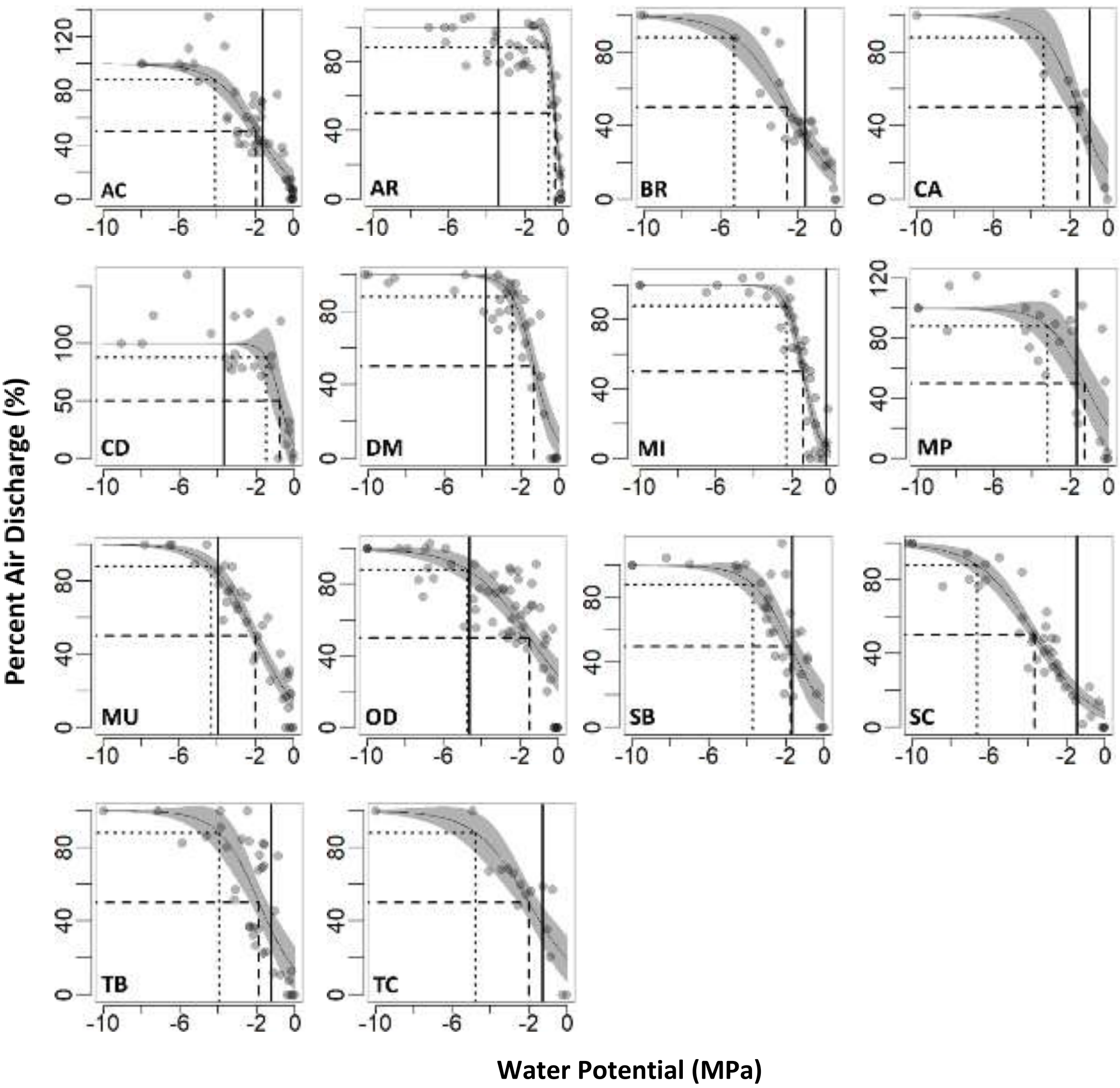

Figure S3: Hydraulic vulnerability curves for the species considered in this study from the drier site in the Northern Western Ghats. Dotted line corresponds Ψ_{88}, dashed line Ψ_{50}, solid line Ψ_{min}. Points correspond to data for all replicates. The grey band corresponds to the confidence interval. The species ID is indicated in each graph and corresponds to the species as described in TableS1.

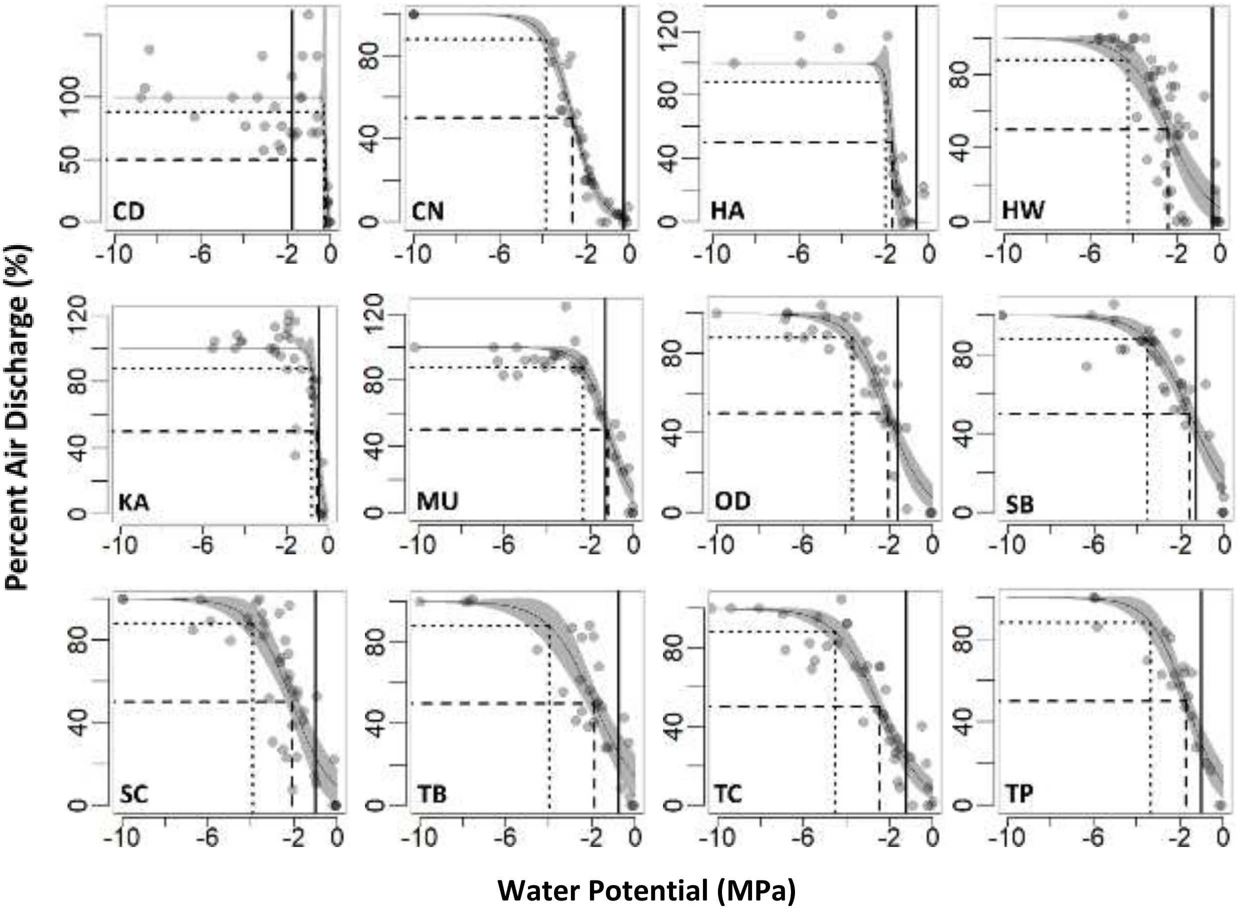

Figure S4: Hydraulic vulnerability curves for the species considered in this study from the wetter site in the Central Western Ghats. Dotted line corresponds Ψ_{88}, dashed line Ψ_{50}, solid line Ψ_{min}. Points correspond to data for all replicates. The grey band corresponds to the confidence interval. The species ID is indicated in each graph and corresponds to the species as described in TableS1.

Chapter 4

Beyond turgor loss point, resistance to loss of cellular function and rate of water loss represent independent ecologically important hydraulic strategies for dry tropical forest trees

Abstract

Large variation in species responses contribute to the complex mortality patterns observed in forests as a result of recent drought events. In trying to understand variation in species drought tolerance, the focus has been on a few key traits, but the multi-dimensional nature of plant responses to drought is often overlooked. In addition to resistance to loss of leaf turgidity and shrinkage that are important during the early stages of dehydration, we examined variation in resistance to loss of cellular function and integrity, quantified as the loss of the maximum quantum yield of Photosystem II (F_v/F_m) that represents resistance to severe dehydration, in eighteen coexisting tree species from a seasonally dry tropical forest. Additionally we examined rates of water loss after stomatal closure (g_{min}), a trait that indicates how quickly physiological damage thresholds are approached. We observed large variation in the hydraulic traits examined. Interestingly, resistance to early dehydration quantified by loss of turgidity and shrinkage, resistance to severe dehydration quantified by loss of photochemical function (F_v/F_m), and the rate of water loss (g_{min}) were not related to each other in these eighteen species. Species that had experienced very low leaf water content in the driest time of the year had high resistance to loss of photochemical function. The rate at which species lost water post stomatal closure was higher for deciduous species with larger stomatal pores and lower leaf mass per area. Thus, resistance to early dehydration, resistance to severe dehydration, and the rates of water loss are ecologically important, and may represent independent strategies in dealing with drought in seasonally dry tropical forests. Incorporating these understudied dimensions of response to drought is important to understand drought-induced mortality in these forests.

Introduction

Drought-induced mortality is expected to increase with projected changes in climate, and tropical ecosystem may be particularly vulnerable to these changes (Duffy et al. 2015, Allen et al. 2017). There is wide variation in species responses to drought, and differences in physiological drought tolerances may alter species distributions and community compositions (Engelbrecht et al. 2007, Baltzer et al. 2008, Kursar et al. 2009, Blackman, Brodribb & Jordan 2010). This is likely to have important consequences for the structure and function of tropical ecosystems and it important to understand species-specific drought tolerance to understand how species rich tropical communities will respond to drought.

Experimental studies of physiological and growth responses to drought are labour and time intensive, and not suitable for larger long lived individual individuals. An alternate approach gaining traction is to identify physiological traits with direct mechanistic links to plant responses to drought. Recent work has shown that leaf water potential at wilting or turgor loss point is one such trait that is closely associated with drought tolerance and with species distributions with respect to water availability within and across biomes (Bartlett 2012, Bartlett 2016, Maréchaux 2015). However, turgor loss associated with leaf wilting, shrinkage, and stomatal closure represents early responses of leaves to drought and occurs as a response to mild to moderate dehydration. These early responses are readily reversible if access to water is restored (Trueba 2019).

Our understanding of variation in leaf responses to more severe dehydration, including the loss of mechanical and structural integrity and breakdown of cellular function that ultimately leads to cellular necrosis and death is poor. It is often assumed that tolerance to early dehydration will be related to tolerance to extreme dehydration. Another key process that remains poorly understood is water loss from leaves after stomata have closed (Brodribb 2017). The rate of loss of water will determine how long leaves can avoid irreversible damage due to extreme dehydration in the face of protracted droughts. Again, it is often assumed that variation in rates of water loss is low and thus not ecologically important. In this study we examined how variation in leaf level tolerance to early drought (resistance to turgor loss) is related to tolerance to more extreme drying (resistance to loss of leaf structural integrity and cellular function). We asked how these measures of leaf physiological tolerance are related rate of loss of water post stomatal closure.

For species, the turgor loss point (TLP), the leaf water potential at which wilting occurs has been identified as an important proxy for drought tolerance (Bartlett et al. 2012). Species that can maintain leaf turgidity when faced with low water availability are able to sustain critical processes including hydraulic conductance (Blackman et al. 2010), stomatal conductance, and photosynthetic gas exchange (Brodribb and Holbrook 2003). TLP is known to vary widely, even in species from the wet Amazonian evergreen forests (Maréchaux et al. 2015), is correlated with aridity (Lenz et al. 2006, Bartlett et al. 2012, Zhu et al. 2018), and is useful to predict species distributions across gradients of water availability (Baltzer et al. 2008). Recent studies have shown that leaf shrinkage can be used as a reliable indicator for the failure of leaf hydraulic conductance (Scoffoni et al. 2014, Scoffoni et al. 2017). Shrinkage during dehydration results from the loss of water and ultimately the loss of leaf structural integrity, and is an indicator of the breakdown of the outside xylem hydraulic pathway, disruption of the apoplastic pathway and loss of cell-cell connectivity within in the leaf (Sancho-knepik 2011, Scoffoni et al. 2014, Buckley 2015). Leaf shrinkage has been used in crops as an indicator of plant water status (Syvertsen and Levy 1982, McBurney 1992), and Scoffoni et al. (2014) show that resistance to shrinkage is greater in species adapted to drier habitats, indicating the potential of using this trait as a good proxy for physiological drought tolerance.

In moist and wet tropical forests, the ability of species to sustain gas exchanges and carbon assimilation in the face of mild to moderate drought is likely to be very important. In these environments resistance to loss of turgidity and resistance to leaf shrinkage may allow plants to maintain stomatal and hydraulic conductance. However, the decline in physiology and function in response to more negative water potentials that result in loss of turgidity and shrinkage are readily reversible on rehydration (Trueba et al. 2019). The relationship between TLP and resistance to severe drought and mortality has rarely been tested (Delzon 2015), and at least for some species may actually be negatively related to drought tolerance (Sun et al. 2020). Thus though resistance to loss of turgor may represent resistance to mild dehydration, TLP may not be related to mortality due to severe dehydration (Farrell et al. 2017, Guadagno et al. 2017). Conditions that result in severe dehydration are common in seasonally dry tropical forests (SDTF), which forms a significant fraction of the tropical biome and are largely understudied. Additionally predictions of extreme precipitation scenarios warrants for better proxies of leaf dehydration resistance, especially those that can predict plant mortality in SDTFs.

While changes in water transport and the water status of plants are commonly used as indicators of drought stress and tolerance, they can be decoupled from cellular function, meristematic activity and regrowth (Guadagno et al. 2017). In contrast, the breakdown of membrane integrity at the cellular level represents a more direct mechanistic cause of cellular death, and can be monitored by chlorophyll fluorescence to quantify cellular function and breakdown during dehydration. Leaf photochemical performance assessed by Chlorophyll fluorescence is widely used to quantify cellular damage and resistance to different forms of abiotic stress in plants (Zulini et al. 2007, Woo et al. 2008, Baker 2008, Ow et al. 2011, Sastry and Barua 2017). Though not commonly used for drought, recent work suggests that measures of chlorophyll *a* fluorescence are reliable indicators of irreversible cellular damage caused by severe drought (Guadagno et al. 2017, Cardoso et al. 2018, Trueba et al. 2019), and it has been suggested that these measures can be used to operationally define death arising from drought stress (Guadagno et al. 2017). The maximum quantum efficiency of photosystem II (PS II) can be quantified as the ratio of dark-adapted variable to maximum fluorescence (F_v/F_m) and is an indicator of the light harvesting function of PS II (Baker 2008). There is large variation in the relative water content that results in the in breakdown of PS II function (Trueba et al. 2019). Thus, understanding leaf photochemical responses to changing leaf water status may be important in assessing resistance to severe dehydration, and will allow us to compare how these responses are related resistance to relatively milder dehydration assessed by quantifying TLP and leaf shrinkage.

While stomatal closure results in a large decrease in transpiration and water loss, plants continue to lose water after stomates are completely closed due to incomplete closure or leaky stomata, or via cuticular conductance (Schreiber & Riederer, 1996). This rate of loss of water determines how long a plant can avoid long-term damage caused by embolism in the face of protracted droughts. In using measures of physiological resistance to dehydration to rank species vulnerability to drought, we make an implicit assumption that rates at which species lose water are similar across species, or at least that differences among species are small enough not to alter rank orders for vulnerability to drought. However, the magnitude of water loss after stomatal closure can be significant and varies considerably among species (Schreiber & Riederer 1996, Duursma et al. 2019), and can potentially alter species rankings that are based on measures of physiological resistance to dehydration. Indeed, a number of recent studies have suggested that the rates of drying are important in determining the time to death (Fig. S2) (Gleason et al. 2014, Blackman et al. 2016, Martin-StPaul et al. 2017). We still understand little about this important hydraulic trait (Duursma et al. 2019, Brodribb 2020), and we do not know how rates of drying are related to resistance to loss of turgidity, structural integrity and cellular function.

To examine variation in species responses to mild dehydration we quantified leaf resistance to turgor loss and shrinkage, and to more severe dehydration we quantified resistance to loss of chlorophyll fluorescence. These were quantified in eighteen coexisting tree species from a seasonally dry tropical forest in peninsular India. Additionally, we measured leaf minimum conductance (g_{min}) to understand the rate at which leaves from these species lose water after stomatal closure. We monitored the time it took for each species to reach critical thresholds: loss of turgidity, breakdown of structural integrity, and breakdown cellular function. In addition to these measurements, we monitored leaf phenology in the field, and quantified leaf water status during the driest time of the year (RWC_{min}). This allowed us to address the following questions:

1. Is resistance to early dehydration (estimated using Ψ_{TLP}, $RWC_{TLP,}$ $RWC_{thick50}$) related to resistance to severe dehydration (estimated using RWC_{flbrk})? Are these differences related to rate to water loss (g_{min})?
2. How are these measures of resistance to dehydration related to: a) stomatal traits; b) modulus of elasticity of the tissue; and, c) leaf capacitance? How are these measures related to commonly examined functional traits LMA, LDMC, SSD and LA?
3. Does rate of water loss during dehydration determine the time to reach critical RWC thresholds that results in loss of turgidity, structural integrity and cellular function?
4. Are these measures of resistance to early and severe dehydration and water loss rate of these species related to their functioning in their natural habitat with respect to: a) deciduousness; b) leaf water status in the driest time of the year?

Table 1: List of traits examined in the study, their abbreviations and their units. RWC stands for 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
		RWC at 50 % leaf thickness	$RWC_{thick50}$	%
b)	Resistance (severe dehydration)	RWC at 50 % decrease in F_v/F_m	RWC_{fl50}	%
		RWC at 5 % decrease in F_v/F_m	RWC_{flbrk}	%
c)	Rate of water loss	Minimum cuticular conductance	Log g_{min}	$mmol \cdot m^{-2} \cdot s^{-1}$
d)	Dehydration time to physiological threshold	Time to RWC_{TLP}	$Time_{TLP}$	Hours
		Time to $RWC_{thick50}$	$Time_{thick50}$	Hours
		Time to RWC_{fl50}	$Time_{fl50}$	Hours
		Time to RWC_{flbrk}	$Time_{flbrk}$	Hours
e)	Stomatal traits	Pore size	Pore size	µm
		Guard cell length	Guard size	µm
		Stomatal density	StomDen	mm^{-2}
		Stomatal Pore Index	SPI	
		Max. stomatal conductance – water	Log g_{wmax}	$mol \cdot m^{-2} \cdot s^{-1}$
f)	Capacitance	Capacitance per leaf area at full turgor	Log CFT_{abs}	$mol \cdot m^{-2}$ MPa^{-1}
		Relative capacitance at full turgor	Log $CFT_{relative}$	MPa^{-1}
		Relative capacitance - zero turgor	CTLP	MPa^{-1}
g)	Leaf water status	Saturated water content	Log SWC	$g \cdot g^{-1}$
		Osmotic potential at full turgor	Ψ_O	MPa
h)	Cell wall rigidity	Modulus of elasticity at full turgor	e	MPa
i)	Morphological functional traits	Leaf area	Log LA	cm^2
		Leaf dry matter content	LDMC	$g \cdot g^{-1}$
		Leaf mass per area	Log LMA	$g \cdot m^{-2}$
		Specific stem density	SSD	$g \cdot cm^{-3}$
j)	On field Parameters	Average canopy loss	Canopy Loss	%
		Minimum relative water content	RWC_{min}	%
		Minimum water potential	Ψ_{min}	MPa

Methods

Plant material: We selected eighteen angiosperm tree species based on the relative abundance from a seasonally dry tropical forest near Nigdale, Maharashtra, India. This region in the northern extremes of the Western Ghats range in peninsular India has a mean annual precipitation of 2266 mm and an eight month dry period with rainfall below 100 mm (New et al. 2002). Mature individuals were chosen, and sun exposed branches collected for bench drying assays.

Minimum cuticular conductance: For the selected species, 2nd or 3rd order branches containing fully expanded and matured leaves were collected from six replicate individuals between March and May of 2017. Collected branches were placed in a darkened plastic bag, and the bags sealed with moist paper towels in the bag to keep the air in the bag water saturated. The branches were brought back to the laboratory, and leaves placed in beakers with petioles immersed in water for rehydration. For each replicate individual, one fully expanded mature leaf was selected and used to quantify minimum cuticular conductance (g_{min}). The selected leaf was weighed to measure the saturated fresh weight (SFW) (±0.01mg), thickness measured using a digital micrometer (±0.002mm, Mitutoyo), and scanned (CanoScan LIDE 110- Canon) to quantify leaf area (Using image analysis software ImageJ (version 1.47v; National Institutes of Health). The leaf was then allowed to dry in the laboratory in low irradiance, under controlled conditions. The average relative humidity in the room was 38 %, and the average temperature was 27 °C. After the initial reading the leaf was weighed after the 1st, 2nd, 3rd, 6th, 9th and 13th hour and then after every 12 hours. This was done so that more frequent measurements were taken during the early drying period. A table fan was used to minimise boundary layer conductance. The drying was continued till the dark-adapted fluorescence measurements (described in the next section) reached zero, indicating complete loss of cellular function. At the end of the bench drying exercise, leaves were oven-dried for 72 hours at 75°C and dry-weight (DW) and thickness were measured. The relative water content (RWC) corresponding to every time point (t) for a given leaf was calculated as:

$$\mathrm{RWC(t)} = \frac{[\mathrm{FW(t)-DW}]}{[\mathrm{SFW-DW}]} \qquad (1)$$

where FW(t) corresponds to the leaf weight measured at time t.

Estimates of minimum cuticular conductance (g_{min}) were determined using protocols described in Sack et al. (2003) and Sack and Scoffoni (2010). The first hour of measurements was omitted to allow for complete stomatal closure, and the slope of the subsequent linear portion of the RWC-time measurements was used to calculate g_{min}.

Leaf shrinkage: The same leaf that was subject to bench drying for estimating g_{min} was used for quantifying leaf thickness using a digital micrometre (±0.002mm, Mitutoyo) to measure shrinkage during dehydration (Scoffoni et al. 2014). Thickness for each leaf was normalized to the maximum thickness measured when the leaf was fully water saturated. Data from how shrinkage progressed as a function of relative water content of leaves from the six replicate individuals were pooled. A three parameter exponential decay curve (parameters *c*, *d* and *f*) was fit to this data using the following model:

$$y = c + (d - c) * \left(\exp^{-\frac{x}{f}}\right) \qquad (2)$$

where *y* corresponded to the relative leaf thickness at water content *x*, *x* corresponded to 100-RWC of the leaf, *d* to an initial relative thickness of 1, *c* was the final relative thickness that the leaves attain towards the end of the dehydration process which was fixed to reflect the average final relative thickness computed for each species and *f* corresponded to the steepness of the decay. The relative water content of leaves that resulted in a 50 % decrease in relative leaf thickness ($RWC_{thick50}$) was used as a measure of leaf resistance to shrinkage during dehydration. In order to quantify how species differed in the time taken to lose leaf thickness during dehydration we fit the data for how relative leaf thickness changed as a function of time for each species to a similar three parameter exponential model where *x* corresponded to time. The time at which the species lost 50% of its relative leaf thickness ($Time_{thick50}$) was estimated from this model as a measure of time to shrinkage for these species.

Chlorophyll fluorescence: The same leaves that were subjected to dehydration above were used to measure the chlorophyll a fluorescence. We measured the maximum quantum yield of PS II as the ratio of dark adapted variable fluorescence to maximum fluorescence (F_v/F_m), where $F_v = F_m - F_0$, and F_m and F_0 are the maximum and basal fluorescence, respectively.

