

Thermal plasticity and limitations in tropical trees

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Abstract

Tropical forests are the most carbon dense and biodiverse terrestrial biome on earth. In a time of global warming and biodiversity crisis, their preservation must be of high priority. At the same time, they likely operate close to their thermal limits because of a historically and seasonally stable environment. With climate change, thermal limits may be crossed more regularly, leading to increased physiological heat stress and tree mortality, which may decrease forest carbon storage. This thesis explores how physiological strategies dealing with climate change differ between tropical tree species, giving them a chance at survival, and how flexible different species are in their strategies.

A variety of tree species were studied at differing temperatures along an experimental elevation gradient in Rwanda as well as in controlled climate chambers. Drought effects were included in some studies.

Thermal acclimation was found for both leaf physiological and water-use traits. With an increase in growth temperature, the photosynthetic optimum temperature (T_{opt}) increased, while a strong downregulation of leaf respiration capacity acted to maintain constant or even decreased respiration rates (R). Traits relevant for tree water use responded to warmer climate by decreasing hydraulic conductance (K_{plant}) and leaf minimum conductance (g_{min}), while leaf osmolality and stomatal conductance did not change. In comparison with studies from other biomes, the acclimation in T_{opt} of photosynthesis was lower and not even statistically significant in the field study. The downregulation in respiratory capacity was stronger than in trees from other biomes. Leaf osmolality, a trait related to water acquisition and status, showed no increase, contrary to results from drought studies from other biomes. Acclimation in traits related to water transport (K_{plant} and g_s) and minimum leaf water loss (g_{min}) was similar to studies from other biomes.

Successional groups were found to make use of highly differing physiological strategies. Early successional (ES) species had high water use and reached high rates of photosynthesis to facilitate fast growth. Late successional (LS) species were more conservative in water use and lower in defoliation, reflecting a low growth, resource saving strategy. Tree mortality due to warming was higher in the LS species. With their low transpiration rates, excessive overheating occurs more frequently than in ES species, leading to photosynthetic heat stress and potential carbon starvation. The ES species showed higher sensitivity to drought, likely due to wasteful water-use strategies, increasing the risk for hydraulic failure.

The work in this thesis improves our understanding on thermal acclimation capacities of tropical trees and species differences in the susceptibility to heat and drought. It shows a generally lower acclimation capacity of tropical trees compared to trees from other biomes and contrasting heat and drought sensitivity in ES and LS species. This is valuable information for models predicting the future of tropical forests under climate change. If the interspecific differences in climate sensitivity observed here lead to corresponding shifts in tree community composition, this would greatly impact the carbon storage and biodiversity of this biome.

Keywords

acclimation, African tropical forest, chlorophyll fluorescence, climate change, drought, hydraulic, montane, photosynthesis, respiration, successional strategies, temperature, tropical trees, warming

Sammanfattning

Tropisk skog har högre kollagringsförmåga och biodiversitet än något annat biom i världen. I en tid av klimatförändring och biodiversitetskris är skyddet av dessa skogar av hög prioritet. Samtidigt lever tropiska träd sannolikt nära sina temperaturgränser då tropikerna har ett stabilt klimat, både historiskt och under årets gång. Klimatförändringen kan därför leda till att dessa temperaturgränser överskrids oftare, vilket leder till stress och ökad dödlighet hos träden, vilket i sin tur kan sänka skogarnas kollagringsförmåga. Den här avhandlingen undersöker hur fysiologiska strategier att hantera klimatförändring skiljer sig mellan trädarter, och hur flexibla dessa är när klimatet ändras.

Olika trädarter exponerades för olika temperaturer längs en naturlig höjdgradient i Rwanda samt i ett kontrollerat experiment med klimatkammare. Effekter av torka studerades i några av fältstudierna.

Resultaten visade att tropiska träd kan acklimatisera både bladens fysiologi och hela trädets vattenanvändning. I ett varmare klimat justerade träden upp fotosyntesens optimala temperatur (T_{opt}) medan en kraftig nedreglering av bladens respirationskapacitet ledde till att respirationshastigheten (R) hölls konstant eller till och med sjönk. Egenskaper som är relevanta för trädens vattenanvändning svarade på ett varmare klimat genom att den hydrauliska konduktansen (K_{plant}) och bladens minimala konduktans (g_{min}) minskade medan bladens osmolalitet och stomatakonduktans var oförändrad. Jämfört med studier från andra biom så var flexibiliteten i fotosyntesens temperaturoptimum lägre, och inte ens statistiskt signifikant i fältstudien. Nedregleringen i respirationskapaciteten var starkare än hos träd från andra biom. Bladens osmolalitet, som är relaterad till vattenupptag och -status, justerades inte upp i ett varmare klimat, till skillnad från tidigare resultat från torkstudier i andra biom. Acklimatiseringen i vattentransport (K_{plant} and g_s) och bladens minimala vattenförlust (g_{min}) var lik studier från andra biom.