Leaves were dark adapted for 20 minutes and fluorescence measurements made on the adaxial side with a PAM 2500 fluorometer (Walz, Effeltrich, Germany). This measure of maximum quantum yield of PS II is an index of leaf photochemical performance and reduction of the same represent irreversible breakdown of the photosynthetic machinery and cellular function. We fit a five parameter logistic sigmoid curve (parameters *b*, *c*, *d*, *f* and *g*) to the chlorophyll *a* fluorescence (F_v/F_m) values measured across the range of leaf relative water content using the R package ‘drc’(Ritz and Streibig 2005), of the form

$$y = c - \frac{d-c}{\left(1+exp^{(b*(log(x)-log(g)))}\right)^{f}} \qquad (3)$$

where *y* corresponded to F_v/F_m at the leaf relative water content *x*, and *d* the upper asymptote. The parameter *c* that corresponded to the lower asymptote was set to zero. We used these curves to estimate the RWC that resulted in the initial 5 % decrease (RWC_{flbrk}), and a 50 % decrease (RWC_{fl50}) in F_v/F_m. To quantify variation among the species in time taken to reach RWC_{flbrk} and RWC_{fl50} we fit the data for F_v/F_m as a function of time for each species using the same four parameter model. From these models we estimated the time to 5 % decrease in F_v/F_m ($Time_{flbrk}$) and time to 50 % decrease in F_v/F_m ($Time_{fl50}$) for each of the species.

Resistance to turgor loss: A second bench drying assay for generating leaf pressure-volume curves was carried out during November-December in the years 2017 and 2018 (as in Sack and Pasquet-Kok 2011). Briefly, sun-exposed, upper canopy leaves were collected from five individuals of every species, rehydrated overnight, and allowed to bench dry while measuring its weight (±0.01mg) and water potential (PMS pressure chamber -Model 1515D). As the leaf dried, we quantified water potential at intervals of 0.2 to 0.3 MPa till a water potential of -3 MPa was reached. The leaf was subsequently oven dried for 72 hours before measuring the dry weight that was needed to estimate the RWC of the leaf. These curves represent the change of water potential with a change in RWC, and were used to estimate water potential at turgor loss point (Ψ_{TLP}), RWC at turgor loss point (RWC_{TLP}), modulus of elasticity, and capacitance. It was not possible to monitor time while measuring leaf weight and leaf water potential to generate a species level pressure-volume curve. We hence used RWC_{TLP} information for each species generated from the pressure-volume curves and used it to estimate the time to loss of turgidity ($Time_{TLP}$) from species level curves corresponding to loss of leaf RWC as a function of time. To quantify variation in time taken by each species to reach their turgor loss point during the dehydration assay, we fit a three parameter exponential decay as in equation (2), for the change in leaf RWC with time.

Stomatal traits: Six mature and sun-exposed leaves were collected from six individuals of every species, and nail polish varnish imprints obtained from the central portion of the abaxial side taking care to avoid the midrib and major veins. Stomatal density, guard cell length, and pore size were quantified from images of the imprints using ImageJ (Schneider et al 2012). Stomatal density (StomDen) was estimated as the number of stomates in a 20X magnified field of view, and stomatal pore size and guard cell length measured from 40X magnified images. The stomatal pore index was calculated as StomDen * (Guard Cell length)^2. The maximum stomatal conductance (g_{wmax}) for water was calculated using the following equation (Franks and Farquhar 2001, Franks et al. 2009):

$$\mathrm{gwmax} = \frac{\mathrm{d} * \mathrm{StomDen} * \mathrm{amax}}{\mathrm{v}\left(\mathrm{l} + \frac{\pi}{2} * \left(\frac{\mathrm{amax}}{\pi}\right)^{-1}\right)} \qquad (4)$$

where *d* is diffusivity of water in air (m^2 s^{-1}), *amax* is the mean maximum stomatal pore area (m^{-2}) calculated as π*(0.5*pore size)^2, *v* is molar volume of air (m^3 mol^{-1}) and *l* is depth of the stomatal pore which was approximated as 0.25*Guard cell length.

Morphological functional traits: At least five mature and sun-exposed leaves were collected from six individuals of every species for quantifying leaf area, leaf dry matter content and leaf mass per area (Pérez-Harguindeguy et al. 2013). Leaves were rehydrated overnight by immersing the petioles in a beaker of water. The saturated fresh weight was measured for the rehydrated leaves and then scanned with a desktop scanner to quantify leaf area. The leaves were then oven-dried for 72 hours and their dry weight measured. Leaf dry matter content was calculated as leaf dry weight per saturated fresh weight (g·g^{-1}), and leaf mass per area as leaf dry weight per leaf area (g·m^{-2}).

Stem specific density (SSD) was estimated from three different branches for three individuals for each 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. These segments were debarked and the volume estimated by the water-displacement method (Pérez-Harguindeguy et al. 2013). The segments were subsequently oven dried to constant weight (three to five days at 70°C) and the dry weight quantified. SSD was calculated as the dry weight per volume ($g{\cdot}m^{-3}$).

Field minimum water status: To measure the leaf water status of the species in the driest time of the year, sun-exposed upper canopy leaves were collected at the end of the summer between 1230 hours to 1500 hours in May 2018. Leaf RWC quantified for 5 or more mature leaves from 6 replicate individuals of each species. The collected leaves were individually stored in small previously weighed, darkened, and sealed plastic bags for transport. The bag and leaf were weighed within three hours of collection, and the fresh weight of the leaf estimated by deducting the known weight of the plastic bag. This allowed us to account for any losses in weight that might have occurred during transportation. The leaves were then rehydrated overnight by immersing the petioles in a beaker of water. The saturated fresh weight (SFW) was measured for the rehydrated leaves measured the next morning, and the leaves oven dried for 72 hours for measurement of the dry weight (DW). The relative water content (RWC) for these leaves were calculated as in equation (1).

To quantify stem xylem water potential at the driest time of the year (Ψ_{min}), midday leaf water potential was measured with a pressure chamber (Model 1515D, PMS Instruments Co., Albany, OR, USA) at the end of the dry season in May 2019. Mature sun exposed leaves collected between 1230-1500 hours were used for quantification of Ψ_{min}, assuming that stem xylem water potential equals leaf water potential. Ψ_{min} was measured for five fully expanded mature leaves collected from the sun exposed upper canopy for three individuals of a subset of twelve species.

Phenology: Leaf phenology was quantified for 15-30 established and mature individuals of each species. The total canopy of individuals was scored by direct observation in a semi-quantitative manner on a scale from 0 to 100 % in steps of 10 %. A value of zero represents complete absence of leaves while a score of 100 represents a full canopy. Observations were conducted monthly for a period of four years between 2014 and 2017. The average canopy maintained by individuals over the year was estimated and 100 - average canopy, a measure of the average canopy loss was used as a quantitative measure of deciduousness.

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, excluding stomatal traits and SSD. We also excluded *Ficus racemosa* from the PCA since data for RWC_{min} was not available for this species. All analysis were conducted using R (version 1.2.5033, The R Foundation for Statistical Computing, Vienna, Austria) and STATISTICA (version 9.1, Statsoft, Tulsa, OK, USA).).

Results

There were large differences across species, in all traits considered to quantify resistance to early dehydration (RWC_{TLP} and Ψ_{TLP} – Fig. 1A, $RWC_{thick50}$ –Fig. 1B), severe dehydration (RWC_{flbrk} –Fig. 1C), rate of water loss (Fig. 1D), and dehydration time to these points of physiological breakdown (Table S1). RWC_{TLP} ranged between 78.6 % (*Callicarpa tomentosa*) and 95 % (*Memecylon umbellatum*). Meanwhile Ψ_{TLP} ranged from -2.51 MPa for *Flacourtia indica* to -0.58 MPa for *Xantolis tomentosa*. These ranges are similar to what is seen in tropical species (Fig. S1). $RWC_{thick50}$ ranged from 70.8 % for *Actinodaphne angustifolia* to 90. 4% for *Mangifera indica.* Species also varied widely with respect to

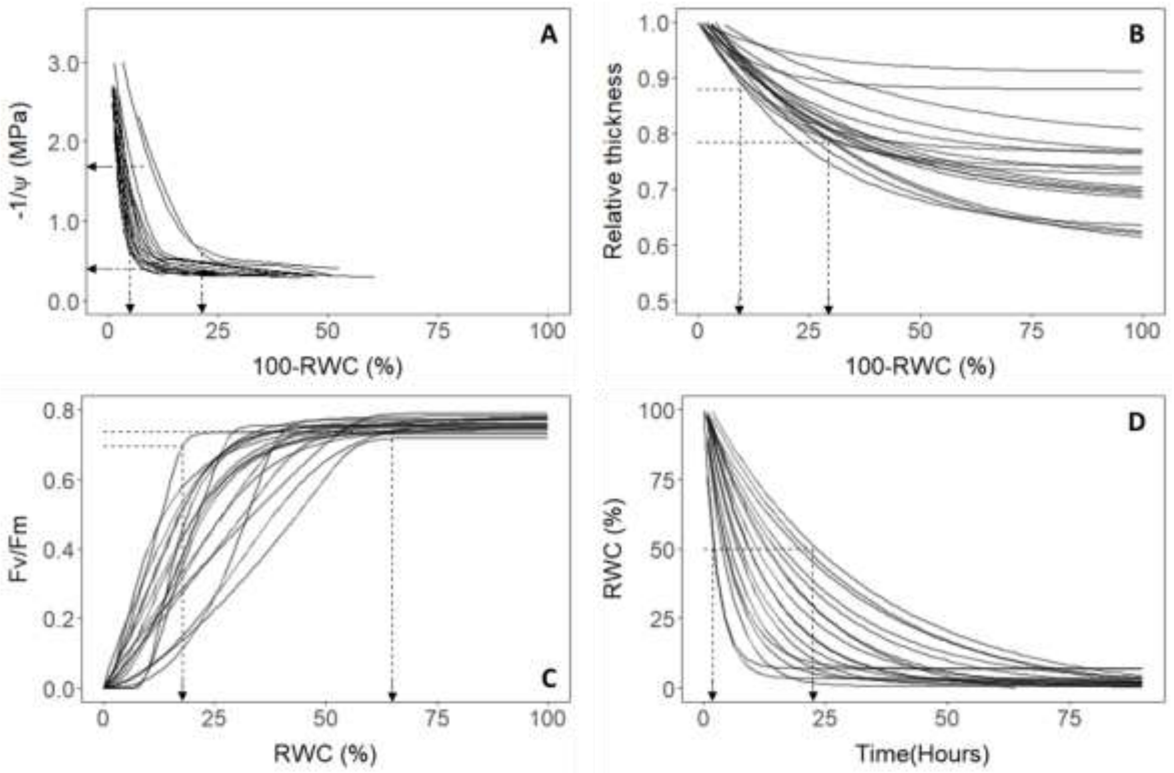

Figure 1: Variation in the four critical function traits examined through the bench drying based leaf desiccation assays A) RWC at turgor loss point (shown here in the pressure-volume curve as 100- RWC_{TLP}) B) RWC at which leaf thickness shrinks by 50% ($RWC_{thick50}$) C) RWC at which Photosystem II function begins to breakdown (RWC_{flbrk}) and D) time taken to reach 50% RWC, studied in 18 coexisting species. Each curve corresponds to a species. Dashed line indicates species with minimum and maximum trait value.

shrinkable thickness, expressed as a percentage of the fully saturated average leaf thickness, from 42 % for *Callicarpa tomentosa* to 9 % for *Olea dioica*. Of the three physiological thresholds examined, RWC_{flbrk} varied the most from 26.5 % for *Xantolis tomentosa* to 65.5 % for *Mangifera indica.* The rate of water loss, estimated as g_{min} ranged from 1.21 mmol m^{-2} s^{-1} for *Syzygium cumini* to 7.41 mmol m^{-2} s^{-1} for *Bridelia retusa*. With respect to dehydration time species ranges varied as much as 34.3 hours for $Time_{flbrk}$, with 14.3 hours for $Time_{thick50}$ and 4.8 hours for $Time_{TLP}$. Interestingly, species with high resistance to early dehydration were not the same as those with resistance to more extreme dehydration, and also not related to species that had the lowest rates of water loss.

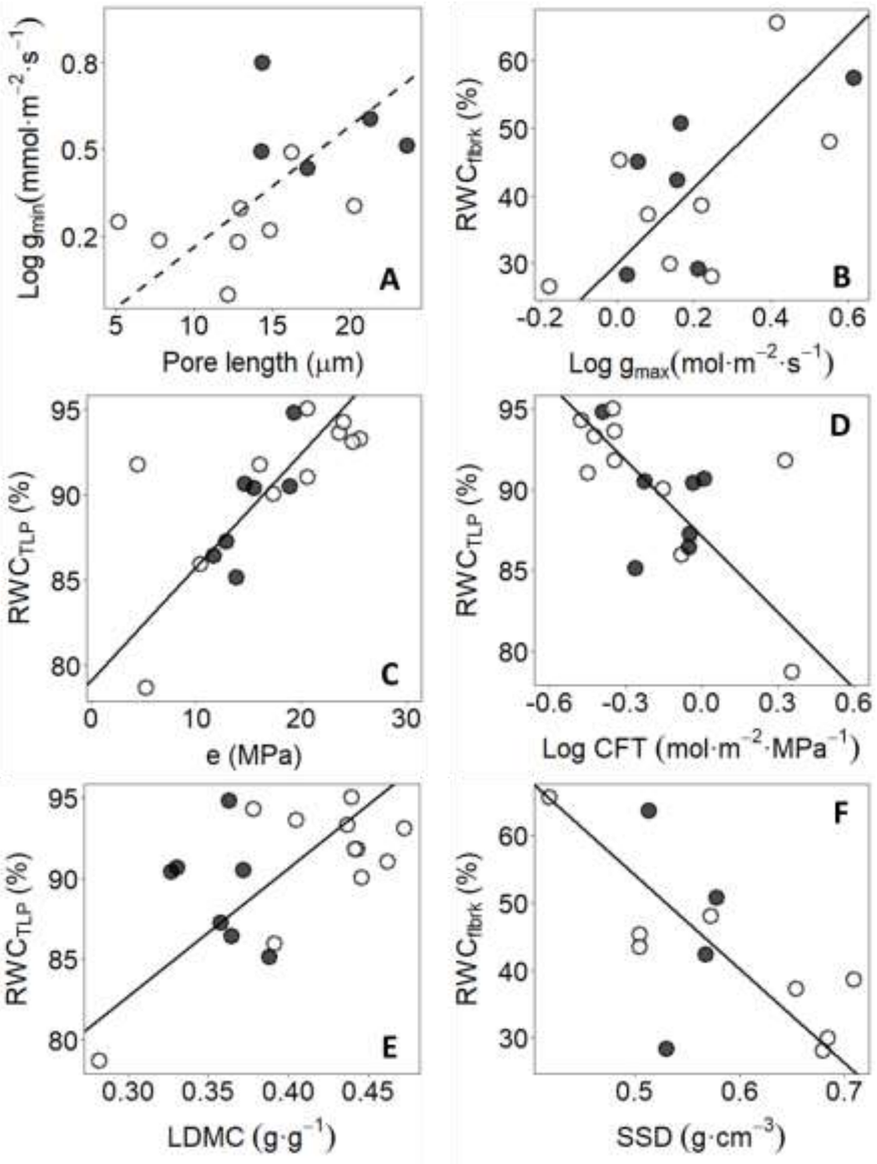

Figure 2: Relation between the 4 traits examined and (A,B) stomatal traits, (C) modulus of elasticity at full turgor, (D) absolute capacitance per leaf area at full turgor and (E,F) morphological functional traits for the 18 study species. Each point corresponds to a species (filled- deciduous, empty- evergreen). Dashed line corresponds to a relationship with Pearson's correlation coefficient corresponding to a $P<0.1$ and solid line corresponds to $P<0.05$.

In explicitly testing relationships between these traits which proxies for resistance to early and severe dehydration we found that these were not related to each other (Fig. S4). Further, g_{min} was not related to resistance to loss of turgidity or cellular function and was weakly related to RWC$_{thick50}$ ($r^2= 0.42$, $p<0.1$). Thus how quickly a species lost water after stomatal closure was independent of the differences in their RWC threshold points.

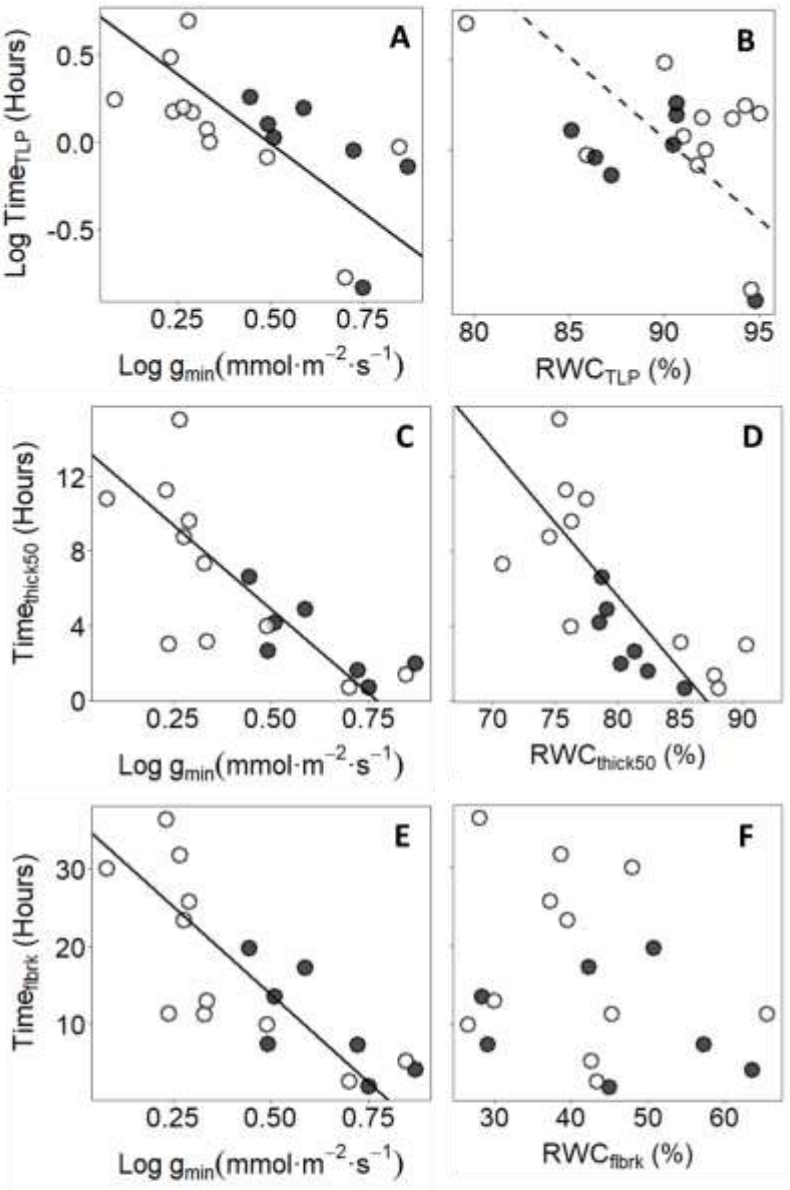

Figure 3: Relation between the four critical function traits examined and the time taken to reach each of these critical breakdown points; Log Time$_{TLP}$ (A, B), Time$_{thick50}$ (C, D) and Time$_{flbrk}$ (E, F) in the 18 species studied. Each point corresponds to a species (filled- deciduous, empty- evergreen). Dashed line corresponds to a relationship with Pearson's correlation coefficient corresponding to a P<0.1 and solid line corresponds to P<0.05.

Species with large stomatal pores had higher g_{min} (Fig. 2A). RWC$_{flbrk}$ was correlated with stomatal density and thus also to g_{max} such that species that had high potential g_{max} had low resistance to breakdown of functional integrity (Fig. 2B).

Both RWC$_{TLP}$ and Ψ_{TLP} were related to modulus of elasticity of the cell wall and capacitance (Fig. 5). Species with high resistance to turgor loss were those that had flexible cell walls that helped sustain turgidity (low modulus of elasticity). Moreover they also had higher leaf hydraulic capacitance (Fig. 2C-D). While RWC_{TLP} was related to LDMC (and weakly to LMA and LA), RWC_{flbrk} was related to SSD (Fig. 2 E-F).

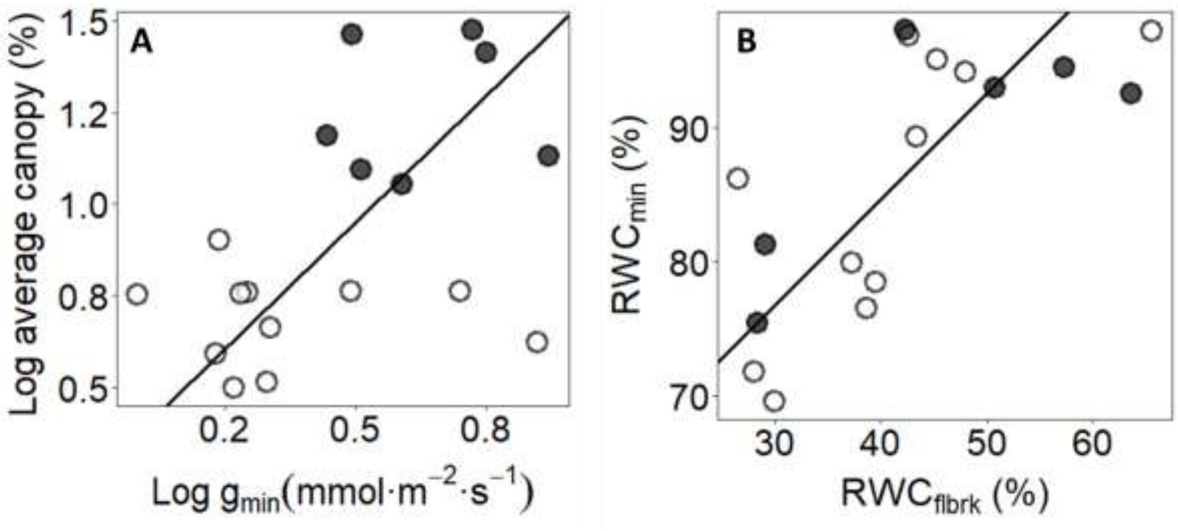

Figure 4: Behaviour of the 18 species studied as examined in the study site in Nigdale, India. Relation between A) leaf phenology (Log average canopy) and Log g_{min} and B) leaf RWC the species experience in the driest time of the year (RWC_{flbrk}) and RWC_{flbrk} in the 18 species studied. Each point corresponds to a species (filled- deciduous, empty- evergreen). Solid line corresponds to a relationship with Pearson's correlation coefficient corresponding to a $P<0.05$.

Though $RWC_{thick50}$ was related to $Time_{thick50}$ (r^2= -0.72, $p<0.05$), RWC_{TLP} was only weakly related to $Time_{TLP}$ (r^2= -0.45, $p<0.1$), while RWC_{flbrk} was not related to $Time_{flbrk}$ (Fig. 3). The rate of water lost after stomatal closure was strongly related to all the three time thresholds (Fig. 3), such that species with low g_{min} took more time to reach $Time_{TLP}$, $Time_{thick50}$ and $Time_{flbrk}$. Differences in LA, which is often thought to affect dehydration time, was not related to any of these three measures.

Table 3: Pearson's correlation coefficients for the relation between the measures of critical threshold, rate of water loss and time thresholds (a), morpho-physiological traits (b-e), and measures of on field performance (f). ** corresponds to $P<0.05$ while * corresponds to $P<0.1$. All abbreviations are as in Table 1.