Arter som dominerar under olika successionsstadier i ett skogsbestånds utveckling använde sig av mycket olika fysiologiska strategier. Arter som dominerar tidigt under successionens gång (early-successional, ES) hade hög vattenförbrukning och fotosyntes för att uppnå högre tillväxt. Arter som dominerar senare under successionen (late-successional, LS) var mera konservativa i sin vattenförbrukning och tappade färre blad vid torka, vilket ligger i linje med en strategi med låg tillväxt och sparsamhet med resurser. Dödligheten orsakad av värme var högre i LS-arter. Med deras låga transpiration kan bladtemperaturen inte hållas ner lika effektivt som hos ES-arterna, vilket leder till högre värmestress i fotosyntesen och potentiell kolsvält. ES-arterna, å andra sidan, var mer känsliga för torka, troligen för att deras högre vattenförbrukning ökar risken för hydraulisk kollaps.

Den här avhandlingen förbättrar vår kunskap om i vilken grad tropiska trädarter kan acklimatisera sig till ett varmare klimat samt hur olika arter skiljer sig åt i sin känslighet för värme och torka. Resultaten visade att förmågan till acklimatisering är generellt lägre hos tropiska träd än hos trädarter från andra biom. De visade även på en skillnad i klimatkänslighet mellan ES- och LS-arter. Denna kunskap är värdefull för modeller som förutsäger tropiska skogars framtid i ett förändrat klimat. Om skillnaderna i klimatkänslighet mellan olika artgrupper leder till motsvarande förändringar i skogens artsammansättning så kan kollagringsförmågan och biodiversiteten bli allvarligt påverkad.

List of papers

- I. **Wittemann M**, Andersson MX, Ntirugulirwa B, Tarvainen L, Wallin G, Uddling J (2022) Temperature acclimation of net photosynthesis and its underlying component processes in four tropical tree species. *Tree Physiology* 00: 1-15. doi: 10.1093/treephys/tpac002.
- II. Dusenge ME, **Wittemann M**, Mujawamariya M, Ntawuhiganayo EB, Zibera E, Ntirugulirwa B, Nsabimana D, Way DA, Uddling J, Wallin G (2021) Limited thermal acclimation of photosynthesis in tropical montane tree species. *Global Change Biology* 27: 4860-4878. doi: 10.1111/gcb.15790.
- III. Mujawamariya M, **Wittemann M**, Dusenge ME, Manishimwe A, Ntirugulirwa B, Zibera E, Nsabimana D, Wallin G, Uddling J Contrasting warming responses of photosynthesis in early- and late-successional tropical trees. Submitted manuscript.
- IV. Tarvainen L, **Wittemann M**, Mujawamariya M, Manishimwe A, Zibera E, Ntirugulirwa B, Ract C, Manzi OJL, Andersson MX, Spetea C, Nsabimana D, Wallin G, Uddling J (2022) Handling the heat–photosynthetic thermal stress in tropical trees. *New Phytologist* 223: 236-250. doi: 10.1111/nph.17809.
- V. Mujawamariya M, **Wittemann M**, Manishimwe A, Ntirugulirwa B, Zibera E, Nsabimana D, Wallin G, Uddling J and Dusenge ME (2021) Complete or overcompensatory thermal acclimation of leaf dark respiration in African tropical trees. *New Phytologist* 229: 2548-2561. doi: 10.1111/nph.17038.
- VI. **Wittemann M**, Mujawamariya M, Zibera E, Tarvainen L, Wallin G, Uddling J Species variability and thermal acclimation potential of water-use traits in African tropical trees. Manuscript.