	Traits	**RWC$_{TLP}$**		**Ψ$_{TLP}$**		**RWC$_{thick50}$**		**RWC$_{flbrk}$**		**Log g$_{min}$**	
a) Time to loss of critical function	Time$_{TLP}$	-0.45	*	-0.15		-0.55	**	-0.07		-0.64	**
	Time$_{thick50}$	0.17		-0.05		-0.72	**	-0.21		-0.77	**
	Time$_{flbrk}$	0.13		-0.07		-0.60	**	-0.28		-0.79	**
b) Stomatal traits	Pore size	-0.24		0.15		-0.02		-0.42		0.49	*
	Stomatal density	-0.23		-0.46		0.20		0.58	**	-0.46	
	Log g$_{wmax}$	-0.30		-0.56	**	0.32		0.65	**	-0.43	
c) Rigidity	e	0.72	**	-0.41	*	0.19		0.28		-0.34	
d) Capacitance	Log CFT	-0.65	**	0.47	**	-0.32		-0.26		0.16	
e) Morphological functional traits	Log LA	-0.45	*	0.04		0.12		0.32		0.03	
	LDMC	0.57	**	-0.14		0.13		-0.15		-0.20	
	Log LMA	0.44	*	-0.01		-0.13		-0.10		-0.22	
	SSD	0.27		0.29		-0.42		-0.69	**	-0.35	
f) Behaviour on field	Canopy loss	-0.22		-0.08		0.30		0.26		0.49	**
	RWC$_{min}$	-0.04		0.01		0.25		0.73	**	0.29	

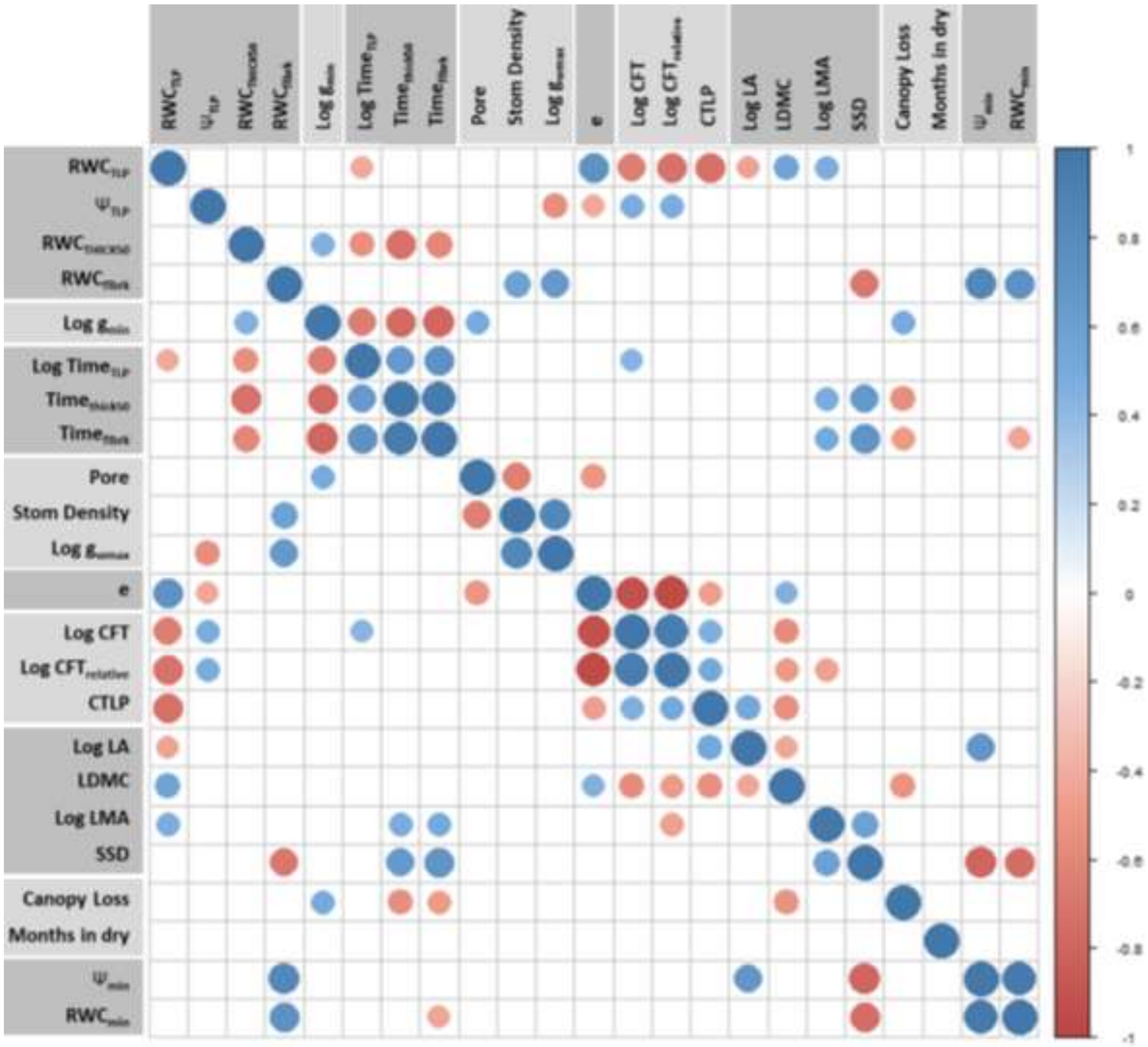

Figure 5: Correlation matrix showing Pearson's correlation coefficient of the traits examined in the study. The magnitude of the coefficient is indicated by size of each point. As indicated by the legend guide, positive relation is blue in colour and negative relations are red in colour, while weaker relations are closer to white. Those relations having $P<0.1$ is shown (others are omitted here). Abbreviations as in Table 1.

Interestingly, species that had high resistance to breakdown of PSII function during dehydration were those that experienced lower RWC during the driest time of the year (Fig. 4B). RWC_{min} was not related to Ψ_{TLP}, RWC_{TLP}, $RWC_{thick50}$ or g_{min}.

Evergreen and deciduous species did not differ in the measures of resistance to physiological breakdown, with the exception of RWC_{TLP} (Table S1) which was lower for the deciduous species than the evergreen. This was consistent with the positive/negative relationship observed between Ψ_{TLP} and the quantitative measure of deciduousness (Table 3). However, species with higher deciduousness and higher average canopy loss also had high g_{min}, likely associated with large stomatal pore size (Fig. 4A). Given the strong relation between g_{min} and time to loss of structural integrity and cellular function, species that had higher indices of deciduousness reached $RWC_{thick50}$ and RWC_{flbrk} sooner than more evergreen species (Fig. 5).

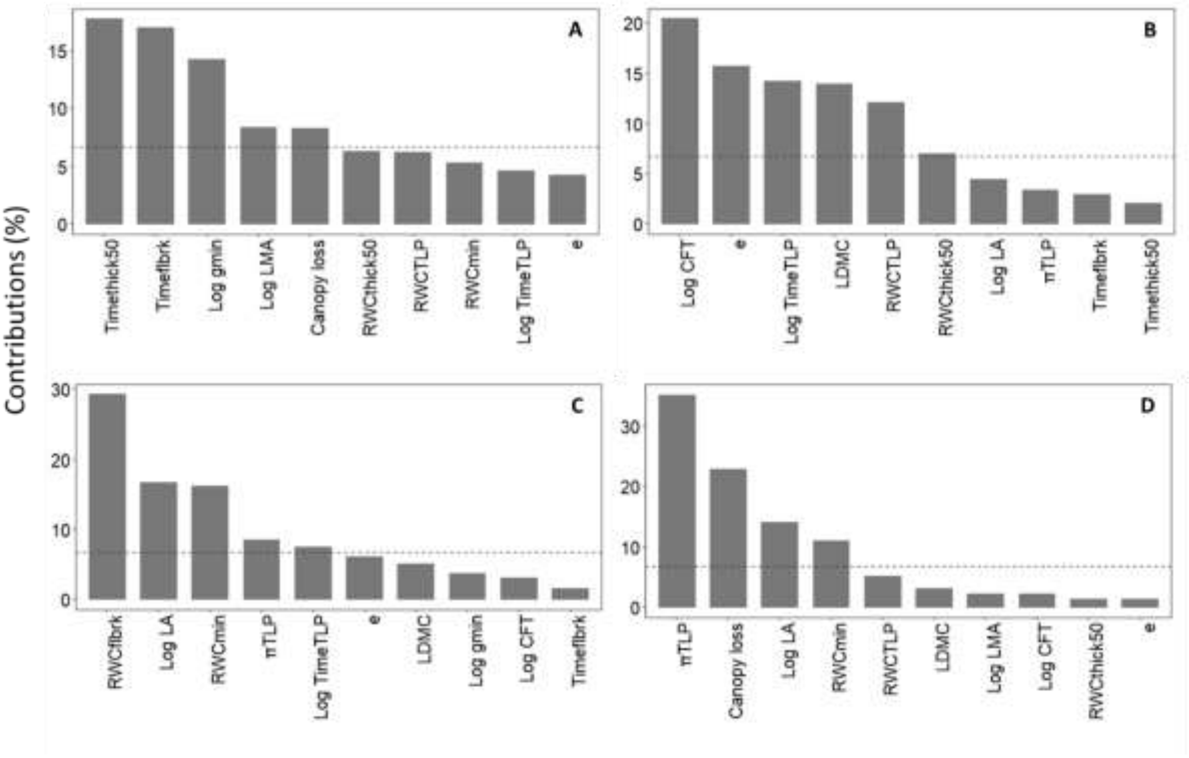

Figure 6: Contribution of variables to each of the first four principal component axes; A) dimension 1, 2) dimension 2, C) dimension 3 and D) dimension4. The dotted line is a reference value which represents the expected values if the contributions of the variables were uniform. Variables above the reference line is considered as important in contributing to the dimension. Abbreviations as in Table 1.

The first four dimensions explained 78.8% of the total variation with the first, second, third and fourth principal component (PC) axes corresponding to 30.65%, 25.18%, 13.87% and 9.18% of the total variation, respectively. Each of these axes were associated with traits related to rate of water loss in PC1, resistance to mild desiccation in PC2 and resistance to severe desiccation in PC3 (Fig. 7). The most important dimension (PC1) represented a spectrum which on one end had high LMA evergreen species, which were slow to reach their shrinkage and their cellular functional breakpoint and had low g_{min}, while on the other extreme were low LMA deciduous species with high g_{min} (Table S2). Leaf capacitance (CFT), $Time_{TLP}$, LDMC, modulus of elasticity and RWC_{TLP} loaded heavily on PC2, representing variation in resistance to early dehydration (Fig. 6). RWC_{flbrk}, RWC_{min} and LA loaded heavily on PC3, representing variation in resistance to severe dehydration (Fig. 6).

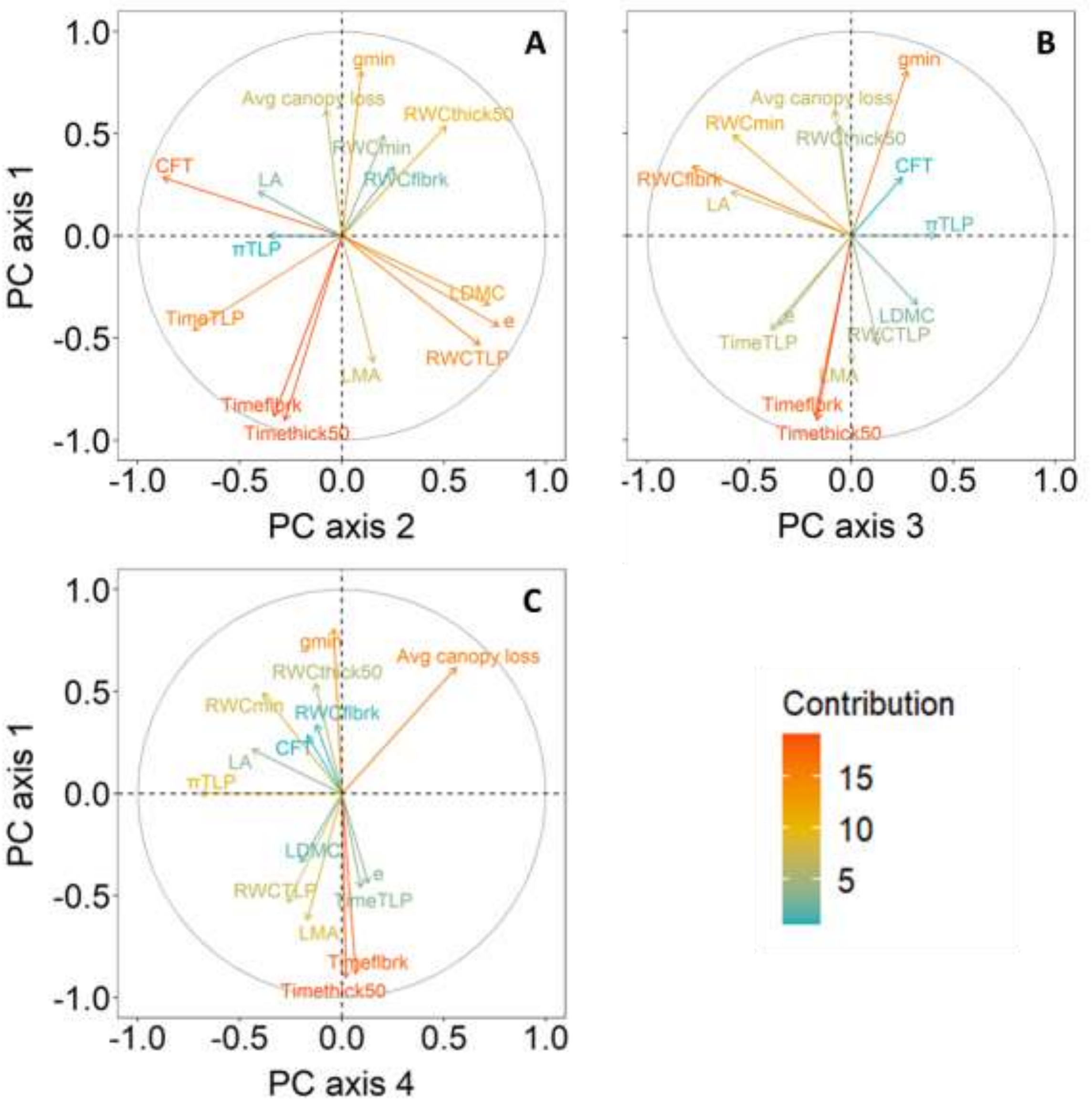

Figure 7: Variables biplot of the principal component analysis computed for 15 traits for the 18 species of A) PC axes 1 and 2 and B) PC axes 1 and 3 C) PC axes 1 and 4. Solid arrows indicate the weighing and the direction of the traits considered. Trait abbreviations can be found in Table 1.

Discussion

We found large variation in leaf responses to dehydration in 18 coexisting tree species from a seasonally dry tropical forest. The rates of water loss differed substantially in these species and resulted in important differences in the time that species took to reach critical, physiologically important RWC thresholds on dehydration. These results highlight the importance of minimising cuticular conductance and increasing resistance to severe dehydration as independent strategies in addition to the increasing resistance to turgor loss during early dehydration. In seasonally dry tropical forests where water availability is highly limited, and variable in space and in time, combinations of multiple strategies are important to facilitate species coexistence.

Loss of cellular turgidity occurs at early stages of dehydration and is good indicator of drought tolerance (Bartlett 2012, Bartlett 2016, Maréchaux 2015). In agreement with previous findings, RWC_{TLP} along with $Time_{TLP}$, CFT, e, LDMC formed an important component of PC2 that explained 25.2% of the total variation between our study species. Species with high resistance to turgor loss (RWC_{TLP}) and low LDMC, had more flexible cell walls and had higher capacitance. They also took more time to lose leaf turgor.
A recent meta-analysis (Bartlett et al. 2012) showed that Ψ_{TLP} may be a better indicator than RWC_{TLP} for biome level differences in drought tolerance. As is seen in this study as well, Ψ_{TLP} has a physiological basis to this given its relation with e and CTLP. RWC_{TLP} is also an important measure of resistance to early or moderate dehydration as it is related to leaf hydraulic conductance, stomatal conductance and loss of rehydration capacity (Trueba et al. 2019). However, as reported for a larger number of species (Bartlett et al. 2012), our results show that RWC_{TLP} was not related to Ψ_{TLP}. The underlying mechanisms for RWC_{TLP} and Ψ_{TLP} may be independent of each other. Interestingly, in our study species, RWC_{TLP} (PC2) explained more of the variation than Ψ_{TLP} (in PC4).

Our results are in accordance with previous studies that show that loss of leaf PS II function occurs at very low leaf RWC (Trueba et al. 2019), and therefore likely to be a result of more severe dehydration. The 18 species studied here had large variation in the RWC that resulted in the initial breakdown of PS II function. We found that RWC_{flbrk} was not related to RWC_{TLP} nor $RWC_{thick50}$ (Fig. S4 B, E). Further RWC_{flbrk} is not related to the rates at which species lost water post stomatal closure (Fig. S4F). Thus RWC_{flbrk} which is indicative of resistance to irreversible damage to cellular function (Dreyer et al. 1992, Guadagno et al. 2017), is an important trait that represents resistance to severe dehydration.

Interestingly, we observed large variation in RWC_{min} in these species. Importantly, we found that species that experienced very low RWC in their natural environment in the driest time of the year had higher resistance to breakdown of cellular function as assessed by RWC_{flbrk}.

Thus, species that typically experience lower RWC may require greater resistance to severe dehydration. Conversely, species that able to maintain higher RWC, due to other traits like strong stomatal regulation, lower loss of water post stomatal closure, may not need to invest in greater resistance to breakdown of cellular function as a response to severe dehydration. Indeed, RWC_{flbrk} and RWC_{min}, along with Leaf area, formed an axis corresponding to variation in resistance to severe dehydration (PC3), which explained 14.2% of the total variation in this study, independent from PC1 that represented rate of water loss and PC2 that represented resistance to early dehydration. This perhaps alludes to a greater importance of traits associated with resistance to severe dehydration in these forests that experience severe water limitations during the long and intense, eight month dry season, in addition to large variation in spatio-temporal water availability. These results suggest RWC_{flbrk} may be an important traits in understanding drought response of tropical species.

The rate of water loss after stomatal closure as determined by g_{min} was not related to the measures of early dehydration or severe dehydration. Further, g_{min} formed an important component of PC1 along with $Time_{thick50}$, $Time_{flbrk}$, deciduousness and LMA and explained 30.65 % of the total variation (Fig. 7A). Resisting dehydration by minimising water loss through cuticular conductance, post stomatal closure, may thus be an independent strategy to deal with drought. This axis also corresponds to the strategy associated with the leaf economic spectrum, as along this strategy axis deciduous species with low LMA and large stomatal pores had high g_{min}, which resulted in shorter $Time_{thick50}$ and $Time_{flbrk}$. Our results indicate that in a scenario of longer dry periods, deciduous species might take lesser time to hit the point of loss of function, for instance RWC_{flbrk}, primarily because of its low resistance to loss of water, post stomatal closure.

Our results showed that differences in the rates at which species lose water during dehydration are important, and corroborate recent studies that have questioned the assumption that the rates of dehydration are likely to be similar (Blackman et al. 2016, Martin-StPaul et al. 2017, Duursma et al. 2019). The variation in g_{min} among the 18 coexisting species, corresponded to the variation in time to loss of turgidity, structural integrity and cellular function. This implies that species ranking based solely on threshold points such as RWC_{TLP} and RWC_{flbrk} may not necessarily correspond to the species risk of drought induced mortality, if other factors related to time to death such as rate of water loss (g_{min}) are not accounted for. Though in other studies, ‘time to death’ is also a function of capacitance (Borchert and Pockman 2005, Pfautsch and Adams 2013) and leaf area (Gleason et al. 2014), neither showed up as important covariates of time in this study.

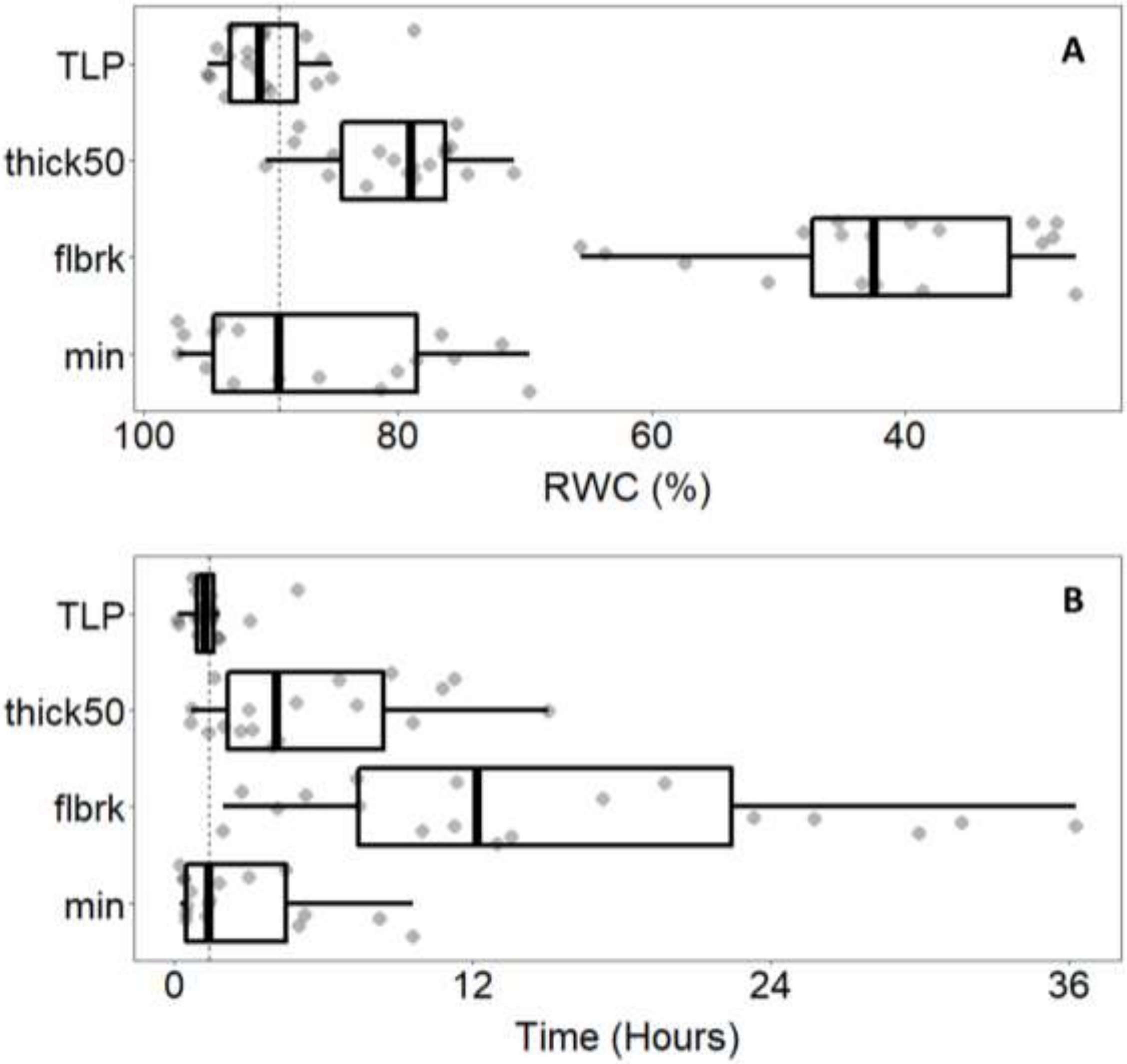

Figure 8: Order of occurrence of each of the critical function breakdown points examined with respect to A) RWC of the drying leaves and B) drying time as examined in the bench drying leaf desiccation assay carried out on 18 species. Time of 0 hours and RWC of 100% corresponds to beginning of the desiccation assay with water saturated leaves. The dashed line corresponds to the RWC_{min} in (A) and time at which this value is reached ($Time_{min}$) in (B). Standard box and whisker plot is shown. Each point corresponds to a species. TLP- turgor loss point, thick50 – 50% of leaf thickness, flbrk- 5% decrease in Fv/Fm indicating initial breakdown of PSII function, fl50- 50% decrease in Fv/Fm, min- leaf RWC the species experience in the driest time of the year.

Of the three physiological responses to dehydration examined, loss of turgor occurred first followed by 50% shrinkage of leaf thickness and only at a much lower RWC did the initial loss of PSII function occur. The median threshold RWC values for these processes were

90.82%, 78.9% and 42.47% respectively. The order of events with respect to time, reflected the order with respect to RWC. There was large variation in the time to 50 % shrinkage (from 30 minutes for *Ficus racemosa* to 15 hours for *Memecylon umbellatum*) and in the time to loss of PSII function in the species (ranging from 2 hours for *Ficus racemosa* to 36 hours for *Psydrax dicoccos*). This larger variation in $\text{Time}_{\text{thick50}}$ and $\text{Time}_{\text{flbrk}}$ indicated the compounded effect of variation due to both its independent variables; $\text{RWC}_{\text{flbrk}}$ or $\text{RWC}_{\text{thick50}}$ and g_{min} (Fig. 8B, Table 3).

In the driest time of the year, species in this SDTF operated at a RWC (median RWC_{min} 89.4%) that is very close to or even lower than their turgor loss point. Though turgor loss point is an important trait that determines optimal leaf function especially with respect to early dehydration, our results indicate that during the dry summers of this SDTF system, mechanisms other than resistance to loss of turgor becomes important to respond to seasonal drought.

Since loss of PSII function occurs at very low RWC values, it has been argued that this trait may not be ecologically relevant as these RWC values represent dehydration levels representative of complete loss of leaf hydraulic conductance (Trueba et al. 2019). However we observed here that $\text{RWC}_{\text{flbrk}}$ was correlated with the minimum RWC experienced by species which is an ecologically relevant trait, and argue that $\text{RWC}_{\text{flbrk}}$ may perhaps be more important than previously thought. Thus while resistance to turgor loss is perhaps relatively more important in moist systems where response to moderate drought can affect carbon assimilation, growth and thus survival, in drier systems like our study site responses to severe drought is crucial and better captured by resistance to severe drought represented by $\text{RWC}_{\text{flbrk}}$.

Conclusion

In conclusion, we highlight that in SDTF, where water availability is highly limited, and spatially and temporally variable, species have evolved diverse complex strategies to facilitate drought tolerance. At the leaf level, we show that resistance to loss of water through minimising cuticular conductance, resistance to early dehydration and resistance to severe dehydration are three important and independent strategies. Further, we call attention to the understudied axis corresponding to minimising g_{min} and thus time to dehydration as an important strategy to resist drought induced mortality (Bréda et al. 2006, Blackman et al.

2016). We show here that the large variation in g_{min} contributes significantly to the large differences between species in the time to reach physiologically important RWC thresholds. Importantly RWC_{flbrk} is an ecologically relevant physiological trait that is related to species ability to deal with the seasonal water stress experienced in the driest time of the year. While RWC_{TLP} is related to early response to drought, RWC_{flbrk} is an important trait that captures response to severe drought. Further how species vary in RWC_{flbrk} is independent of g_{min} and $RWC_{TLP.}$ The independence of these strategies may allow for complex combinations of trait values to evolve as a response to drought. Integrating these multiple dimensions is essential to accurately predict species variation in response to climate change associated drought.

Contributions

Ron Sunny and Deepak Barua designed the experiment. Ron Sunny collected data for the traits with help from Malavika Venu with the bench drying assays, and Bandaru Peddiraju with quantifaction of stomatal traits. The phenology data was collected by Souparna Chakrabarty.

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Supplementary figures and tables

List of tables and figures

Table S1: Summary table with F statistic for ANOVA for effect of leaf habit (evergreen and deciduous) and species (nested in leaf habit) on the traits examined for the 18 study species. * indicates P<0.05. Abbreviations used as described in table 1. Time$_{TLP}$ (estimated from the species level RWC vs Time equation using species level RWC$_{TLP}$ measures) and Time$_{thick50}$ (species level curves were used, since insufficient data at individual level to fit a model) were not included in this analysis since individual level data was not available. N indicates number of species while n indicates number of replicates per species

	Traits	**Leaf habit**	**Species | Leaf habit**	**N**	**≥n**
a) Critical thresholds	RWC$_{TLP}$	17.3 *	19.3 *	18	3
	Ψ_{TLP}	0.7	18.2 *	18	3
	RWC$_{thick50}$	0.2	7 *	18	5
	RWC$_{fl50}$	2.6	9.9 *	18	4
	RWC$_{flbrk}$	3.6	4.4 *	18	4
b) Water loss rate	Log g$_{min}$	99.2 *	14 *	18	6
c) Time thresholds	Time$_{fl50}$	68.5 *	18.5 *	18	4
	Time$_{flbrk}$	58.1 *	17.1 *	18	4
d) Stomatal traits	Pore size	340.3 *	78.9 *	15	3
	Guard size	203.9 *	69.9 *	15	3
	Stomatal density	57.1 *	97.3 *	15	3
	SPI	34.7 *	78.6 *	15	3
	Log g$_{wmax}$	0	84.8 *	15	3
e) Leaf water status	Log CFT	4.5 *	8.6 *	18	3
	Log CFT$_{relative}$	12.8 *	23.1 *	18	3
	CTLP	17.4 *	5.3 *	18	3
	Log SWC	58.8 *	21.5 *	18	3
	Ψ_{O}	2.1	9.4 *	18	3
f) Cell wall rigidity	e	12.5 *	11 *	18	3
g) Morphological functional traits	Log LA	16.9 *	54.8 *	18	5
	LDMC	87.7 *	12.3 *	18	5
	Log LMA	7.1 *	21.6 *	18	5
	SSD	8.4 *	13.4 *	17	3
h) On field parameters	RWC$_{min}$	13.7 *	22.9 *	17	5
	Ψ_{min}	7.4 *	9.6 *	14	3

Table S2: Pearson's correlation coefficient for relation between each of the traits examined and the first three principal component axes that together explains 73.4% of the total variation. Indicated in brackets is the proportion of variation explained by each of the component axes. * corresponds to a statistically significant correlation with $P<0.05$. The values in bold are those that load heavily on the axes as indicated by the correlation coefficients.

	Traits	**PC1**		**PC2**		**PC3**		**PC4**	
		30.7%		**25.2%**		**13.9%**		**9.2%**	
a) Critical function traits	RWC$_{TLP}$	-0.53	*	0.68	*	0.13		-0.26	
	Ψ_{TLP}	0.00		-0.36		0.42		-0.70	*
	RWC$_{thick50}$	0.54	*	0.51	*	-0.06		-0.13	
	RWC$_{flbrk}$	0.34		0.26		-0.78	*	-0.13	
	Log g$_{min}$	0.81	*	0.10		0.27		-0.04	
b) Time to loss of critical function	Log Time$_{TLP}$	-0.46		-0.73	*	-0.39		0.09	
	Time$_{thick50}$	-0.90	*	-0.28		-0.17		0.02	
	Time$_{flbrk}$	-0.88	*	-0.33		-0.18		0.07	
c) Cell wall rigidity	e	-0.44		0.77	*	-0.36		0.13	
d) Capacitance	Log CFT	0.29		-0.88	*	0.25		-0.17	
e) Functional traits	Log LA	0.22		-0.41		-0.59	*	-0.44	
	LDMC	-0.34		0.73	*	0.32		-0.20	
	Log LMA	-0.62	*	0.16		0.00		-0.17	
f) Behaviour on field	Canopy Loss	0.62	*	-0.08		-0.08		0.56	*
	RWC$_{min}$	0.49	*	0.21		-0.58	*	-0.39	

Table S3: Species list, taxonomic affiliation, species ID and trait values of the 18 study species from the seasonally dry tropical forest located in Nigdale, Maharashtra, in the Northern Western Ghats in India. Trait abbreviations as listed in Table 1.

Species	**Family**	**ID**	**RWC$_{TLP}$**	**Ψ$_{TLP}$**	**RWC$_{thick50}$**	**RWC$_{fl50}$**	**RWC$_{flbrk}$**	**g$_{min}$**	**Time$_{TLP}$**	**Time$_{thick50}$**	**Time$_{flbrk}$**	**Time$_{fl50}$**
1. *Actinodaphne angustifolia*	Lauraceae	AC	91.0	-2.20	70.8	22.0	45.3	2.14	1.2	7.3	11.3	15.7
2. *Atalantia racemosa*	Rutaceae	AR	93.6	-1.81	76.3	12.6	37.3	1.96	1.5	9.6	25.8	40.6
3. *Bridelia retusa*	Phyllanthaceae	BR	87.2	-1.73	80.3	33.3	63.6	7.41	0.7	2.0	4.1	5.4
4. *Psydrax dicoccos*	Rubiaceae	CD	90.0	-2.29	75.8	18.1	28.1	1.71	3.0	11.3	36.3	46.1
5. *Macaranga peltata*	Euphorbiaceae	CH	85.9	-1.72	87.8	28.9	42.7	7.03	0.9	1.4	5.3	7.8
6. *Catunaregam Spinosa*	Rubiaceae	CS	86.4	-2.02	82.5	13.2	29.1	5.29	0.9	1.6	7.4	12.2
7. *Callicarpa tomentosa*	Lamiaceae	CT	78.7	-1.70	74.5	14.2	39.6	1.90	4.9	8.7	23.3	31.9
8. *Diospyros montana*	Ebenaceae	DM	90.5	-2.14	78.6	10.8	28.3	3.24	1.1	4.2	13.5	21.2
9. *Flacourtia indica*	Salicaceae	FI	85.1	-2.51	81.4	18.2	57.4	3.11	1.3	2.7	7.4	13.6
10. *Ficus racemosa*	Moraceae	FR	94.8	-1.29	85.4	25.0	45.0	5.62	0.1	0.7	2.0	4.7
11. *Mangifera indica*	Anacardiaceae	MI	93.3	-1.92	90.4	32.7	65.6	1.73	1.5	3.0	11.3	19.2
12. *Mallotus phillipensis*	Euphorbiaceae	MP	93.1	-2.19	88.1	14.4	43.4	5.02	0.2	0.7	2.7	4.5
13. Memecylon umbellatum	Melastomataceae	MU	95.0	-1.67	75.3	12.5	38.6	1.85	1.6	15.1	31.7	50.4
14. *Olea dioica*	Oleaceae	OD	91.8	-1.58	85.1	10.1	29.9	2.17	1.0	3.1	13.0	22.8
15. *Syzygium cumini*	Myrtaceae	SC	94.3	-1.73	77.5	20.4	48.0	1.21	1.7	10.8	30.0	41.9
16. *Terminalia bellirica*	Combretaceae	TB	90.6	-1.52	79.1	17.8	42.3	3.88	1.6	4.9	17.3	25.2
17. *Terminalia chebula*	Combretaceae	TC	90.4	-1.61	78.7	19.9	50.8	2.79	1.8	6.6	19.7	25.6
18. *Xantolis tomentosa*	Sapotaceae	XT	91.8	-0.59	76.3	7.6	26.5	3.10	0.8	4.0	10.0	16.0

Table S4: Species ID (as in supplementary table S3), leaf habit (LH; E – evergreen and D – deciduous) and trait values of the 18 study species from the seasonally dry tropical forest located in Nigdale, Maharashtra, in the Northern Western Ghats in India. Trait abbreviations as listed in Table 1.

	Sp ID	**LH**	**Pore size**	**Guard size**	**Stom Den**	**SPI**	**g_{wmax}**	**LA**	**LDMC**	**LMA**	**SSD**	**$CFT_{relative}$**	**CTLP**	**CFT_{abs}**	**SWC**	**Ψ_O**	**e**	**Canopy Loss**	**RWC_{min}**
1.	AC	E	13.0	20.3	268.7	0.111	1.01	44.8	0.462	119.1	0.50	0.041	0.148	0.358	1.09	-1.81	20.6	3.3	95.1
2.	AR	E	5.2	8.1	800.2	0.053	1.21	25.2	0.405	123.4	0.65	0.036	0.120	0.454	1.85	-1.52	23.7	5.8	80.0
3.	BR	D	-	-	-	-	-	42.1	0.358	117.6	0.51	0.069	0.203	0.897	1.53	-1.44	12.9	13.5	92.6
4.	CD	E	12.8	19.2	493.3	0.183	1.77	49.7	0.446	198.6	0.68	0.044	0.143	0.708	1.32	-1.75	17.3	3.9	71.8
5.	CH	E	-	-	-	-	-	133.0	0.391	118.2	-	0.081	0.184	0.837	1.97	-1.37	10.4	4.2	96.8
6.	CS	D	-	-	-	-	1.62	15.6	0.364	111.1	-	0.069	0.157	0.893	1.72	-1.57	11.7	29.9	81.3
7.	CT	E	-	-	-	-	-	126.3	0.282	73.3	-	0.170	0.227	2.277	2.82	-1.26	5.2	5.7	78.5
8.	DM	D	23.6	31.7	180.7	0.181	1.06	29.3	0.372	99.7	0.53	0.048	0.187	0.599	1.64	-1.82	19.0	12.4	75.5
9.	FI	D	14.3	22.0	1074.0	0.517	4.13	33.1	0.388	86.7	-	0.057	0.205	0.547	1.56	-1.94	13.8	29.0	94.5
10.	FR	D	14.3	19.5	313.7	0.119	1.13	43.2	0.363	115.2	-	0.042	0.074	0.408	2.22	-1.04	19.4	25.8	-
11.	MI	E	7.8	18.6	749.3	0.259	2.60	60.7	0.437	131.9	0.42	0.037	0.139	0.380	1.13	-1.70	25.6	8.0	97.2
12.	MP	E	-	-	-	-	-	31.8	0.472	88.0	0.50	0.035	0.131	0.232	1.15	-1.78	24.9	5.8	89.4
13.	MU	E	14.9	25.4	350.9	0.227	1.66	19.2	0.439	204.4	0.71	0.032	0.047	0.448	1.10	-1.13	20.6	3.2	76.5
14.	OD	E	20.2	27.0	276.2	0.199	1.37	34.7	0.443	123.4	0.68	0.052	0.175	0.456	1.14	-1.28	16.0	4.6	69.6
15.	SC	E	12.2	19.6	949.6	0.357	3.57	46.8	0.378	103.8	0.57	0.034	0.096	0.336	1.18	-1.41	24.0	5.7	94.1
16.	TB	D	21.3	29.0	268.5	0.223	1.44	79.3	0.331	142.5	0.57	0.065	0.226	1.024	1.77	-1.33	14.6	11.3	97.3
17.	TC	D	17.3	23.1	340.3	0.183	1.47	58.8	0.327	127.6	0.58	0.068	0.217	0.927	1.65	-1.41	15.5	15.4	92.9
18.	XT	E	16.3	19.6	183.0	0.070	0.67	25.1	0.442	98.0	-	0.162	0.107	2.141	1.88	-0.43	4.4	5.8	86.2

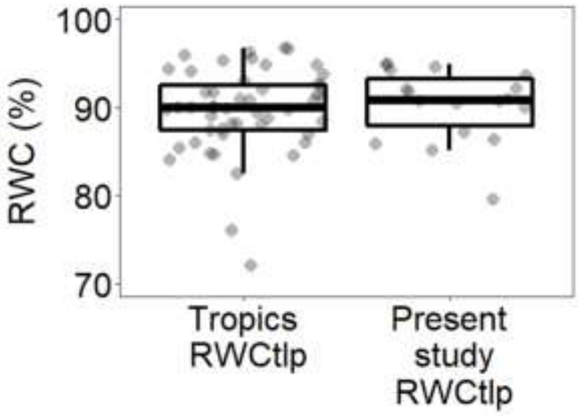

Figure S1: Comparison of the range and median of the RWC_{TLP} data measured for 50 species from across the tropics (as compiled in Bartlett 2012) and the RWC_{TLP} measured for the 18 species in this study. Each point corresponds to a species. Standard box and whisper plot is shown.

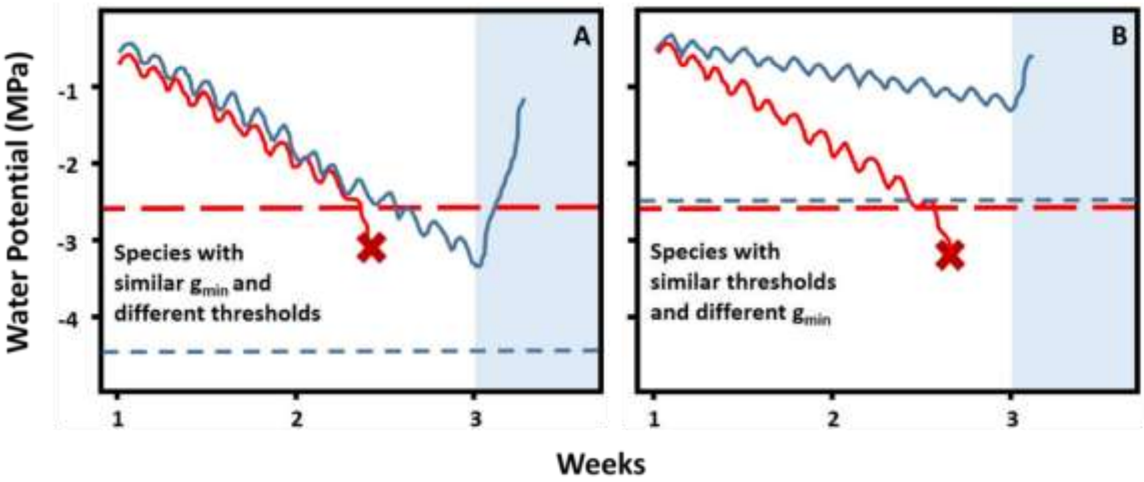

Figure S2: A schematic representation decreasing water potential with the onset of a dry period, to demonstrate two different strategies that can help species deal with drought. Each color denotes a species. The horizontal line corresponds to the threshold of physiological damage such as water level corresponding to loss of turgidity, structural integrity or cellular function. The slope of each curve is a function of the rate of water loss (g_{min}). The light blue shaded area corresponds to a wet phase due to onset of rain while the white area corresponds to the dry phase. A species (blue curve) can avoid physiological damage if it has a higher threshold of physiological damage (A) or a lower rate of water loss (B). (Modified from Brodribb et al. 2020)

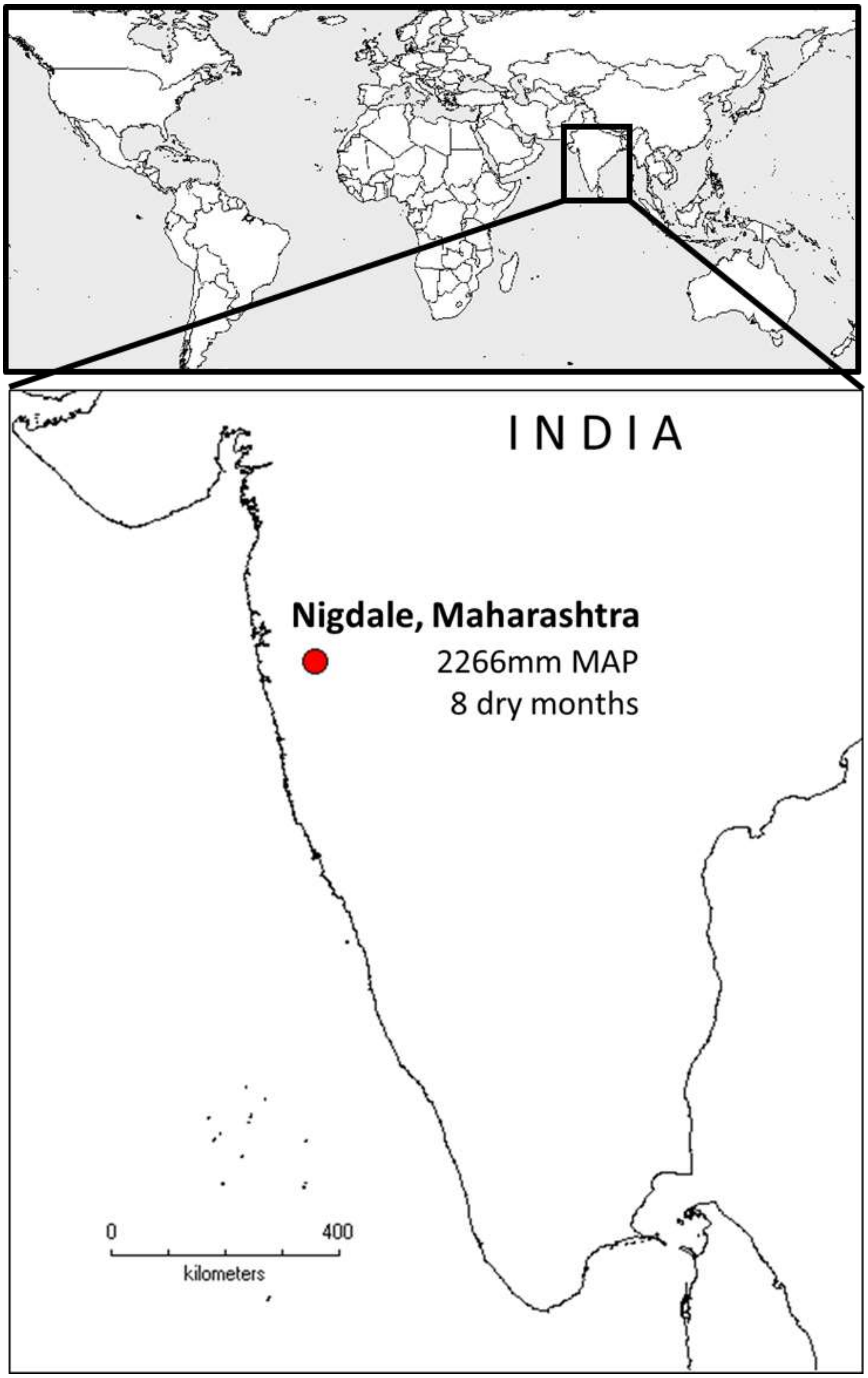

Figure S3: Map showing location of the seasonally dry tropical forest located in Nigdale, Maharashtra, in the northern part of the Northern Western Ghats in India. 18 dominant species were sampled from this forest for the present study.

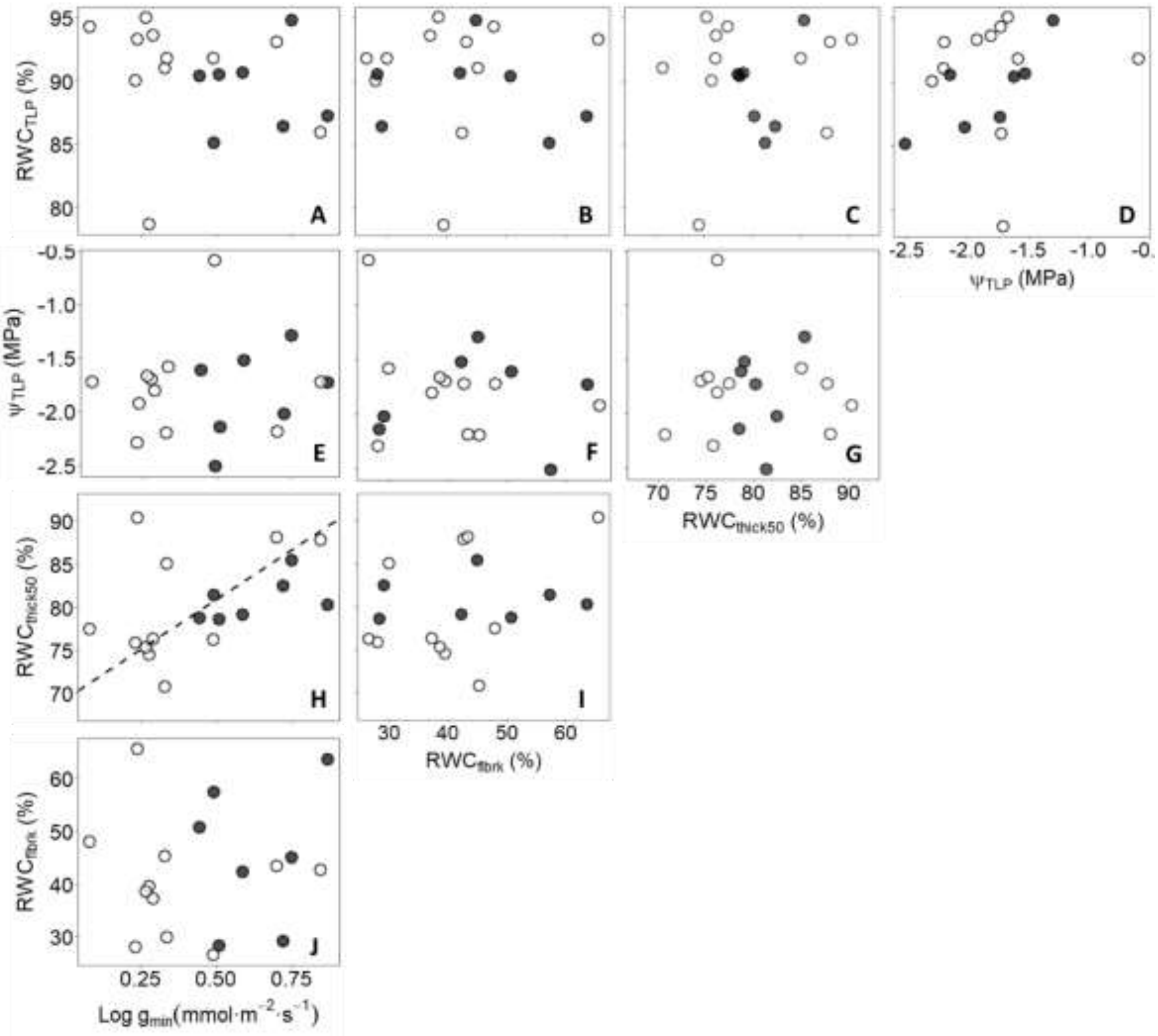

Figure S4: Relation between the resistance to loss of turgidity, structural integrity and cellular function and rate of leaf water loss examined for the 18 study species; $\mathrm{RWC}_{\mathrm{TLP}}$ (A,B,C,D), Ψ_{TLP} (E,F,G,D), $\mathrm{RWC}_{\mathrm{thick50}}$ (H,I,G,C) $\mathrm{RWC}_{\mathrm{flbrk}}$ (J,B,F,I) and Log g_{min} (A,E,H,J). Each point corresponds to a species (filled- deciduous, empty- evergreen). Dashed line corresponds to a relationship with Pearson's correlation coefficient corresponding to a P<0.1.