Abbreviations

A_n	Net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
A_{gross}	Gross photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
A_{growth}	Net photosynthesis at growth temperature ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
C	Carbon
C_a	Ambient air CO_2 concentration
C_i	Leaf intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$)
DBI	Double bond index; average number of double bonds in the thylakoid membrane fatty acids
DGDG	Digalactosyldiacylglycerol (fatty acid)
ES	Early-successional
g_{min}	Leaf minimum conductance
g_s	Stomatal conductance for water vapor ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
J	Photosynthetic electron transport rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)
J_{max}	Maximum rate of photosynthetic electron transport ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)
K_{plant}	Plant hydraulic conductance ($\text{mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$)
LS	Late-successional
MGDG	Monogalactosyldiacylglycerol (fatty acid)
PG	Phosphatidylglycerol (fatty acid)
PPFD	Photosynthetic photon flux density ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)
PSII	Photosystem II
ψ_{leaf}	Leaf water potential (MPa)
ψ_{soil}	Soil water potential (MPa)
Rwanda TREE	Rwanda TRopical Elevation Experiment
R	Leaf respiration rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
R_{dark}	Leaf dark respiration rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
SQDG	Sulfoquinovosyldiacylglycerol (fatty acid)
T_{air}	Air temperature ($^{\circ}\text{C}$)
T_{crit}	Critical temperature at which chlorophyll fluorescence starts to rapidly increase ($^{\circ}\text{C}$)
T_{growth}	Growth temperature ($^{\circ}\text{C}$)
T_{leaf}	Leaf temperature ($^{\circ}\text{C}$)
T_{max}	Maximum daily leaf temperature ($^{\circ}\text{C}$)
T_{opt}	Optimum temperature ($^{\circ}\text{C}$)
T_{20}	Temperature at which the maximum efficiency of PSII is reduced by 20% ($^{\circ}\text{C}$)
T_{50}	Temperature at which the maximum efficiency of PSII is reduced by 50% ($^{\circ}\text{C}$)
V_{cmax}	Maximum velocity of Rubisco carboxylation ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)

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1 Tropical trees in a changing climate

Climate change impacts on forest ecosystems in general, and tropical rainforest in particular, are one of the most pressing issues we are facing in the coming years. Tropical regions contain the largest amount of aboveground carbon (C) of any terrestrial biome (Roy, Saugier and Mooney, 2001) and structurally intact tropical forests comprise a strong C sink (Pan *et al.*, 2011). With increasing tree mortality, this carbon sink may change into a source, releasing massive amounts of CO₂ into the atmosphere. In fact, the C sink of the Amazon forest has been found to decline and is predicted to turn into a C source within the next 15 years (Hubau *et al.*, 2020). For African tropical forests, the C sink has been stable so far, but is predicted to decline with continued climate change in the coming decades.

Tropical ecosystems have been found to be more sensitive to warming than temperate or boreal forests (Way and Oren, 2010). This has been attributed to a very stable climate both historically – tropical ecosystems did not experience ice ages – and seasonally, since seasonality is low or absent in the moist tropics (Janzen, 1967; Way and Oren, 2010). Tropical plant species are therefore expected to currently experience temperatures close to their thermal optimum (T_{opt} , Huang *et al.*, 2019). As a consequence, global warming may be more detrimental to these extremely species-rich and high-C storage ecosystems than to regions of higher latitude, even though temperature increases more rapidly closer to the poles (IPCC, 2018).

Despite the urgency of these issues, we know little about the mechanisms and processes leading to tree mortality and the intensities of heat and drought that different species can tolerate and survive (McDowell *et al.*, 2008). Accordingly, this thesis explores how physiological strategies dealing with heat and drought differ between tropical tree species, giving them a chance at to grow and survive, and how flexible different species are in their strategies when climate changes.

1.1 Leaf physiology

Leaves are the main location of carbon fixation and are thus central to biomass accumulation and plant growth. However, a considerable part of the total amount of fixed C (A_{gross}) is used already in the leaves via respiration (R), supporting leaf metabolism. The remaining difference between A_{gross} and R is termed net photosynthesis (A_n). Integrated over time, it determines how much of the fixed carbon can be used elsewhere in the plant. The study of both photosynthesis and leaf respiration is thus important for understanding leaf and plant C balance.

1.1.1 Photosynthesis

The instant temperature response of A_n is bell-shaped, peaking at an T_{opt} and decreasing with higher and lower temperatures (Berry and Bjorkman, 1980; Sage and Kubien, 2007). The rate of acclimation in plants grown at different temperatures can be evaluated using different methods. Often, A_n is measured at a standard temperature of 25 °C or at growth temperature (T_{growth}), which allows for a comparison of photosynthetic capacity or prevailing photosynthesis rates, respectively. Another widely used method to study acclimation is the determination of T_{opt} of A_n in plants grown at different T_{growth} (Way and Yamori, 2014). A warm acclimated plant will show an increase in T_{opt} with warming, i.e. at higher T_{growth} (Fig. 1a). Across biomes, meta-analyses have shown that this shift is, on average, between 0.34 and 0.38

$^{\circ}\text{C}$ change in T_{opt} per $^{\circ}\text{C}$ change in T_{growth} (Yamori, Hikosaka and Way, 2014 (value for C_3 plants); Kumarathunge *et al.*, 2019; Crous, Uddling and De Kauwe, 2022).

High-temperature limits for photosynthetic biochemistry are difficult to study using gas exchange methodology, since low stomatal conductance (g_s) complicates determination of maximum rates of photosynthetic capacity at high temperatures. Instead, chlorophyll fluorescence can be measured to estimate the potential quantum yield of photosynthetic light reactions. Light energy absorbed by chlorophyll molecules will be used in three ways: it can be dissipated as heat or chlorophyll fluorescence, or it can be converted into chemical energy. Since these three processes will always add up to the incoming absorbed energy, we can approximate photosystem II (PSII) activity from the amount of energy that goes into fluorescence (Maxwell and Johnson, 2000). Two commonly used measures to express high-temperature limits of A_n are T_{crit} , the critical temperature at which the minimum chlorophyll fluorescence increases rapidly, and T_{50} , the temperature at which the quantum yield (i.e. the light use efficiency) of PSII is reduced by 50% relative to unstressed values. In tropical tree species in the northern Andes, T_{50} increased by only $0.08\text{ }^{\circ}\text{C}$ per $^{\circ}\text{C}$ of temperature increase along an elevation gradient (Feeley *et al.*, 2020). This is lower than the T_{crit} increase by $0.34\text{ }^{\circ}\text{C}$ per $^{\circ}\text{C}$ seasonal increase in T_{growth} in an Australian study with species from different biomes (Zhu *et al.*, 2018). If plants do not acclimate in their upper thermal limits, the temperature response curves and therefore the range over which A_n can be realized will get more narrow (Fig. 1b).

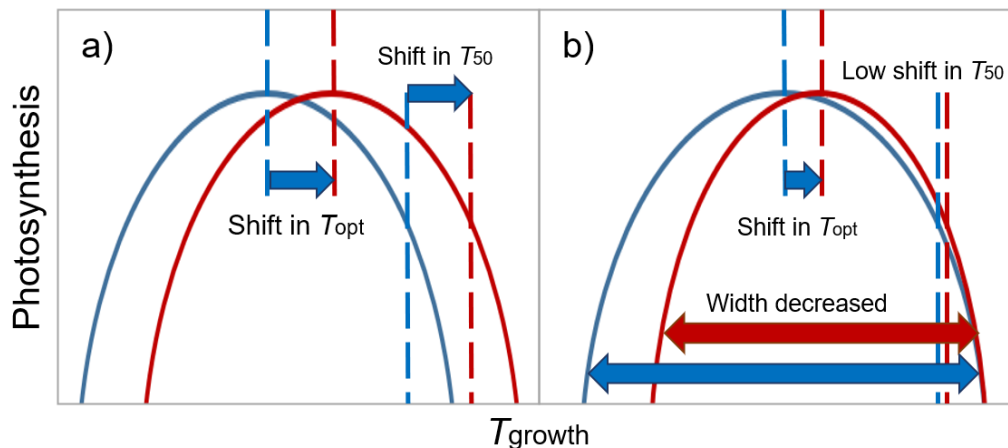


Fig. 1: Traits used to quantify acclimation capacity in A_n . Shifts in T_{opt} and T_{50} with fixed shape of the temperature response curve (a) and decreased curve width (b).

Studies on the acclimation capacity of A_n in tropical trees have led to highly differing results. While a study on four subtropical species showed increased A_{growth} with temperature (Li *et al.*, 2020), other studies showed a decrease in A_{growth} (Doughty, 2011; Cheesman and Winter, 2013). Acclimation in the T_{opt} of A_n was found in some studies (Kositsup *et al.*, 2009; Slot and Winter, 2017), but not in others (Smith and Dukes, 2017; Crous *et al.*, 2018). These differences may be attributed to differing acclimation capacities in the component processes of A_n . These include the two potentially rate limiting reactions of photosynthesis – the maximum rate of photosynthetic electron transport, J_{max} , and the maximum velocity of carboxylation by Rubisco, V_{cmax} . According to the Farquhar model, these two processes can be estimated by measurements of A_n at varying CO_2 concentrations at light-saturating conditions (Farquhar,

von Caemmerer and Berry, 1980). Next to these reactions, also leaf respiration (R) and g_s determine the rate of A_n .