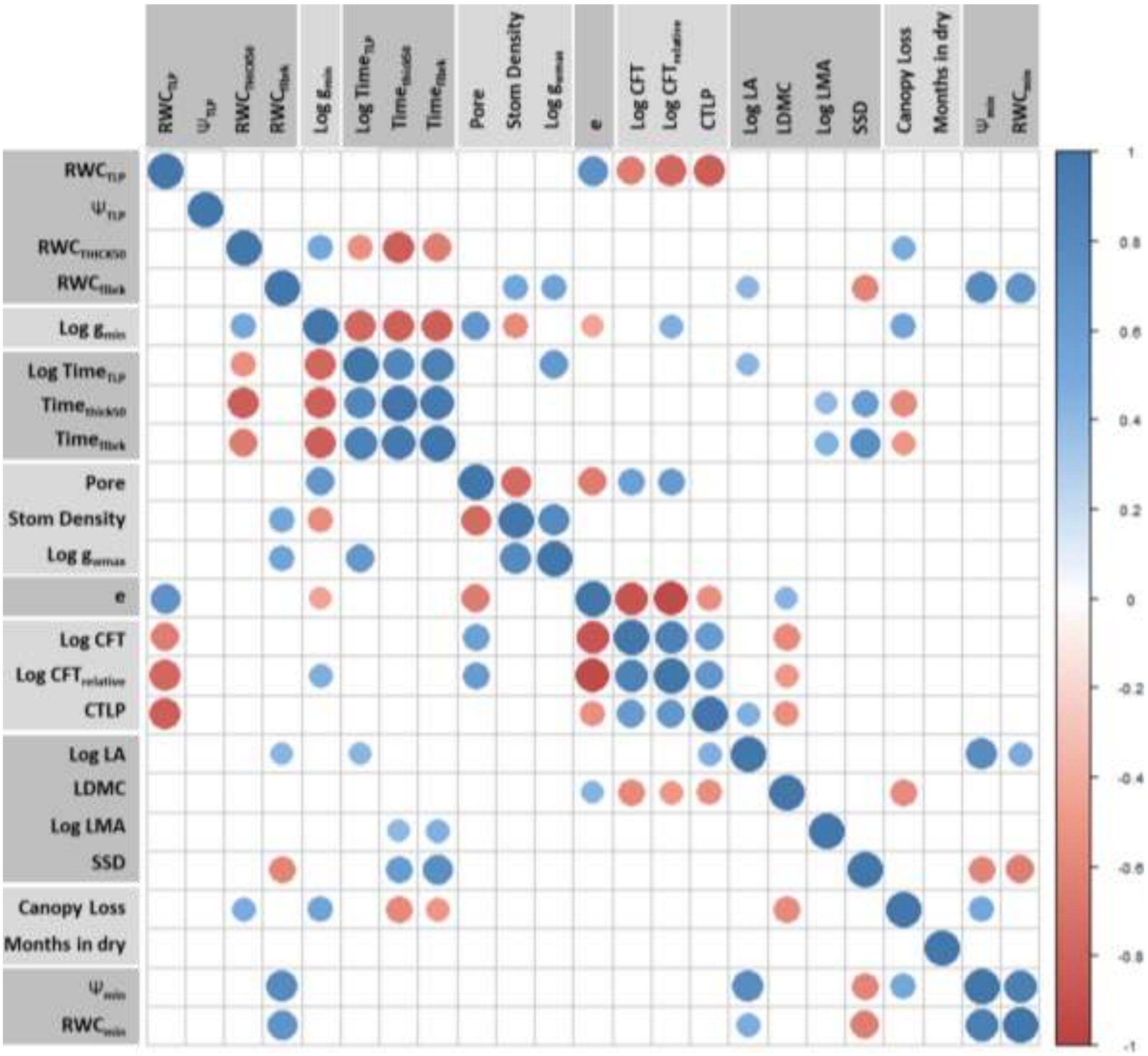

Figure S5: Correlation matrix showing Spearman’s correlation coefficient of the traits examined in the study. The magnitude of the coefficient is indicated by size of each point. As indicated by the legend guide, positive relation is blue in colour and negative relations are red in colour, while weaker relations are closer to white. Those relations having $P<0.1$ is shown (others are omitted here). The rectangular boxes indicate the three independent strategy axes as seen in the PCA; Yellow- PC1 corresponding to delay in time to desiccation, Green – PC2 corresponding to resistance to mild desiccation and Red- PC3 corresponding to resistance to severe desiccation. Abbreviations as in Table 1.

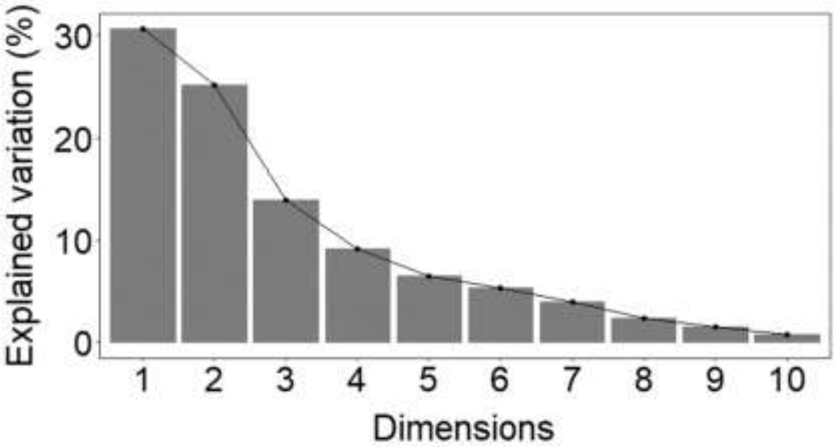

Figure S6: Scree plot of the principal component analysis showing the explained variation of each of the dimensions for the 18 species in this study. The first four dimensions explained 78.88% of the total variation.

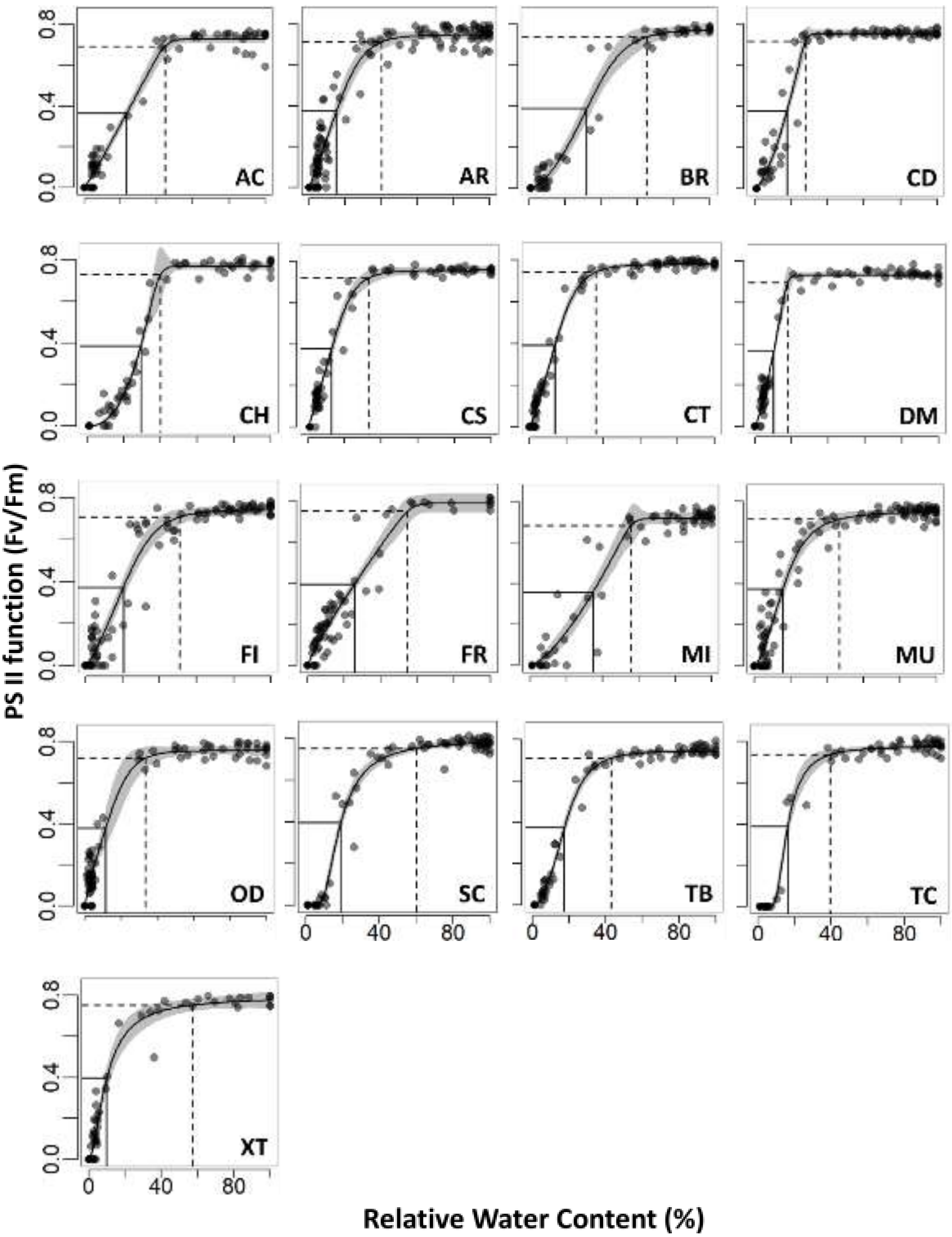

Figure S7: Dehydration response curves for leaf PSII function (Fv/Fm) that corresponds to dark-adapted chlorophyll A fluorescence efficiency for the 18 species studied. Water status of the leaf in terms of the relative water content (RWC) ranges from 100% (well hydrated) to 0% (completely dry). Each graph is data pooled from at least 5 replicate individuals. A five parameter log-logistic function fit to the data is shown with grey confidence interval band. The lines correspond to the RWC at which 5% loss of PSII function occurs (dashed) and the RWC at which 50% loss of PSII function occurs (solid). The abbreviations for the species are as in table S4.

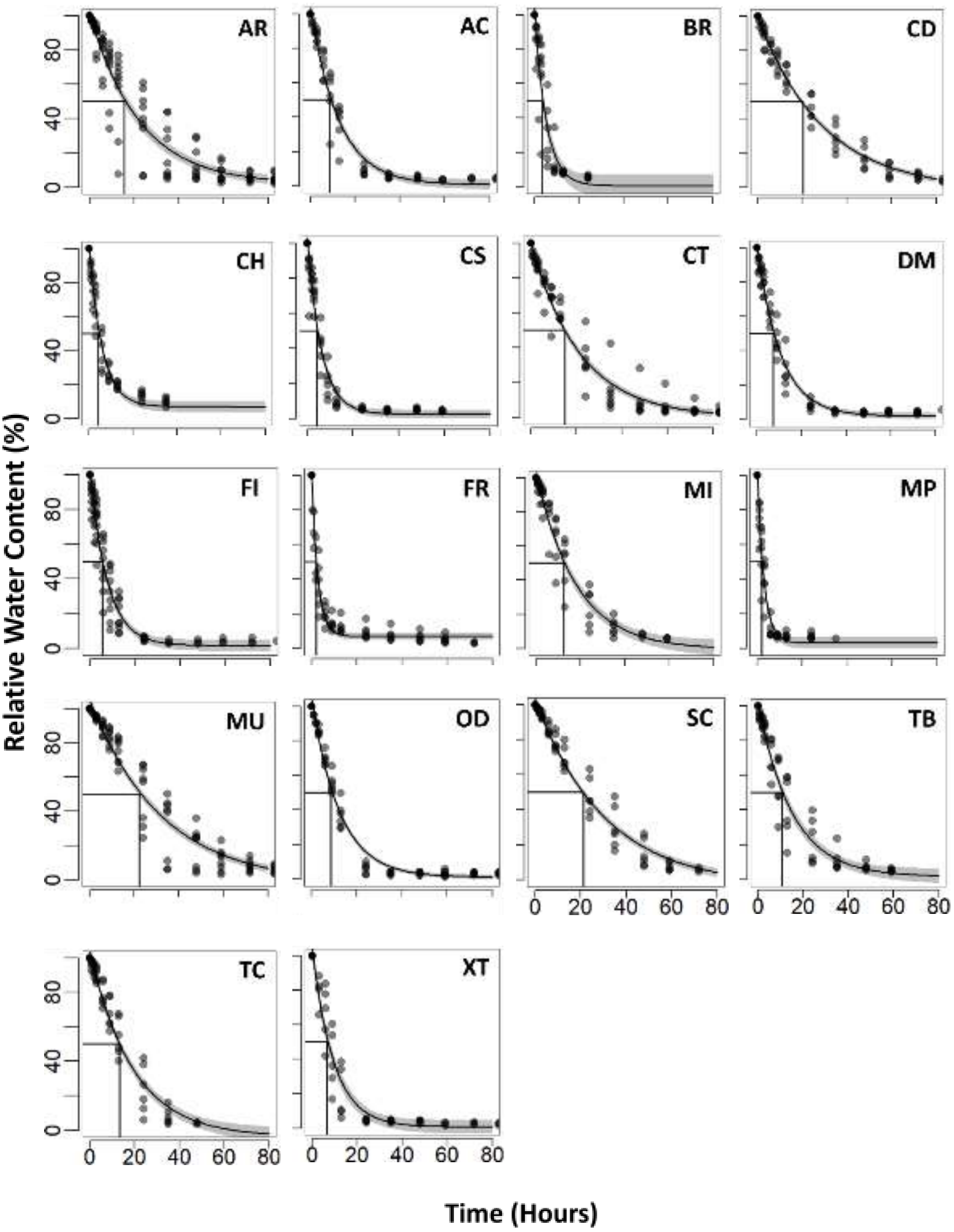

Figure S8: Time to leaf dehydration for the 18 species studied from its water saturated stage as represented by leaf relative water content lost over time in hours. Water status of the leaf in terms of the relative water content (RWC) ranges from 100% (well hydrated) to 0% (completely dry). Each graph is data pooled from at least 5 replicate individuals. A three parameter exponential decay function fit to the data is shown with grey confidence interval band. The line correspond to the time at which the species lost 50% of its leaf relative water content. The abbreviations for the species are as in table S4.

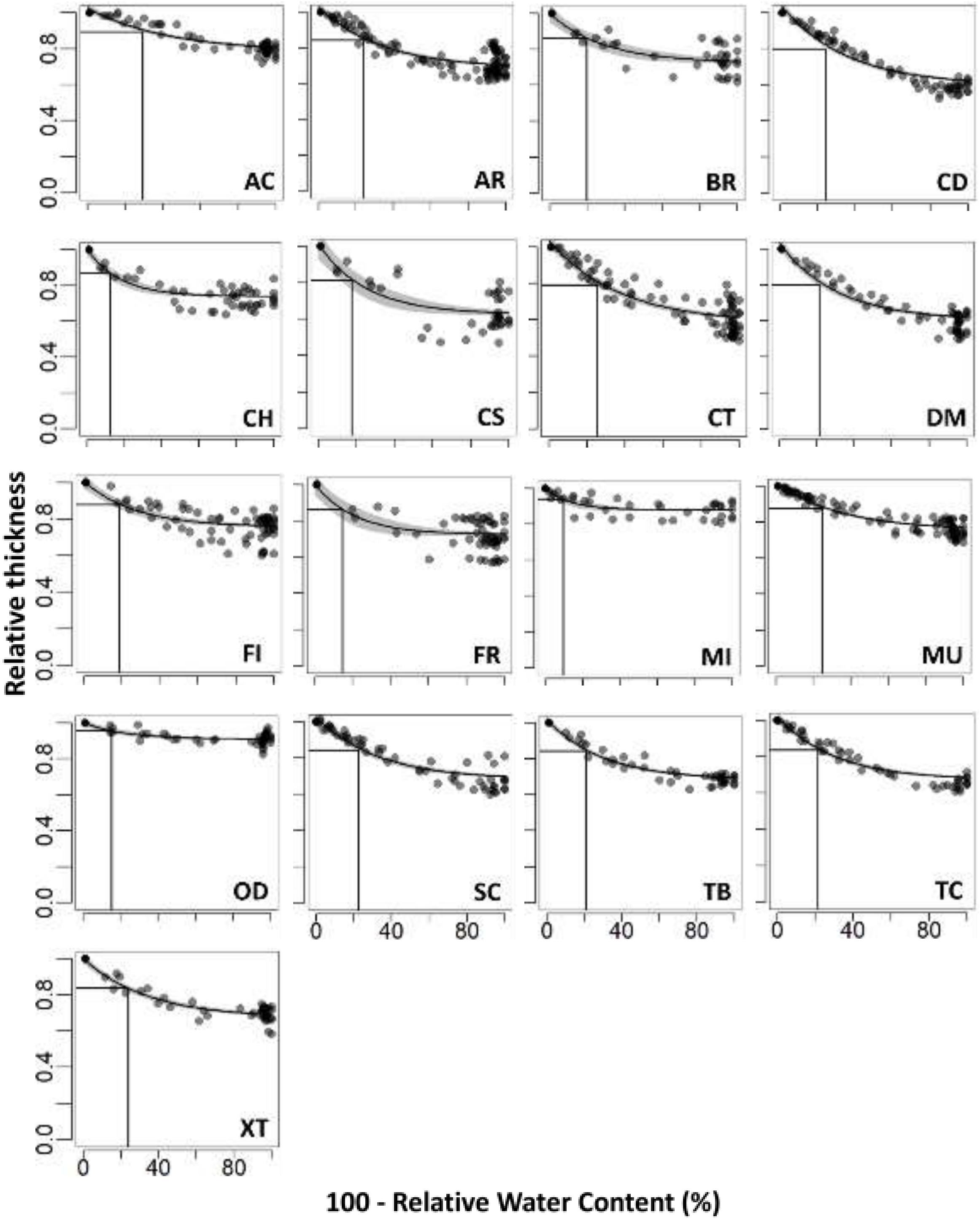

Figure S9: Dehydration response curves of the leaf relative thickness for the 18 species studied. Water status of the leaf in terms of leaf relative water content (100 - RWC) ranges from 0% (well hydrated) to 100% (completely dry). Each graph is data pooled from at least 5 replicate individuals. A two parameter exponential decay function fit to the data is shown with grey confidence interval band. The line corresponds to the leaf water status at which the species lost 50% of its shrinkable thickness. The abbreviations for the species are as in table S4.

Chapter 5

Response of whole plant water use to limiting light and water conditions are independent of each other in seedlings of seasonally dry tropical forests

Abstract

How co-occurring species vary in the utilization of the shared and limited supply of water, especially in the context of other limiting resources like light, is essential for understanding processes that facilitate species coexistence and community assembly. For seedlings in a seasonally dry tropical forest that experience large heterogeny in light and water conditions, how water use, leaf physiology, and subsequently plant growth, is affected by limited water and light availability is still not well understood. In a controlled common garden experiment with four co-existing and commonly occurring dry tropical forest species, we examined how whole plant water uptake, responds to limiting water and light conditions and whether these responses are reflected in leaf physiology, and translated to growth. Water use varied dramatically in seedlings of the four species with a five-fold difference in well-watered plants grown in full sunlight. Species varied in their response to shade, but did not differ in responses to the low water treatment, possibly resulting from the strong selective force imposed by the very low water availability and the long dry period in these seasonally dry forests. Interestingly, species response in water use, physiology, and growth in limiting water conditions was independent of light. Thus, species response to both these limiting conditions may evolve independently of each other. Responses in water use was largely congruent with responses in leaf physiology and growth. However, while magnitude of changes in leaf physiology were largely driven by light conditions, changes in whole plant water use and growth were influenced to a greater degree by the water treatment. This highlights the need to measure whole plant water use to better understand plant growth responses in these seasonally dry tropical forests.

Introduction

Growth, reproduction and survival of plants strongly depends on its ability to acquire water (Santiago et al. 2004, Poorter et al. 2010, Fan et al. 2012). This is a challenge for co-occuring species that share this resource pool, since water is often a highly limited resource especially in seasonally dry tropical forests where water availability is restricted both spatially and temporally. There is large variation in species water use (Wullschleger et al. 1998, Ehleringer et al. 1999, Collins and Bras 2007) and quantifying this variation, understanding how it relates to leaf level physiology, and translates to growth is important to understand competition between co-occurring species, coexistence and further community assembly. Additionally, for tropical forests seedlings that have to cope with heterogeneous forest structures and light environments, in addition to seasonal rainfall regimes, it is important to understand how water use responds to varying water and light availability (Kitajma et al. 1994, Holmgen et al, 2012). This becomes particularly important given current changing rainfall patterns and increased fragmentation due to climate change and anthropogenic disturbances (Feng et al. 2013, IPCC 2014).

Light is an important limiting factor that is often negatively correlated with water availability in many natural systems including tropical forests. Large statured trees with dense canopies in wet forests result in diminished light availability in the understory, and conversely, low plant cover and open canopies allow greater availability of light in dry forests (Kitajma et al. 1994, Johnson et al. 2017, Comita et al. 2014). It has been proposed that plants face a trade-off in responding to limiting water and light as a result of contrasting physiological and morphological adaptations required for dealing with each of these limiting resources separately (Smith and Huston 1989, Holmgren et al. 2012, Kupers et al. 2019). However, results from other studies suggest that plant responses to limiting water may be independent of light (Sack et al. 2004, Sánchez-Gómez et al. 2006). In fact, morphological and physiological adaptations can simultaneously reduce requirements for both light and water, and this would allow greater drought and shade tolerance (Holmgren 2000, Holmgren et al. 2012), and coordinated whole plant resource acquisition (Reich 2014). Thus, growth responses to limiting water and light availability are still not understood and could be negatively related (trade-off between responses to limiting water and shade tolerance), positively related (facilitation and coordinated resource acquisition), or independent of each other.

The primary mechanisms that might explain a trade-off between resistance to low water and tolerance to shade is with respect to differential allocation to leaf and root. An increased leaf area fractions and a corresponding decrease in root allocation would increase the efficiency of light capture but might also increase sensitivity to low water availability (Smith and Huston 1989). In contrast, conservative strategies might select for traits which result in a reduced demand for resources including light and water (Reich 2014). This might allow plants to overcome potential trade-offs to exhibit coordinated whole plant resource acquisitions strategies and positive interspecies relationships between responses to limiting light and water. This understanding of the relationship between responses to limiting water and light has important implications for niche partitioning and species interactions in habitats with spatial and temporal variation in light and water availability.

Water use by plants involves uptake by roots from the soil, flux through the stem and leaf xylem vessels and transpiration loss through the stomata to the atmosphere. Studies of water use that have quantified stem xylem conductance (He et al. 2020), leaf xylem conductance (Nardini and Luglio 2014), and leaf stomatal conductance (Cavaleri and Sack 2010) suggest that water use is important in understanding association with habitat specific water availability. However, inferences from these studies may be limited because quantification of water use is confined to scales that are smaller than the whole plant level (Meinzer et al. 2010). Organ-level studies may not be able to account for the ability to mitigate limiting water availability by other means including deep roots (Paz 2003, Pérez-Ramos et al. 2013, Paz et al. 2015, Brum et al. 2019), minimizing leaf area (Bucci et al. 2005), or using stem water reserves (Wolfe and Goldstein 2017). A more comprehensive understanding of how water use responds to limiting resource availability requires whole plant level integrated studies (Reich 2014).

Light and water are key abiotic factors limiting growth and establishment of seedlings in seasonally dry tropical forests like our study system in the Northern Western Ghats range of peninsular India (Pascal 1988). Water availability in this region is highly seasonal with a long dry season that extends for eight months (CRU TS version 4.1). There is large spatial heterogeneity in forest cover which ranges from open savanna like matrices with less than 30% cover, to closed canopy tall statured forests with 100% cover. Thus these systems have spatially and temporarily variable light and water conditions that may allow co-existence of trees with differing water use strategies (Sterck et al. 2011). In this study, we examined variation in whole plant water uptake in experimentally grown seedlings using the gravimetric method to overcome limitations of organ level proxies. For seedling of four commonly occurring and co-existing dry tropical forest tree species, we examined: 1) How water uptake responds to limiting water and light conditions; 2) If species responses in water uptake to the light and water treatments are reflected in leaf physiology and whole plant biomass allocation; and, 3) If these responses are translated to growth.