1.1.2 Respiration

A considerable part of the C fixed in A_{gross} is used by respiration in the leaves, both during day and night, to create energy for leaf metabolic processes (O’Leary *et al.*, 2019). The size of R determines how much C is available to other plant organs for maintenance, defensive mechanisms and growth. This may lead us to expect high growth rates in species with low R . However, dark respiration (R_{dark}) has been found to be strongly related to A_n (Dusenge, Duarte and Way, 2019). Likely, this is due to high R not just being ‘wasteful’ but also needed to achieve high concentrations and activities of enzymes (O’Leary *et al.*, 2019) and phloem loading (Cannell and Thornley, 2000), stimulating photosynthesis and growth. Consequently, early successional (ES) species with high growth rates have been found to have both high rates of A_n and R_{dark} (Ziegler *et al.*, 2020).

With a short-term increase in temperature, R_{dark} increases exponentially, restricting the export of C (Atkin and Tjoelker, 2003; O’Sullivan *et al.*, 2013). Therefore, acclimation to long-term warming is common in this trait, across biomes (Atkin *et al.*, 2015; Smith and Dukes, 2017). Usually, acclimation is partial, with lower R_{dark} in warm-grown trees when measured at the same temperature, but higher R_{dark} when measured at prevailing T_{growth} (Slot and Kitajima, 2015; Vanderwel *et al.*, 2015; Smith and Dukes, 2017). In tropical trees, however, R_{dark} has even been found to acclimate completely, to similar rates at different T_{growth} (Slot and Winter, 2018). This may be linked to substrate limitation in plants with severely restricted photosynthesis at high temperatures (Dusenge, Duarte and Way, 2019).

1.1.3 Stomatal conductance

CO_2 enters the leaf through stomata, but while high g_s allows for high C fixation, it also increases transpiration and water loss. Stomatal regulation is therefore the core process that determines the balance between two risks: carbon starvation and hydraulic failure.

Studies on the acclimation capacity of g_s in tropical trees had differing results, with warming increasing (Li *et al.*, 2020) or decreasing g_s measured at T_{growth} (Slot and Winter, 2017; Fauset *et al.*, 2019). Higher temperatures typically lead to an increased atmospheric demand for water. Unless g_s is decreased, this results in increased plant transpiration, drying soil and higher tensions in the stem.

1.2 Plant water-use traits

To avoid hydraulic failure, plants have developed different strategies connected to water acquisition, transport and loss. Traits that influence water accessibility to plants include rooting depth, the negative pressure that can be tolerated by the xylem water column, and leaf osmolality. Trees lift water from the soil using negative matric potentials in the leaf cell walls as water evaporates. A higher amount of solutes in the mesophyll cells supports turgor pressure at drier soil and higher transpiration rates.

Water loss outside severe drought is governed by g_s , which in turn is closely coordinated with the capacity for water transport, plant hydraulic conductance (K_{plant} , Brodribb, McAdam and Carins Murphy, 2017). If g_s and therefore water loss from the leaves is higher than water supply facilitated by K_{plant} , the leaf water potential (ψ_{leaf}) will fall until the turgor loss point is reached.

Under severe drought, water loss is determined by the residual leaf conductance when stomata are closed as much as possible (g_{\min}). Drought-deciduous trees also use defoliation to minimize water loss. A completely defoliated tree will only lose water via residual transpiration through the bark.

Knowledge about water-use traits in tropical trees is limited, and thermal acclimation has not been studied at all, except for g_s . A global meta-analysis on leaf osmolality showed limited acclimation capacity in this trait, accounting for only 16% of post-drought osmolality (Bartlett *et al.*, 2014). Studies on drought acclimation of K_{plant} in tropical trees had contrasting results, with high (Schuldt *et al.*, 2011) or no plasticity (Bittencourt *et al.*, 2020). A global study on g_{\min} showed high flexibility under drought, with a downregulation in the trait by on average 70% (Duursma *et al.*, 2019).