Methods

Species selection and study area: The study species were selected from a set of 80 species commonly found in seasonally dry tropical forest of the Northern Western Ghats range, India (19.1320°N, 73.5540°E). Based on data for species dominance, species abundance and leaf functional traits (Table S1, Barua unpublished), we identified representative species that span the range of leafing behaviour and leaf functional trait values observed in this region, and selected the following species for this experimental study: *Terminalia chebula* and *Diospyros melanosylon* are relatively fast growing deciduous species with low leaf mass per area (LMA) and high leaf nitrogen content (LNC), *Syzygium cumminii* is a semi-evergreen species with moderate LMA and LNC, while *Memecylon umbellatum* is a slow growing evergreen species with high LMA and low LNC. These four species account for 54% of the dominant species in this forest (Barua unpublished).

Table 1: The study species with taxonomic affiliation, leaf mass per area (LMA, g·m^{-2}), leaf habit (LH; E - evergreen, D - deciduous) and preferred habitat in seasonally dry tropical forest in Nigdale, Maharashtra in the Northern Western Ghats of India. LMA was classified as low, medium and high based on the distribution of traits values across 80 species in these forests.

Species	**Family**	**LMA**	**LH**	**Habitat**
Syzygium cumini	Myrtaceae	Medium (149.8)	E	Open, Edge
Terminalia chebula	Combretaceae	Medium (173)	D	Open, Edge
Diospyros Montana	Ebenaceae	Low (121.5)	D	Open
Memecylon umbellatum	Melastomataceae	High (243.3)	E	Open, Edge, Closed

The climate in this region is highly seasonal, and most of the annual rainfall of 2266mm falls between June and September. The long dry period extends from October to May. The landscape is topographically diverse with valleys carved out by rivers and their tributaries. The top of these valleys consist of flattened ridges. Soil depth varies from very shallow (<

10cm) in these flattened ridges to relatively deep (>100cm) in the mid slopes and valleys (Jazeera AM 2016). These ridge tops (henceforth open habitats) with low soil depth, low water availability and high light availability are characterized by scrub/savanna vegetation consisting of stunted trees, shrubs and lianas with a high percentage of deciduous species. At the other extreme the valley forests (henceforth closed habitats) with greater soil depth, higher soil water availability, and low light availability are dominated by tall statured evergreen tree species. The transition zones on the slopes (henceforth edge habitats) consist of the smaller patches of fragmented forests that contain a mixture of evergreen and deciduous species with some overlap with both the open and the closed forests. Tree heights in the open habitats range from 3-5 m, in the edge habitats from 10-15 m, and in the closed habitats from 15-20 m (Jazeera AM 2016).

Common garden experimental design and light and water treatments: The common garden experiment was carried out in an experimental plot at the Indian Institute of Science Education and Research, Pune, India. Seedlings of the four study species were obtained from a local nursery, J.E. Farms in February 2015. These 2.5 year old seedlings were transferred to 19 Litre mini-lysimeter pots (20 cm diameter and 60 cm length) with 18 kg of uniformly mixed dry red alfisol (pH 7.2) supplemented with organic manure (1:50 v/v) and urea (0.05 $g{\cdot}kg^{-1}$ soil). Seedlings were allowed to acclimate to the lysimeter pots for a period of 30 days. A full factorial design of two levels of light (open sun and shade), and water (control and low water) was imposed for a period of 50 days starting from May 2nd, 2015. We selected 15 % of full sunlight, and 40 % of saturated pot water content as the limiting light and water treatments, respectively. These light and water levels were chosen to impose limiting but non-lethal levels based on a literature survey of previous experiments (Sánchez-Gómez, Poorter 1999, Huang et al. 2008, Schumacher et al. 2008, Brown et al. 2014) and also to simulate light in shaded habits and soil moisture content in the early dry season in our study site. Six replicate individuals of each species were used for each species per treatment giving 24 individuals per species and a total of 96 individuals in the experiment.

The shade treatment was imposed using a nylon net that allowed approximately 15 % of the sunlight to pass through it, thus replicating the light conditions observed in shaded habitats in the field. Plants subjected to the open sun treatment were kept in the open in full sunlight. The average photosynthetic photon flux density in the shade treatment was 153.7 ± 68 µmol·m^{-2}·sec^{-1}, and in the open sun treatment was 1109.3 ± 440 µmol·m^{-2}·sec^{-1} (Fig S5). For the watering treatments, control pots were maintained at 85 % of the saturated pot water content, while low water treatment pots were maintained at 45 % of the saturated pot water content. Saturated pot water content was estimated for a subset of the pots at the beginning of the experiment. Pots were weighed at regular intervals (usually every 3 days) during the experiment to determine the amount of water lost in that period and to bring the pot water back to the appropriate treatment levels. A thin plastic sheet was taped to the rim of the pot and tied to the base of the stem of the seedlings to prevent water loss from the soil. Soil moisture content (SMC) was quantified by collecting a soil core from the small holes made on the sides of the pot. Soil cores were weighed for fresh weight, oven dried for three days at 95 °C, and the dry weighed measured. SMC was calculated as: SMC= (FW-DW)/DW.

Quantification of plant water uptake, leaf physiology, biomass allocation and growth: Whole plant water uptake was quantified by weighing all the individual pots every three days during the experiment. Leaf gas exchange parameters were quantified with a LICOR, LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, USA) using the standard broad-leaf cuvette (6 cm^2) fitted with the LICOR-6400-02B LED light source on the first fully expanded and mature leaf of every individual between days 44 to 49. All measurements were made between 9:00-11:00 hrs with the cuvette light, chamber CO_2 concentrations (incoming reference), relative humidity and temperature set at 1400 µmol·m^2·s^{-1} PPFD, 390 + 10 ppm, 50-60 %, and 28-30 °C, respectively. Stem height and stem diameter were measured for all individuals at the start and at the end of the treatment period to quantify growth. Height was measured with a measuring tape from the base of the stem to the tip of the terminal branch, and stem diameter was measured using a vernier caliper at a height of 10cm from the base of the seedling. All plants were harvested at the end of the treatment period to quantify biomass fractions of root, leaf and stem. Leaf area was estimated for all individuals. Leaves that had emerged during the experiment period were marked and this allowed us to measure leaf area increase during the treatment period. Leaf, stem and root tissue were oven dried at 70°C to quantify dry mass.

Statistical analysis: All responses measured were tested for normality and transformed when necessary. The response of the four study species to the light and water treatments were analyzed using a full factorial ANOVA with species, light and water as fixed effects. All analysis were performed with Statistica (version 9.1, Statsoft, Tulsa, OK, USA).

Results

Whole plant water uptake varied nearly five-fold in seedlings of these four co-existing species. Water uptake in the well watered control treatments in full sunlight ranged from around 200 ml per day for *Memecylon* to nearly 1000 ml per day for *Syzygium* (Fig. 1). In addition to inherent differences in water uptake, the species responded differently to the light and water treatments over the course of the experiment.

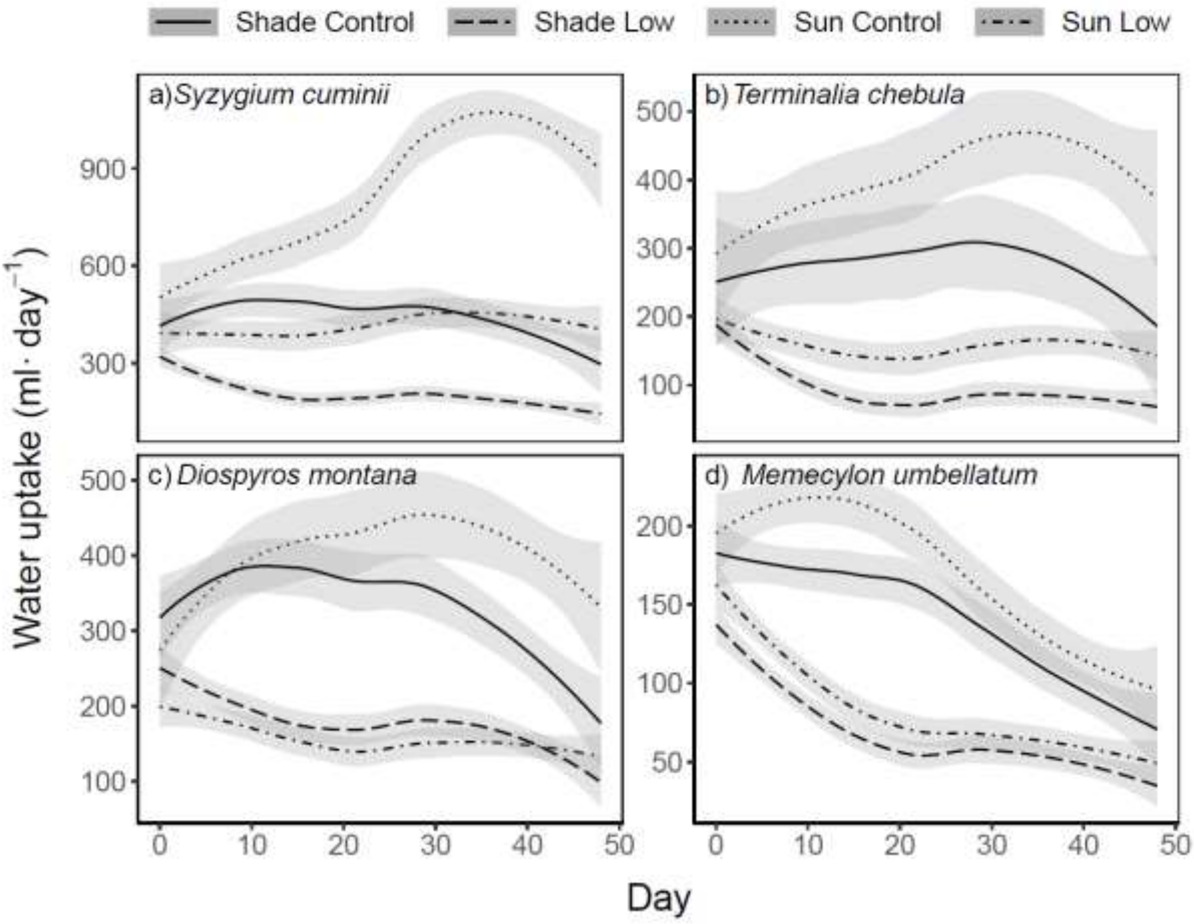

Figure 1: Whole plant water uptake in the four study species during the treatment period for: a) *Syzygium cumini*; b) *Terminalia chebula*; c) *Diospyros montana*; and, d) *Memecylon umbellatum*. Water uptake for 6 individuals for each species was quantified for a period of fifty days. The treatments were a full factorial of two light levels - full sunlight and shade, and two watering regimes - well watered controls and low water. These are depicted by: shade control - solid lines; shade and low water - dashed lines; sun control - dotted lines; sun

and low water- lines with alternating dots and dashes. The grey bands around the lines represent 95% confidence intervals.

As expected reduced water availability resulted in decreased water uptake and this was consistent across all four species (Fig. 2a-d, Table 2). Water uptake was lower for plants in the shade than in the open sun treatment. The reduction in water uptake in the low water treatment was similar for both sun and shade plants indicating that the shade treatments did not alleviate effects of reduced water availability (Table 2). The reduction in water uptake between the open sun and shade treatments differed among species (Table 2), and was lowest for Memecylon and highest for Syzygium.

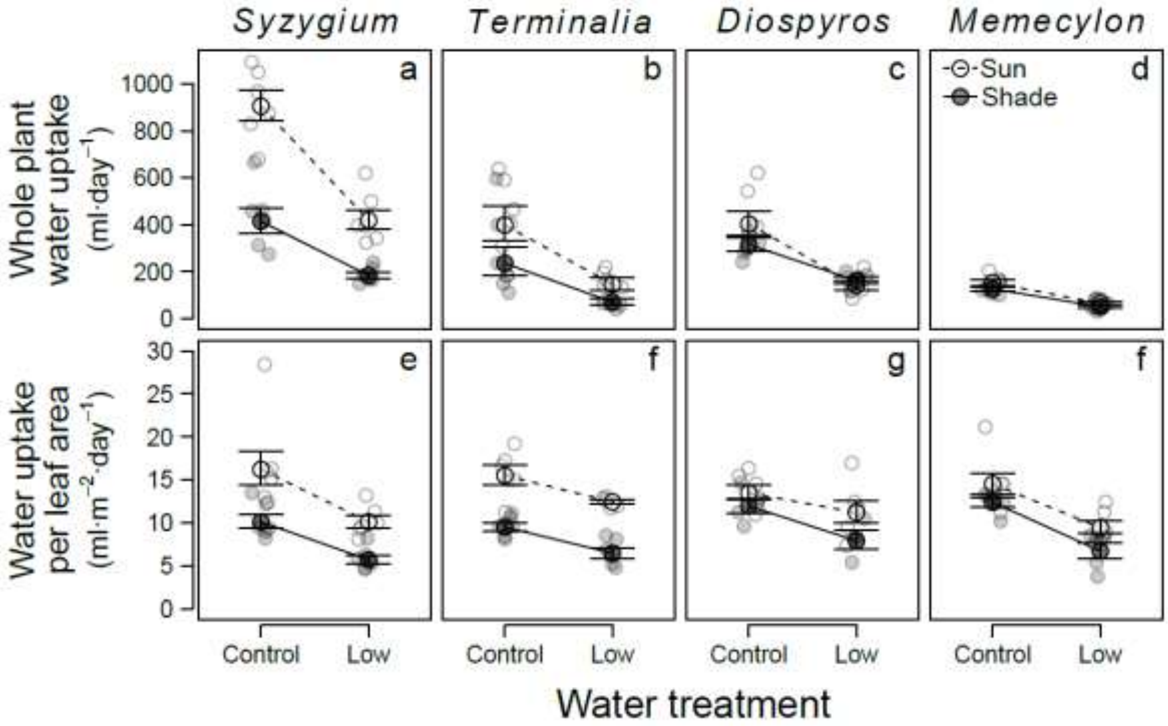

Figure 2: Water uptake for the study species in the experimental light and water treatments: a-d) average daily water uptake per plant; c-f) leaf area normalized daily water uptake. Error bars corresponds to ± 1 standard error. Open symbols and dashed lines represent plants in full sun light, and solid symbols and lines plants in the shade treatment.

Whole plant water uptake was qualitatively similar when water uptake was quantified on a leaf area basis (Fig. 2c-f, Table 2). There was no significant three way interactions, and the reduction in water uptake per leaf area between the open sun and shade treatments was lowest for Memecylon and highest for Syzygium. The decrease in water uptake per leaf area in the low water treatment was not dependent on light, and was similar for all four species as indicated by the lack of light into water, and species into water interactions, respectively (Table 2).

For the most part, leaf physiology measurements mirrored plant water uptake (Fig. 3, Table 2). Photosynthesis, stomatal conductance and transpiration decreased in the shade and low water treatments, but we observed no interaction between the response to shade and water treatments (Table 2). As seen in water uptake, the change in photosynthesis and transpiration with shading differed across species. While the decrease in photosynthesis and transpiration was high in Terminalia, there was no significant change in photosynthesis for Memecylon in the shade.

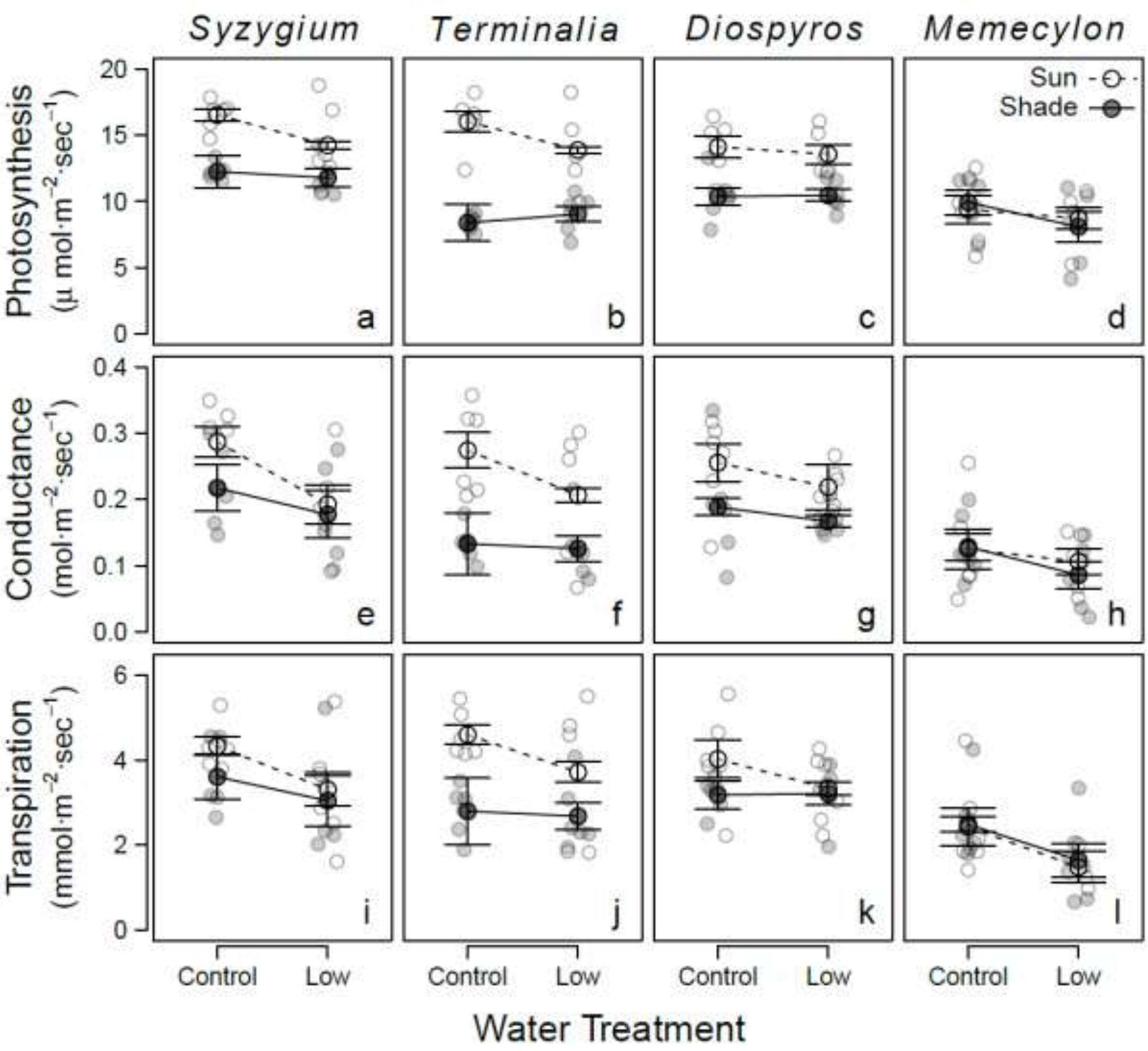

Figure 3: Response of leaf physiology in the study species to the light and water treatments: a-d) Photosynthesis (A_{net}); e-h) Stomatal conductance (g_s); and, i-l) Transpiration (E). Error

bars corresponds to ± 1 standard error. Open symbols and dashed lines represent plants in full sun light, and solid symbols and lines plants in the shade treatment.

The results for leaf photosynthesis and stomatal conductance differed from what we observed for water uptake in an important way in that the magnitude of change was higher for the light relative to the water treatments. This is understandable in that both photosynthesis and conductance are expected to be strongly driven by light intensity. Additionally, unlike with water uptake we did not see a light into species interaction for conductance indicating that the effects of light were similar for all four species.

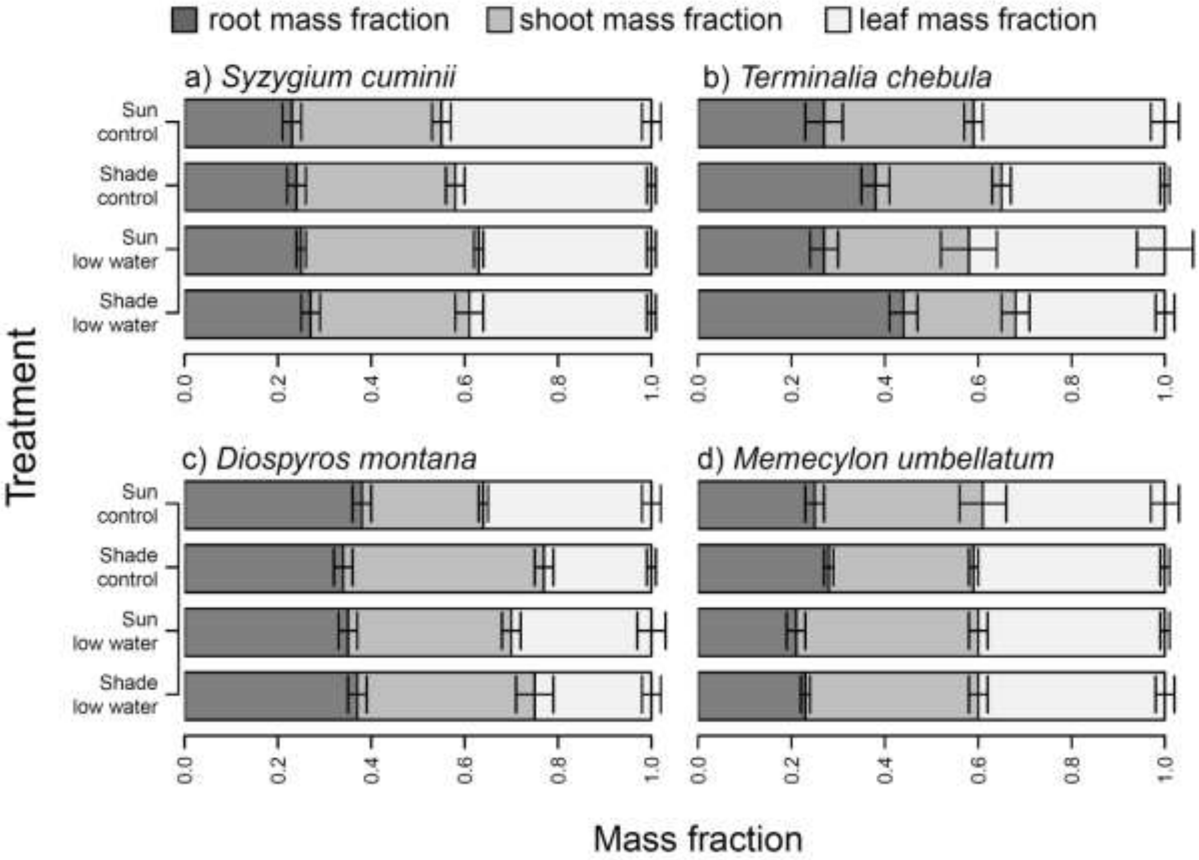

Figure 4: Biomass allocation to leaves, stems and roots in response to the light and water treatments for: a) *Syzygium cumini*; b) *Terminalia chebula*; c) *Diospyros montana*; and, d) *Memecylon umbellatum*. Error bars corresponds to ± 1 standard error. Mass fractions for leaves, stems and roots are presented as the dry weight in these organs as a fraction of the total dry biomass. Leaf mass fractions are shown in dark gray; stem mass fractions in gray; and, root mass fractions in light gray.

Light did not alter biomass allocation (Fig. 4, Table 2). The low water treatment resulted in a decrease in leaf mass fraction and a corresponding increase in root mass fraction in Diospyros and Terminalia, but not in Syzygium or Memecylon (Fig. 4, Table 2).

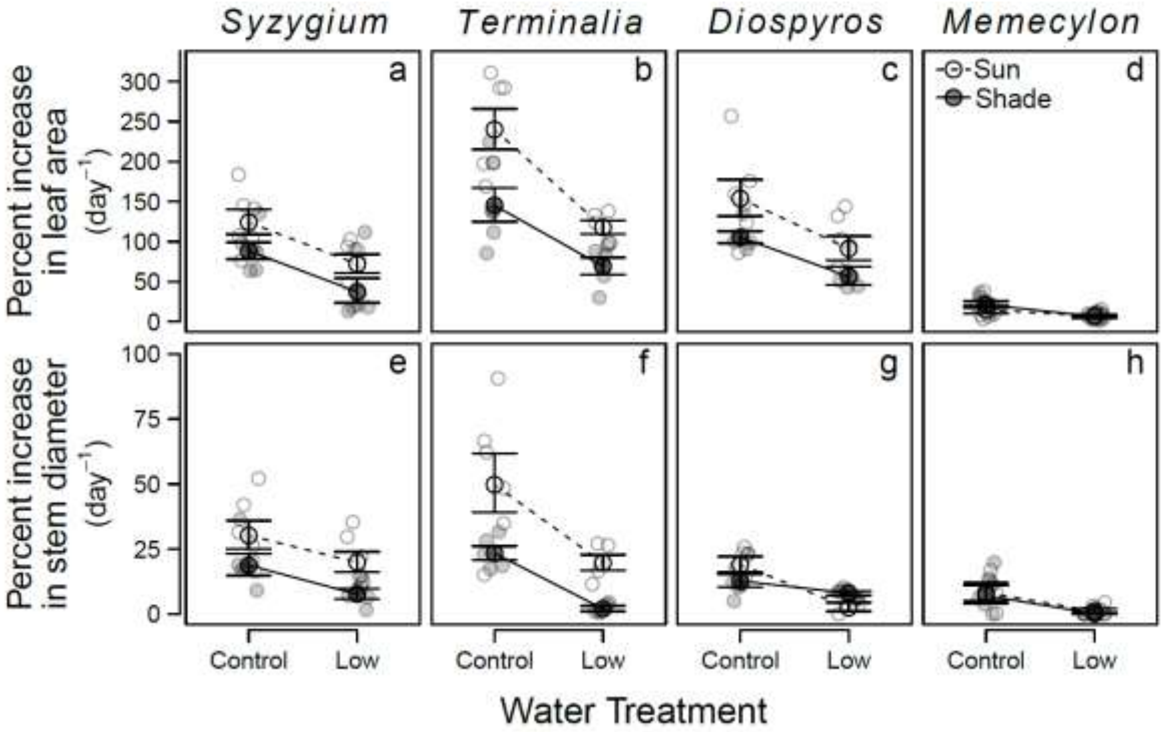

Figure 5: Growth measures for the four species in response to the light and water treatments: a-d) Percent increase in leaf area; and, e-h) Percent increase in stem diameter. Error bars corresponds to ± 1 standard error. Open symbols and dashed lines represent plants in open sun, and solid symbols and lines plants the shade treatment.

Growth responses to the treatments in these four species were consistent with what was observed for plant water uptake and leaf physiology. The low water and shade treatments resulted in a reduction in the percent increase in leaf area and stem diameter (Fig. 5, Table 2). As before, the lack of a light into water interaction indicates that the shade treatment did not alleviate the reductions in growth caused by the low water treatment (Table 2). Mirroring what was observed in whole plant water uptake, species differed in how they responded to the shade but not the water treatments (Table 2). Terminlia and Memecylon showed the highest and lowest changes in growth, respectively.

Reductions in whole plant water uptake in these species in response to reduced water availability and shading were qualitatively similar to water uptake quantified on a leaf area basis. Despite the overall congruency in whole plant water use and leaf area normalized water use, the decrease in the effect size in the water treatment implies a changes in leaf area allocation in response to the limiting water condition. Indeed, we did observe this change in decreased leaf (and increased root) biomass allocation in response to the limiting water treatment, though the responses were species specific.

Table 2: Results for 3-way ANOVA for the effects of light treatment (L), watering regime (W), species (S) and their interactions for: a) water uptake; b) physiology; c) biomass allocation; and, growth. There were two light treatments, two water regimes and four species, with each L x W x S treatment having six replicates. F statistic are presented and statistical significance indicated in bold and by the presence of asterisk (* $P < 0.05$, ** $P < 0.01$).

Processes	**Light**	**Water**	**Species**	**LxW**	**LxS**	**WxS**	**LxWxS**
a) Water Uptake							
Per individual	**37.2****	**170.1****	**85.9****	0.0	**6.2****	0.9	0.8
Leaf area normalized	**79.8****	**86.9****	**15.5****	3.6	**3.9***	1.9	0.1
b) Physiology							
Photosynthesis	**62.6****	**4.7 ***	**21.8****	1.5	**9.4****	0.4	1.1
Conductance	**18.0****	**5.8 ***	**12.6****	0.2	2.1	0.0	0.4
Transpiration	**8.5 ***	**10.3 ***	**14.2****	1.7	**2.6***	0.4	0.2
c) Biomass allocation							
Leaf mass fraction	3.2	**11.2****	**20.3****	0.7	0.6	**3.7***	1.3
Stem mass fraction	0.9	**4.3 ***	**6.5****	0.0	1.1	0.7	0.9
Root mass fraction	0.6	**38.9****	**27.8****	0.7	2.2	**6.2****	3.7
d) Growth							
% leaf area increase	**22.2****	**64.1****	**100.6****	0.0	**4.0****	2.2	0.3
% stem dia. increase	**22.2****	**75.7****	**29.6****	0.0	**6.6****	2.4	1.9

Discussion

Whole plant water uptake varied dramatically in seedlings of these four co-existing species with a five-fold difference in maximum water uptake in well watered conditions in full sunlight. As expected species responded to low water availability by reducing water uptake, but this reduction was similar across species. Shading also resulted in reduced water uptake, but in a species specific manner. The responses to the low water treatment were similar for plants in the sun and shade treatments, and this indicates that low light levels did not alleviate effects of reduced water availability. This also suggests the lack of a trade-off between responses to limiting light and water. Overall, responses of leaf physiology were congruent with what we observed for water uptake, but the relative effect of shade was larger for leaf physiology than water uptake. The reductions in water uptake observed in limiting light and water were reflected in decreased growth.

The large differences among species in whole plant water uptake in the full sunlight and well watered conditions was associated with a two-fold difference in photosynthesis, and 16-fold difference in growth as measured by percent increase in leaf area. However, despite these large inherent differences in resource use, we did not detect any differences in responses to limiting water treatments in these species. This was true for water uptake, leaf physiology and growth, and implies that the more conservative species did not fare better in limiting water conditions. This is contrary to suggested trade-offs in conservative strategies between performances in resource-abundant versus resource limiting conditions (Meinzer et al. 2010). The lack of differences in how these species responded to limiting water conditions may be in part due to strong selective forces imposed by the highly seasonal climate and the long and severe dry season that species have to endure in this region. In contrast to the responses to limiting water, species responses to the shade treatment differed for water uptake, photosynthesis and growth. Memecylon which exhibited the lowest overall change in response to shade is the only species which is dominant in the closed forests, and the observed differences in shade tolerance are likely important in being able to establish in the edge and closed habitats with lower light availability.

The lack of a significant interaction between the light and water treatments for all of the study species (no significant three-way interaction between species, light and water) indicated that responses to limiting water and light were independent of each other. Thus, we did not find evidence for a negative relationship between responses to limiting light and water in support the proposed trade-off between these responses (Smith and Huston 1989, Holmgren et al. 2012, Kupers et al. 2019). Further, though 2 species responded to limiting water condition with a decreased allocation to leaf mass fraction and an increased allocation to root, there was no evidence for the opposite allocation in response to shade, as would be expected in case of a trade-off. The lack of an interaction between light and water also rules out positive relationships between responses to limiting light and water in these species, and suggests that shade did not ameliorate responses to drought (Holmgren 2000, Sack and Grubb 2002) and did not result in coordinated whole plant strategies (Reich 2014). Seedling responses to limiting light and water may be able to evolve independently of each other. This will allow wider ranges of morphological and physiological trait combinations in these species which would allow extensive niche differentiation to effectively explore the highly heterogeneous, seasonally and temporally variable light and water environments in these tropical dry forests.

How plant water uptake changed in response to limiting water and light treatments was largely congruent with change in leaf physiology as well as whole plant growth for the four species examined. However, there were important differences in the relative magnitude of effect sizes for the light and water treatments for the different responses examined. Not surprisingly, the effect of limiting water treatment was more pronounced for plant water uptake. A reduction in this effect size in the case of leaf area normalized water use, indicates that leaf area reduction in a response to limiting water conditions in these species. In contrast, the relative effect of the light treatment was more pronounced for leaf level physiology. Importantly, the effect of limiting water on growth was more pronounced than limiting light. Thus, growth in seedlings of the study species, a measure of overall performance in the face of limiting water and light, was better reflected in whole plant water uptake than in leaf level physiology. The difficulty in quantifying whole plant level water use has often resulted in relying on organ level proxies (Dial 2004, Rodríguez-Gamir et al. 2016).This result highlights the importance of examining whole plant water use to understand plant responses to changing light and water conditions.

Several studies that have examined trade-offs in responses to limiting light and water have often used natural gradients instead of controlled experiments owing to the advantage of exploring patterns in a larger set of species (Kupers et al. 2019). While these studies are able to examine larger numbers of species, this comes at the cost of not being able to tease apart confounding correlated environmental factors that can influence species responses. While this problem is overcome in experimental studies like this one, the number of species examined in this study was limited, and thus, caution should be exercised in extrapolating these results to the larger community. The water and light levels selected for this experimental study were chosen to represent limiting but not lethal conditions to allow us to examine physiologically relevant changes in responses and growth in seedlings of the selected dry tropical forest species. These conditions are ecologically relevant in that the limiting light levels are representative of levels in the understory environments in forests in this region, while the low water levels reflect levels in the forests during the early dry season. However, previous work has indicated the possibility of a non-linear responses to varying light and water conditions (Holmgren 2012). Thus, controlled experiments across wider gradients of light and water availability and more species are essential to disentangle the complex responses and associated trade-offs.

Conclusion

We report large variation in whole plant water use in seedlings of four dominant coexisting species of a seasonally dry tropical forest. Our results demonstrate that seedling responses to limiting light and water conditions were independent of each other and this suggests that responses to limiting water may evolve independently of shade tolerance. This would allow larger potential combinations of light and water responses to allow these species to adapt to light and water niches in dry tropical forests. Growth responses in these species were explained by whole plant water use than leaf physiology, and this highlights the importance of studying whole plant water use responses in co-occurring species to better understand species establishment as well as the complex processes of coexistence and community assembly in seasonally dry tropical forests.

Contributions

Ron Sunny, Deepak Barua and Anirban Guha designed the experiment. Ron Sunny conducted the experiment with help from Asmi Jezeera, Kavya Mohan N, Neha Mohan Babu and Anirban Guha. Ron Sunny performed the analysis.

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Supplementary figures and tables

List of tables and Figures

Table S1: Species habitat preference as indicated by the relative proportion of the species in each habitat. The absolute number of individuals are shown in brackets. This data (D. Barua, unpublished) is collected from 9 plots of a total of 0.36 hectares for each habitat, from a seasonally dry tropical forest in Nigdale, Maharashtra in the northern Western Ghats in India.

	Habitats		
Species	**Closed**	**Edge**	**Open**
Syzygium cumini	9.7 (3)	77.4 (24)	12.9 (4)
Terminalia chebula	3.1 (1)	53.1 (17)	43.8 (14)
Diospyros Montana	0.0 (0)	26.9 (7)	73.1 (19)
Memecylon umbellatum	15.3 (97)	34.8 (220)	49.9 (316)

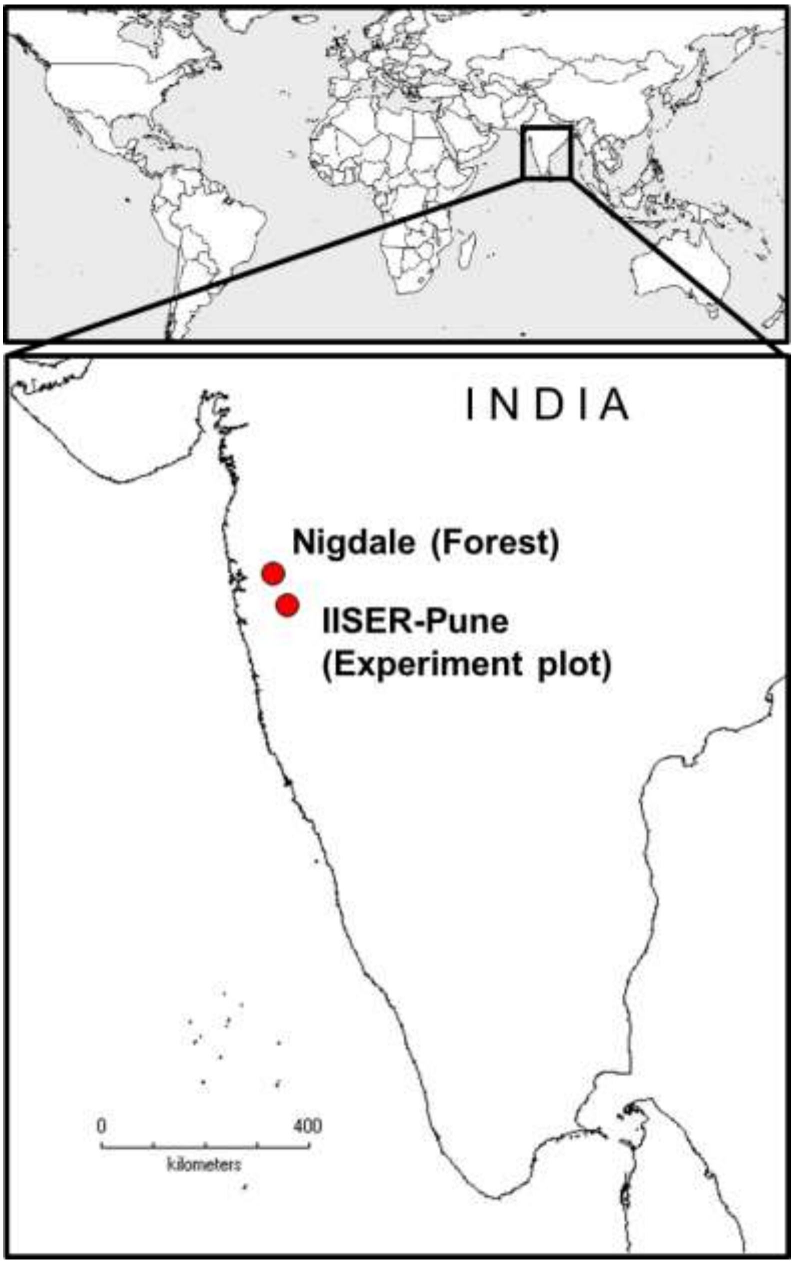

Figure S1: Map showing location of the garden experiment in Pune, and the field site from where species distribution data was collected in Nigdale, Maharashtra located at the northern end of the northern Western Ghats.

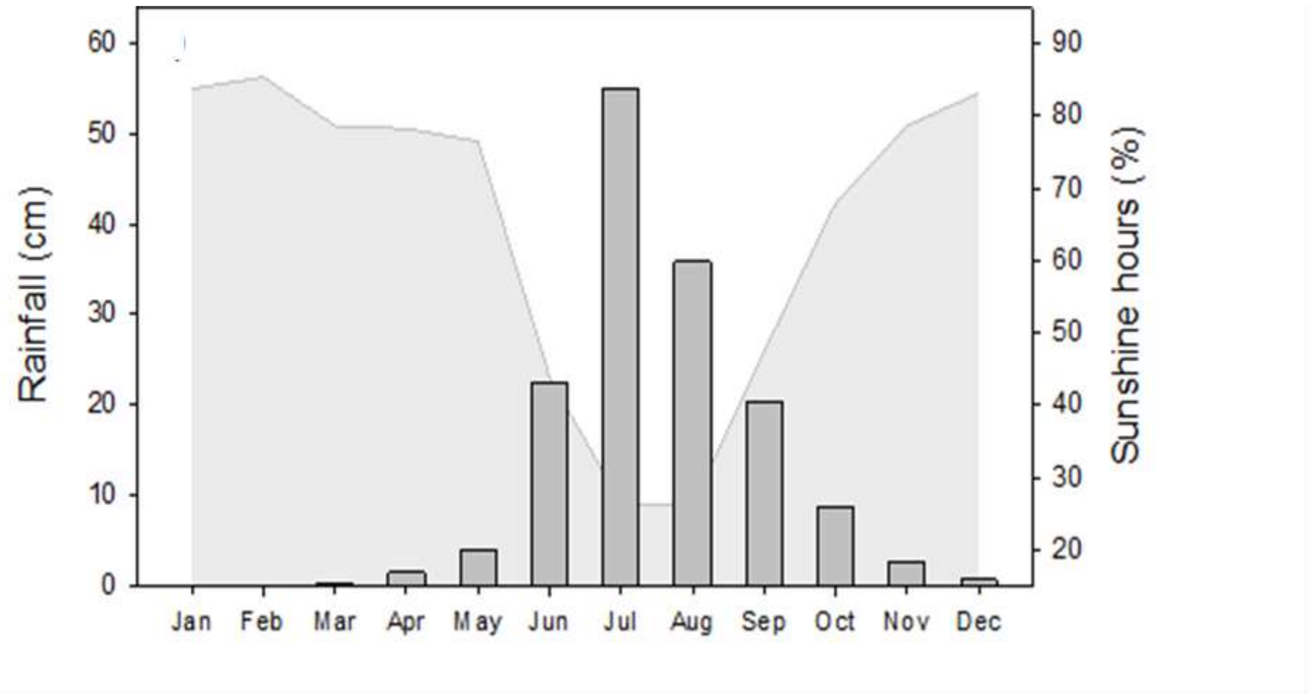

Figure S2: Climate data for the region from where species are abundant (Pune, Maharashtra, India). Monthly averaged precipitation (1961-1990) - Dark grey vertical bars; and, sunshine duration (light grey curve) are from a high resolution global dataset (New et al. 2002). Reproduced from Sastry and Barua (2017).

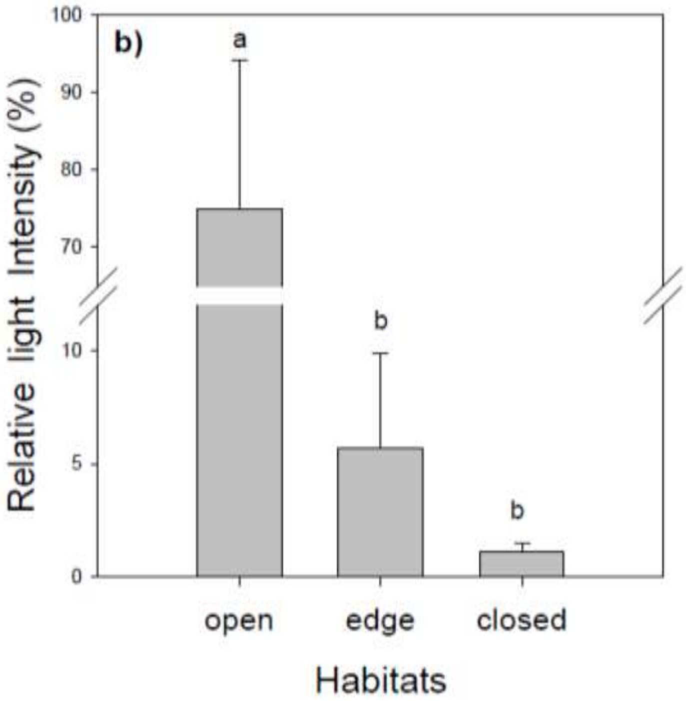

Figure S3: Relative light intensity across the three habitat types (Open fragmented forest patches-grass matrix; transition forests - Edge; and, Closed canopy forests) in Nigdale, Maharashtra in the Northern Western Ghats. This information was used to determine the intensity of the shade treatment. Reproduced from Jazeera AM (2016).

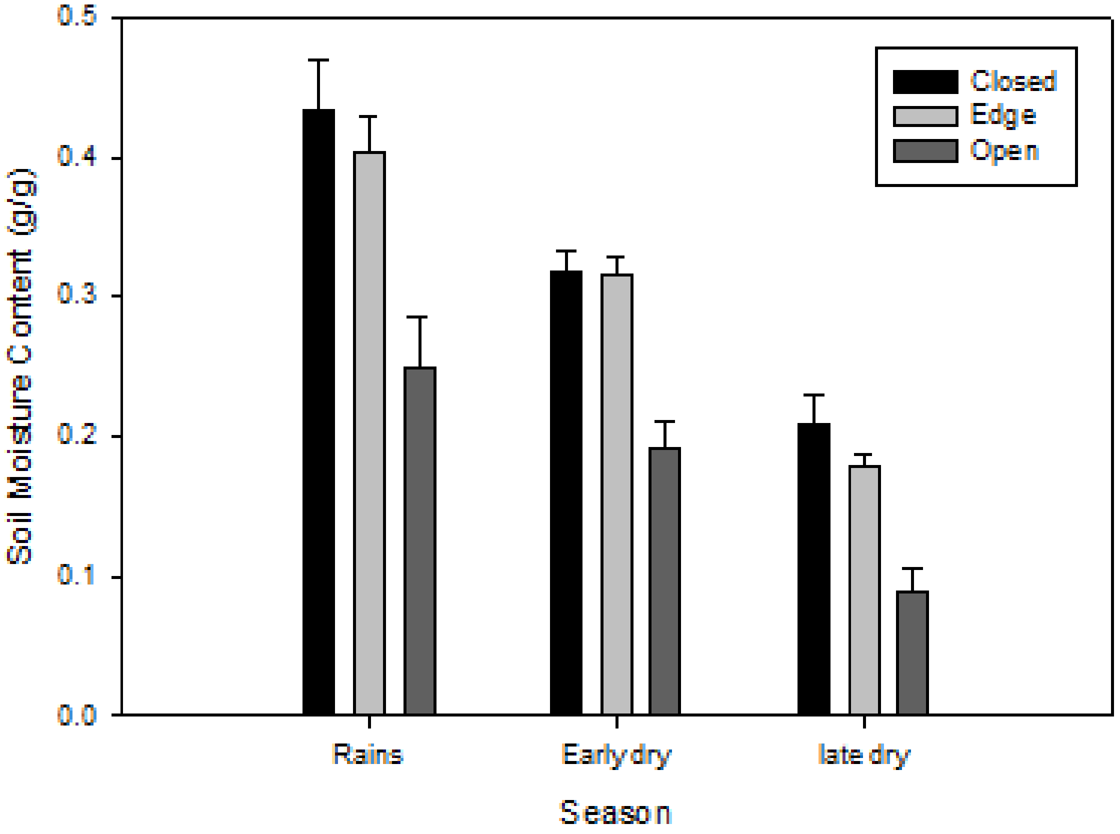

Figure S4: Gravimetric soil moisture content in the habitat types (Closed canopy forests; transition forests - Edge; Open fragmented forest patches-grass matrix) in Nigdale, Maharashtra in the Northern Western Ghats. Measurements were made during the latter part of the rainy season (September); early dry season (December); and late dry season (April) of 2015. This information was used to decide the watering treatment. Reproduced from Jazeera AM (2016).