1.3 Causes of tree mortality

Two mechanisms have been proposed to lead to climate-induced mortality: hydraulic failure and carbon starvation (McDowell *et al.*, 2008). In a warmer and drier climate, transpiration via the stomata will increase if plants do not close stomata. The increased water loss together with low water supply will result in stronger negative pressures in the xylem. Depending on the structure of their xylem, plants can endure highly differing intensities of water tensions until air bubbles form in the cells, a process called cavitation. With the introduction of air in the xylem, the continuous column of water from soil to leaf is broken and water can no longer be transported: hydraulic failure has occurred.

If plants close stomata to avoid low pressures in the xylem, C uptake is inhibited, which can lead to carbon starvation. Recent research has shown the central role of hydraulic failure in causing drought-induced tree mortality, while evidence for carbon starvation is scarce, but also much harder to study (Rowland *et al.*, 2015; Anderegg *et al.*, 2016; Adams *et al.*, 2017). There are, however, indications of an interdependence between the two processes. Hydraulic failure leads to stomatal closure and a decrease in C fixation, while carbohydrates are necessary for embolism repair to prevent hydraulic failure (McDowell *et al.*, 2011).

1.4 Successional strategies and origin elevation

With at least 40 000 tropical tree species known and estimates ranging even higher (Slik *et al.*, 2015), characterizing water-use strategies and carbon fixation in all of them is impossible. Efforts have been made to find similarities in the behavior of species sharing common characteristics, like tree size, age, growth rate and origin elevation. Comparative research has proposed the grouping, or ranking, of species along a slow-safe and fast-risky spectrum (Oliveira *et al.*, 2021).

Early successional (ES) fast-growing species are dominating in the early stages of succession, when light and nutrient limitations are low. ES species' water-use strategies align well with the fast/risky end of the growth rate/risk continuum suggested to determine tree mortality (Oliveira *et al.*, 2021). Late successional (LS) species grow slower and are therefore typically shade tolerant. They usually invest higher amounts of chlorophyll and structural compounds in longer-lived leaves compared to ES species (Poorter *et al.*, 2004). With their longer life spans and less risky strategies they take over the canopy in older stands.

With their need for high growth rates, ES species can be expected to be higher in A_n and g_s than LS species. Next to the provisioning of C for photosynthesis, high g_s in ES species can be beneficial in a warmer climate since transpiration cools down the leaves. In a climate that is also drier, however, plant will not be able to use large amounts of water for leaf cooling. LS species often have low transpiration also under moist conditions and may therefore face higher risks with climate warming. They have been shown to experience leaf temperatures (T_{leaf}) up to 18 °C above T_{air} (Fauset *et al.*, 2018).

Elevation of origin has also been found to explain species' sensitivity to climate change, with high elevation species giving way to lower elevation species in studies along tropical elevation gradients (Duque, Stevenson and Feeley, 2015; Fadrique *et al.*, 2018). This leads to a change in plant communities termed 'thermophilisation'.

1.5 Research questions

The overall aim across the papers included in this thesis is to address the following three research questions

- (1) Do tropical trees have a lower acclimation potential in leaf physiological and water-use traits than trees from other biomes?
- (2) How do water-use traits differ between species with different successional strategy or origin elevation?
- (3) Is hydraulic failure or carbon starvation impairing plant performance in tropical trees growing in a warmer and/or drier climate?

2 Approaches and methods

2.1 Species and study systems

The studies included in this thesis are all conducted on Central-East African tropical tree species that originate from either montane (10 species) or transitional rainforest regions (10 species) and are classified as either LS (8) or ES species (11). One of the species (*Fth*, see species abbreviations in Table 1) was not specified as ES or LS, because it has been reported as both in the literature. In total, 20 species are covered (Table 1).

We conducted focused experiments on a few species to study mechanisms in-depth (**paper I, II and IV**) as well as broader studies covering 16 (**paper III and V**) or all 20 species (**paper VI**), to explore patterns and to be able to generalize our results (Table 2). To quantify species differences in terms of temperature and drought responses, including acclimation capacities, we subjected trees to different temperature and drought intensities in the field (along an experimental elevation gradient) and in controlled indoor chambers (only temperature treatments).

Paper I was a focused study on thermal acclimation of photosynthesis and its component processes in four species, conducted in controlled-climate chambers set to 20, 25 and 30 °C (with night temperatures set 5 °C lower than day temperatures). Tree seedlings were grown from seeds or cuttings imported from Rwanda. Each treatment included six individuals of each species and they were all well-watered and -fertilized.