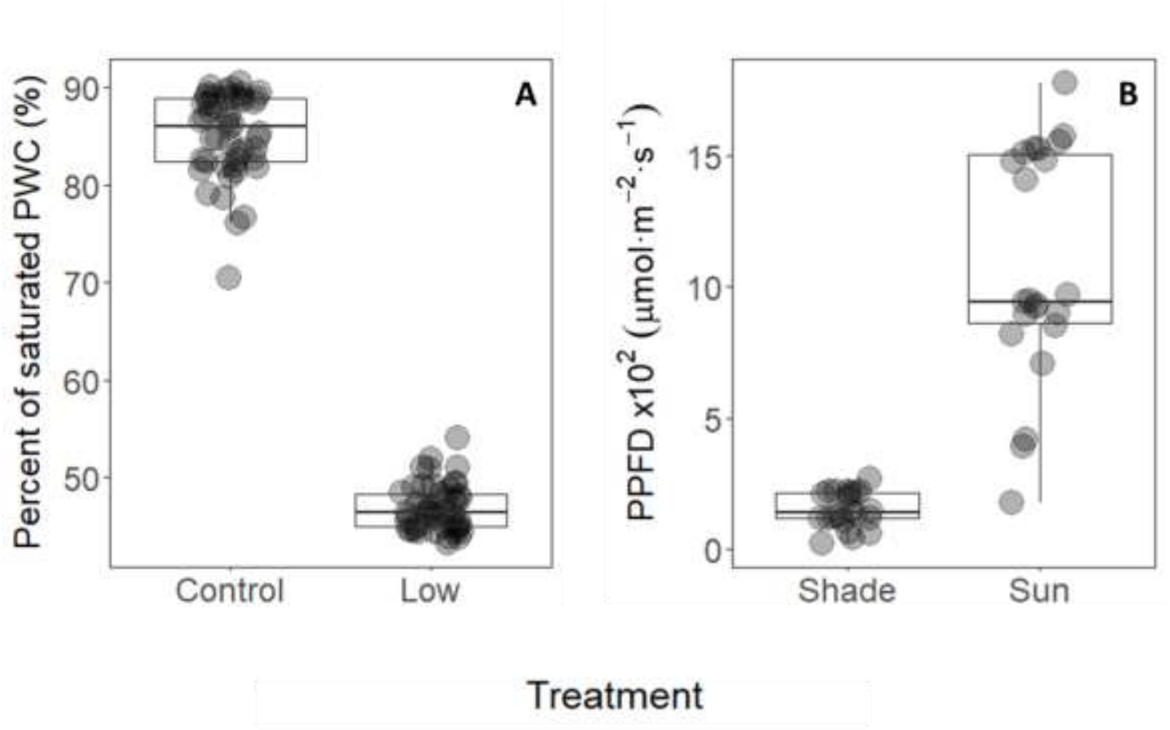

Figure S5: Treatment conditions imposed during the 50 day experiment period starting from May 2nd, 2015. A) Water treatment in terms of pot water content (PWC) corresponding to control (mean= 85% of saturated PWC, mode = 95%) and limiting water level (mean=46%, mode= 43%). Each point corresponds to the PWC of a single pot averaged over the treatment period. The control pots were reset to 95% while the low water treatment pots were reset to 45% every 3rd day. B) Photosynthetically active radiation measures for the shade treatment and sun treatment conditions in terms of average photosynthetic photon flux density (PPFD) measured during the course of the experiment. Each point corresponds to the PPFD of a day measured at 1230 Hrs.

Chapter 6

Conclusions

This thesis highlights considerable variation in hydraulic trait diversity and essential understudied hydraulic strategies in tropical forests that vary in water availability, using Indian forests as a primary study system. For the first time, this thesis also reports key hydraulic trait measurements for species from the Indian peninsula. This characterisation is important, especially given that this region is rich in biodiversity and is home to many endemic species and remains largely understudied. We observed remarkable variation in the hydraulic traits quantified in this thesis, including in g_{min}, HSM-Ψ_{50} (Fig. 2), Ψ_{min} (Chapter 2, Fig. 2A), RWC_{TLP} (Chapter 4, Fig.S1), often having ranges similar to or higher than the ranges observed in the tropical biome.

Chapter 2 took advantage of the recent dramatic increase in tropical species trait data to understand pan-tropical patterns associated with species variation in risk of drought induced catastrophic failure of xylem hydraulic conductance. The study also included the first such dataset from Indian forests. A synthesis of the available data on this safety margin showed that species in wetter sites are at lesser risk of hydraulic failure than drier sites. The drier sites had larger trait variation indicating higher capability to sustain ecosystem function during drought. Notably, the Indian sites had one of the highest reported variation in HSM among the tropical sites. In Chapter 3 we characterised differences between the two Indian forest communities that differ in the rainfall they experience, in their risk of hydraulic failure and showed the importance of accounting for differences in species dominance in determining forest communities' risk to drought. Chapter 4 showed that the popular drought tolerance trait of resistance to turgor loss, which represents resistance to early dehydration, is independent of resistance to severe dehydration and resistance to water loss. The independence of these strategies may allow for complex combinations of these traits to evolve as a response to drought in these systems. In Chapter 5, in the context of the real world multiple stress scenarios experienced by seedlings of coexisting species in a seasonally dry tropical forest, we showed that species response in water use and growth, to limiting water may evolve independently of their tolerance to shade. This independence would permit larger potential combinations of light and water responses, which would allow these species to adapt to the diverse light and water niches in such dry tropical forests. Overall, in this thesis, I show that the large variation in hydraulic traits observed in these tropical forests reflects multiple coexisting hydraulic strategies, which may be an evolutionary outcome of the strong selective pressure that water availability exerts in these systems.

In the following section, I summarise these findings and highlight the new questions that emerge.

Pan-tropical patterns of risk of hydraulic failure

The last study on global patterns of risk of xylem hydraulic failure (Choat et al. 2012) reported a global convergence in risk but also revealed large within biome variation in species hydraulic safety margin. Chapter 2 took advantage of the 8-fold increase in HSM data from the tropics and examined if this variation in risk is related to the precipitation they experience. Despite the large increase in data, this pan-tropical study was in agreement with Choat et al. (2012) in that tropical tree species, in general, are operating with a very low safety margin (Engelbrecht 2012). Almost a third of the species had negative HSM-Ψ_{50}values, indicating that a large fraction of tropical species may be at high risk of hydraulic failure in the future environment. However, contrary to what was reported in Choat et al. 2012 that indicated global convergence, species in drier sites were found to be more at risk when compared to those from wetter sites. This difference was because a change in Ψ_{50} with the mean annual precipitation was less corresponding to the change in Ψ_{min} (Fig. 1), perhaps due to a biological constrain on having very high $\Psi_{50.}$ Interestingly, drier forest sites had higher variation in Ψ_{min}, Ψ_{50} and HSM-Ψ_{50}. A recent study (Anderegg et al. 2018), showed that temperate and boreal forests with higher variation in HSM had greater capability at maintaining ecosystem function during drought. If this is also the case for tropical forests, these results will imply that drier sites would be better at buffering the effects of drought, to sustain ecosystem-level function.

On examining the geographic spread of data availability (Chapter 2, Fig. 1), the lack of data from the African Congo basin and the Indo-Malay basin needs urgent attention, especially since these regions are home to several of the global biodiversity hotspots (Myers et al. 2000). Global coverage is also necessary given that not all sites are predicted to experience drought (IPCC 2014) and accounting for this is necessary to make sound predictions. Though this study helps understand the risk of sites relative to each other, these findings still need to be put in the context of the predicted climate scenario these sites are expected to experience.

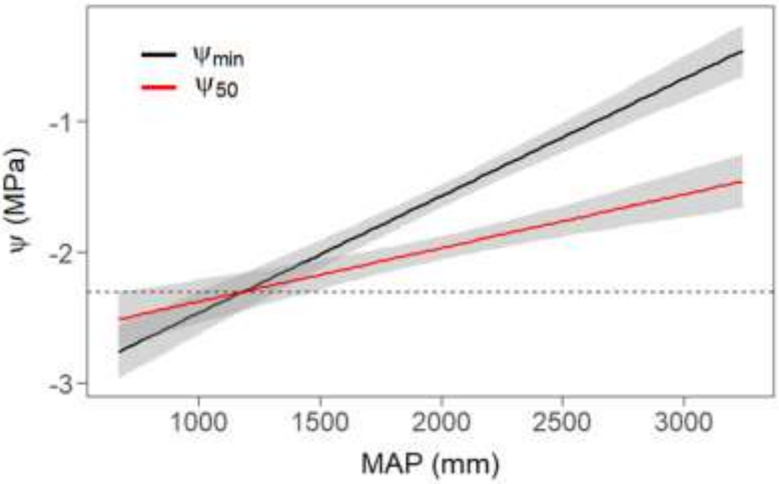

Figure 1: Difference in the relationship between Ψ_{min} and mean annual precipitation (black line) and Ψ_{50} and mean annual precipitation (MAP) (red line) together result in wetter sites being safer than the drier sites in the tropics. The dotted line corresponds to the point where hydraulic safety margin is equal to zero. Almost a third of the species examined have negative HSM and fall below this dotted line. (Redrawn from Chapter 2, Figure 2)

Despite being present in the drier sites, some species can maintain their water potential close to zero and thus not "feel" the stress during the driest time of the year (Chapter 2, Fig. 2A and Chapter 3, Fig. 1). Understanding this ability of plants is vital to understand how future drought scenarios will affect them (Martínez-Vilalta and Garcia-Forner 2017, Brodrick et al. 2019, Martinez-Vilalta et al. 2019). In this regard, remote sensing of canopy water content to assess plant dehydration status on the field and to understand how drought affects plant function is now gaining more attention as a method with great potential in monitoring plant water status at various spatial and temporal scales (Konings and Gentine 2017, Rao et al. 2019, Konings et al. 2019).

Characterising hydraulic vulnerability of two Indian forest communities

Despite the high species diversity in the tropics and large variation in coexisting species in hydraulic traits, there are very limited studies that examine hydraulic trait assemblages at a community scale. In Chapter 3, we compared hydraulic safety margins of the dominant species in two forest communities that differed drastically in their mean annual precipitation and the length of their dry seasons. This is the first study that characterises xylem hydraulic vulnerability and the associated safety margin for the Indian peninsula species. We also examined whether commonly measured functional traits could function as proxies to xylem vulnerability. For the species that managed to occur in both these very different communities, we checked for intra-specific variation in these traits.

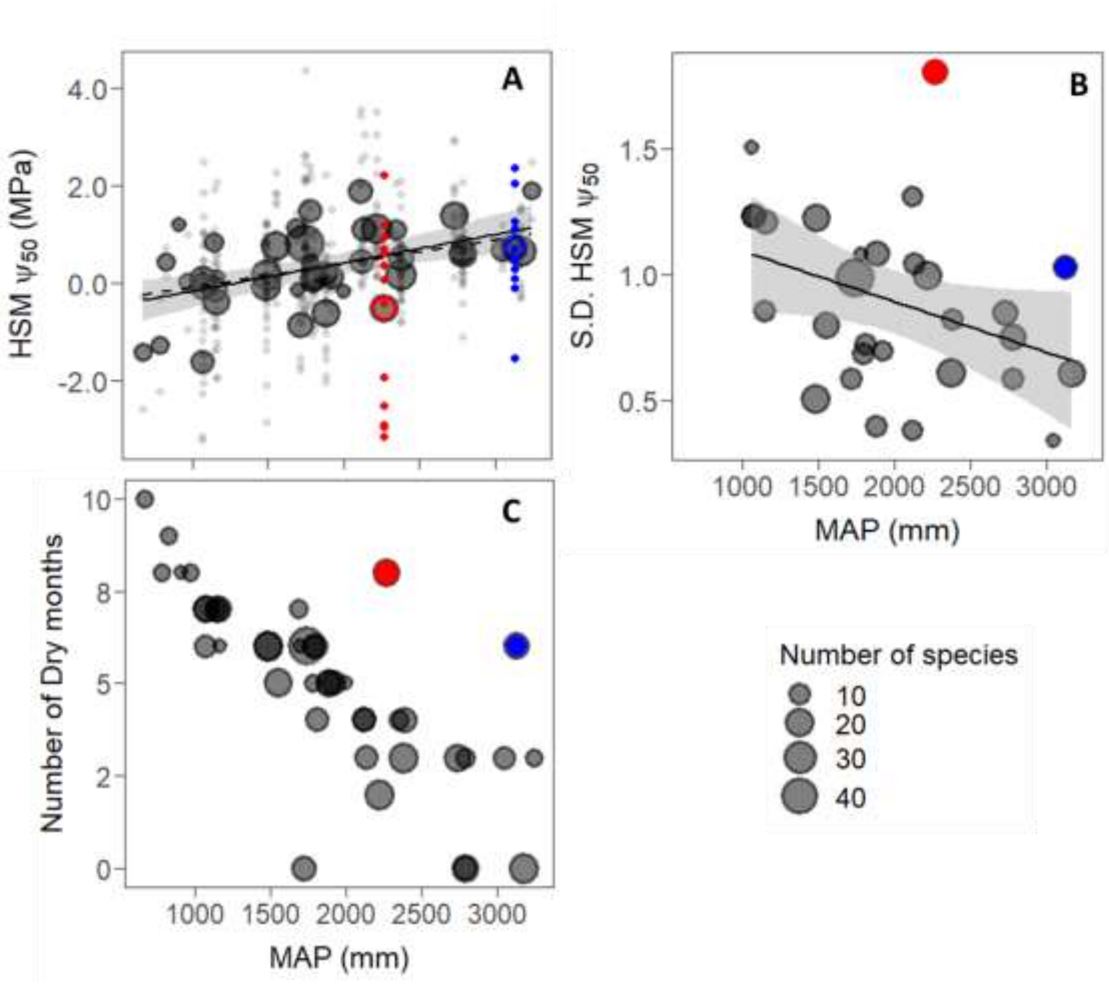

Figure 2: The two Indian sites from the drier Northern Western Ghats (red) and the wetter Central Western Ghats (blue) considered in the context of the pan-tropical data on variation in Hydraulic Safety Margin (HSM) and its relation with mean annual precipitation (MAP). The relationship between mean annual precipitation (MAP) and A) Hydraulic safety margins for Ψ_{50} (HSM-Ψ_{50}) B) standard deviation (S.D.) of HSM-Ψ_{50} and C) the number of dry months with rainfall <100mm for 43 tropical sites. Regression line with 95% confidence band in grey corresponds to significant relationships for sites (solid) and species (dashed line). The smaller symbols in the background represent individual species, and the larger symbols represent site averages. The size of the larger symbols is representative of the number of species examined.

Compared to the tropical sites for which data was available for HSM (Chapter 1), the two Indian sites were in the drier extreme with respect to rainfall seasonality, i.e. with longer dry periods and higher rainfall concentrated to a few months (Fig. 2C). These sites also had a considerable variation in HSM, and associated traits, comparable to the range observed in the tropical biome (Fig. 2A, B). This large variation indicates that these forests maybe some of the most diverse systems in the tropics, with respect to hydraulic strategies. The high within-site variation in these two Indian forests also implies that while some species may be low at risk, some species are at very high risk of catastrophic hydraulic failure, loss of physiological function and subsequent mortality.

Consistent with what was observed in Chapter 1, the wetter CWG site was safer than the drier NWG site, with respect to HSM-Ψ_{50}. Dominance weighted community-level average with respect to HSM-Ψ_{50}, which accounts for species diversity and the variation in traits in co-occurring species, also reflected this trend. Moreover, similar to what was observed in the pan-tropical study, both sites did not differ in their average HSM-Ψ_{88}. However, with respect to community weighted mean HSM-Ψ_{88}, the drier NWG site had a higher safety margin. This study thus highlights that while comparisons based on site-average and community weighted averages are both ecologically meaningful, both methods can give different outcomes on predicting forest response to drought. Community-weighted average accounts for the fact that not all species are equal in their dominance, their trait values and in their ecological roles they perform in these communities. According to the mass-ratio hypothesis (Grime 1998), at the community level, it is the characteristics of the dominant species that are likely to influence ecosystem function and processes (Garnier et al. 2004, Vitra et al. 2019, Barros et al. 2019). Another reason for this contradicting outcome is that for sites that may differ in their average slope, as is in this case, the HSM based on Ψ_{50} and Ψ_{88} can predict different outcomes for risk of hydraulic failure (Chapter 3, Fig. 7). Thus caution needs to be taken in both these aspects while characterising drought associated risk for forest communities.

However, it is to be noted that this study did not have replicate sites for a given rainfall regime, as the primary focus was to maximise species coverage and due to limitation in time and resources to do multiple sites. Thus variation in community weighted estimates could not be accounted for here.

On synthesising data available in the tropics for HSM (Chapter 1), we observed that only nine published studies had reported that data was collected for the dominant species that represent the community. Given that some communities are more diverse than others (Kreft and Jetz 2007, De Cáceres et al. 2012) and that there is a large variation in hydraulic traits among co-existing species observed in many of these communities (Chapter 2, Fig. 2), there is thus an urgent need to have more community-level studies of hydraulic traits, in order to assess the vulnerability of entire communities to drought.

Though they varied considerably, LMA, LDMC and SSD were not related to xylem vulnerability in the species of the two communities, indicating independence in hydraulic and economic traits in these systems (Li et al. 2015). Whether the relation between these two trait spectrums is dependent on the environment considered, is still not well understood (Yin et al. 2018). Though not in itself a hydraulic trait, how Ψ_{min} was related to SSD depended on the environment in this study (Chapter 3, Fig. 5).

Though this study, in its limited scope, examined intra-specific variation in the common species for Ψ_{min} and Ψ_{50}, there is still a considerable dearth of studies that examine intra-specific variation in hydraulic traits in tropical species (Anderegg 2015, Kuppler et al. 2020). Given the considerable variation in water availability in the tropics (Feng et al. 2013), intraspecific variation in hydraulic traits is expected to play a crucial role in facilitating survival in a changing climate scenario.

Other important dimensions of resistance to drought

Species experience drought stress in different organs at different time scales and even to different levels of stress. A sum total of all the responses in all these dimensions determine a whole plant response. To account for strategies that may have evolved in response to severe drought stress, beyond early responses, and strategies that minimise time to functional breakdown, we quantified resistance to loss of PSII function and minimal cuticular conductance for 18 coexisting tree species of a seasonally dry tropical forest. Proxy measures of resistance to mild dehydration (RWC_{TLP}, $RWC_{thick50}$), resistance to severe dehydration (RWC_{flbrk}) and resistance to loss of water (g_{min}) were three ecologically important strategies that were independent of each other.

Resistance to loss of Photosystem II function (RWC_{flbrk}) and associated loss of cellular integrity and function has long been used as a drought tolerance indicator especially for crops (Zulini et al. 2007, Woo et al. 2008, Baker 2008). It is also an indicator of resistance to irreversible damage (unlike the loss of turgor which is reversible) and is related to drought-induced plant mortality (Guadagno et al. 2017). Unlike most previous studies that focused on crops or planted trees and shrubs (Faraloni et al. 2011, Trueba et al. 2019), we report considerable variation in RWC_{flbrk} for dominant coexisting adult tree species from a natural forest setting. Previous studies have reported that loss of PSII function occurs at water levels beyond the loss of the leaf's rehydration capacity and RWC_{flbrk} may not be of relevance (Cardoso et al. 2018, Trueba et al. 2019). However, we argue that these measures of leaf rehydration capacity have been done on detached leaves, while rehydration capacity might be much more robust when the leaf is attached to the plant. Secondly, we observed in our study, that species that could not maintain their water status at a well hydrated state in the driest time of the year (experienced very low RWC_{min} or Ψ_{min}) could resist loss of cellular integrity and function till very low water levels (Chapter 4, Fig 5B). A correlation between RWC_{min} and RWC_{flbrk} may have evolved due to past drought events where individuals with lower RWC_{flbrk} (more resistant to irreversible damage) may have been selected for. Species are likely to experience lower RWC_{min} or Ψ_{min} in drought years to water levels that can cause irreversible damage and subsequently death. We thus argue that RWC_{flbrk} is an important ecologically relevant and useful trait that represents an understudied dimension of plant response to severe drought that can cause irreversible damage and death.

Rate of water loss post stomatal closure (g_{min}) is a strong determinant of time to loss of function during dehydration as is shown in this study (Chapter 4, Fig. 3) as well as several previous studies (Gleason et al. 2014, Blackman et al. 2016, Martin-StPaul et al. 2017). In this study, g_{min} represented an important strategy axis that explained 30.65% of the total variation. On one end of this axis spectrum were high LMA species with small stomates and low g_{min} while on the other extreme were deciduous species with high gmin and low LMA. Importantly, g_{min} was independent of both resistance to loss of turgidity and resistance to loss of cellular and functional integrity. g_{min} was also not related to Ψ_{50} (or Ψ_{88}) for a subset of these species for which data was available ($r^2 = 0.03$, $p > 0.1$, $n = 12$), contrary to expected coordination (Brodribb et al. 2014). We thus highlight that traits that represent physiological thresholds such as Ψ_{TLP}, Ψ_{50} or Ψ_{88}, RWC_{flbrk} may not alone predict the risk of drought-induced mortality and accounting for how quickly these thresholds are approached during a drought in forest mortality models is important (Brodribb et al. 2020).

As g_{min} is gaining traction as a critical determinant of dehydration time, it is also essential to understand whether this trait's relevance is environment-specific. For instance, how do species in the wetter sites differ from those in drier sites in g_{min}? What is the cost associated with high g_{min}? Does variation in g_{min} among coexisting species increase with increasing rainfall seasonality?

The last literature review summarising data available for g_{min} (Duursma et al. 2019), compiled global data for only 247 species. Surprisingly, in this dataset, only 14 tree species were from the tropics. A large fraction of the data was for crop species. Given the increasing understanding of the importance of this trait (Blackman et al. 2016, Martin-StPaul et al. 2017, Schuster et al. 2017, Brodribb et al. 2020), there is an urgent need to measure g_{min} for more species.

While Chapter 4, highlights two important and understudied dimensions of species response to drought, one dimension that has not been addressed in this thesis and understudied in general, is the importance of root in response to drought. Several studies indicate considerable variation in root hydraulic traits that translates to a large variation in water use and drought tolerance (Guha et al. 2018), even in co-existing species (Paz 2003, Collins and Bras 2007, Paz et al. 2015, Chitra-Tarak et al. 2018). Thus, in reality, the sum total of all these organ level responses to multiple stress combinations, integrated over time, will together determine the individual's overall fitness (Rodríguez-Gamir et al. 2016). More recent whole-plant level process-based models that attempt to integrate many of these aspects by incorporating hydraulic traits, to explicitly model hydraulic failure induced tree mortality (Sperry et al. 2016, 2017) is a promising step in this regard.

Whole-plant level water use and response to limiting light and water conditions

Light is perhaps one of the most critical limiting factors in the tropics. Light availability can potentially influence species' response to limiting water conditions, especially in SDTFs. While the previous chapters dealt with understanding the effect of limiting water conditions on leaf turgidity, stem xylem hydraulic conductance, leaf cellular and functional integrity as organ level proxies for effect on plant growth, Chapter 5 examined the effect of limiting water and limiting light on whole-plant water use and growth in seedlings through a controlled garden experiment. To simulate conditions experienced on the field, limiting water treatment imposed in the experiment was similar to the early part of the dry phase that seedlings experience in such forests, corresponding to a non-lethal low water level that affected growth.

Species response to the limiting water condition was independent of the light condition it was grown in. Thus strategies associated with response to limiting light and limiting water conditions can evolve independently, allowing for a wide range of potential light-water niches that these species can occupy. This observed independence is congruent with the broader idea that the stark spatial and temporal limitation in water and light conditions in these systems allows for large trait diversity in these forests (Sterck et al. 2011).

An earlier review (Holmgren et al. 2012) had indicated the possibility of a non-linear effect in response to low water condition with varying light availability. However, this was primarily observed in temperate species and not examined in tropical species due to insufficiency in the number of studies. Given that light and water are critical limiting factors in the tropics, understanding the manner of this response is crucial to understand community assembly processes in these complex forests. Though we show that response to limiting water is independent of the light condition, this is for a narrow range of conditions which could be a small part of a larger non-linear relationship. While there has been an increase in field and greenhouse based factorial design experiments examining species response to varying levels of light and water in the tropics (Kupers et al. 2019), the nature of this relationship in tropical species is yet to be explored.

How co-occurring species differ in their response of water use and growth when subject to limiting light and water conditions is also essential to understand how they use this shared resource of water when growing on a forest floor heterogeneous to light and water. There was a five-fold variation in whole-plant water use in seedlings of four co-existing species when examined in non-limiting conditions. Moreover, though species differed in their response to limiting light conditions, species did not differ in their response to limiting water condition. The lack of variation in response to limiting water condition may be due to a strong selective force imposed by the high rainfall seasonality and the eight month long severe dry season in this region that all seedlings experience, irrespective of where they are in the forest. Accounting for these differences in species water use helps understand species competition for shared resources (Palanisamy and Chui 2014), especially in the realistic context of multiple resource limitations (Everard et al. 2010). Understanding water use differences have also been useful in understanding invasive species dynamics (Davis and Pelsor 2001, Cavaleri and Sack 2010).

Conclusion

Tropical forests contribute disproportionately to global ecosystem services and provide significant ecological, social and economic benefits to a whole range of life forms that directly or indirectly rely on them. Hydraulic traits have helped ecologists over the past few decades to understand the incredible plant diversity in this biome. This thesis examined hydraulic trait diversity in tropical forests, to understand species variation patterns and highlights the need to examine understudied drought response strategies in these systems. Despite the growing interest in hydraulic trait ecology, the tropics remain a highly understudied system, as highlighted in this thesis. With the increasing threat posed by the incredibly rapid pace of climate change and rampant anthropogenic disturbances, such studies are urgent now more than ever. While this thesis focused primarily on response to water availability, in reality, the challenge these species face is much more complex; increased vulnerability to herbivory, co-occurring temperature stress being two other significant factors that threaten these species. A growing momentum in trait ecology based research to understand this complex system, which eventually translates to informed policies, coupled with pro-active measures, is essential to conserve and protect our tropical forests.

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