Paper III-VI were field studies conducted at a large-scale elevation experiment including 20 tree species in Rwanda (Rwanda TREE). Differences in altitude at three different sites (~1300,

1600 and 2400 m above sea level) resulted in differing climate: daytime mean temperature 2018 – 2020 was 5.3 and 6.9 °C higher at the mid- and low-elevation site, respectively, compared to the high-elevation site (17.1 °C), and mean precipitation was 800 and 960 mm lower (2200 mm at high elevation).

Table 1: Tree species covered in the different papers and their abbreviations. ES – early successional, LS – late successional, MS – mixed successional. All species are included in **paper VI**.

	Montane rainforest			Transitional rainforest		
ES	<i>Bridelia brideliifolia</i> (Pax) Fedde	<i>Bbr</i>	III, V	<i>Albizia gummifera</i> (J.F.Gmel.) C.A.Sm.	<i>Agu</i>	
	<i>Harungana montana</i> Spirlet	<i>Hmo</i>	II, III, IV, V	<i>Bridelia micrantha</i> (Hochst.) Baill.	<i>Bmi</i>	III, V
	<i>Macaranga kilimandscharica</i> Pax	<i>Mki</i>	III, V	<i>Croton megalocarpus</i> Hutch.	<i>Cme</i>	I, III, V
	<i>Maesa lanceolata</i> Forssk.	<i>Mla</i>	I, III, V	<i>Dombeya torrida</i> (J.F.Gmel.) Bamps	<i>Dto</i>	III, V
	<i>Polyscias fulva</i> (Hiern) Harms	<i>Pfu</i>	III, V	<i>Harungana madagascariensis</i> Lam. ex Poir.	<i>Hma</i>	III, V
				<i>Markhamia lutea</i> (Benth.) K.Schum.	<i>Mlu</i>	I, III, V
LS	<i>Afrocarpus falcatus</i> (Thunb.) C.N.Page	<i>Afa</i>		<i>Chrysophyllum gorongosanum</i> Engl.	<i>Cgo</i>	III, V
	<i>Carapa grandiflora</i> Sprague	<i>Cgr</i>	III, V	<i>Entandrophragma excelsum</i> (Dawe & Sprague) Sprague	<i>Eex</i>	III, IV, V
	<i>Faurea saligna</i> Harv.	<i>Fsa</i>		<i>Newtonia buchananii</i> (Baker) G.C.C.Gilbert & Boutique	<i>Nbu</i>	
	<i>Prunus africana</i> (Hook.f.) Kalkman	<i>Paf</i>	III, V			
	<i>Syzygium guineense</i> (Willd.) DC.	<i>Sgu</i>	II, III, IV, V			
MS				<i>Ficus thonningii</i> Blume ¹	<i>Fth</i>	I, III, V

¹Reported as both early- and late-successional species in the literature

Table 2: Traits studied in the different papers; traits that were focused on are highlighted in green

		Papers					
		I	II	III	IV	V	VI
Number of species		4	2	16	3	16	20
System		chamber	potted, field	field	field	field	field
Gas exchange traits	A_n	x	x	x	x	x	x
	V_{cmax}	x	x	x		x	
	J_{max}/J	x	x				
	g_s	x	x	x	x		x
	R_{dark}	x	x			x	
Extreme heat tolerance	Chlorophyll fluorescence				x		
	Lipids	x			x		
Water-use traits	g_{min}						x
	Osmolality				x		x
	K_{plant}						x
	Defoliation						x
Biomass/Growth		x	x				x

Trees were planted in December 2017 to January 2018. During the first 1.5 years, tree seedlings were irrigated when needed to avoid water deficiency and to aid tree establishment. Sites were later divided into plots with water and nutrient treatment, but most data included in this thesis were collected before treatments started, thereby exploring responses to warming rather than to drought (but see responses to seasonal drought in 2019 and 2021 in **papers III, V and VI**). **Paper III** was a broad study on A_n acclimation including most of the species (16). **Paper V** was a study on R_{dark} acclimation including the same 16 species. **Paper IV** was an in-depth study on upper heat tolerance in three species with differing successional strategy. **Paper VI** on tree water-use traits and strategies included all species in two of the studied traits and 12 or 16 species in the other three traits.

Paper II was based at the Rwanda TREE field sites, but it was conducted on potted trees in a smaller-scale elevation companion experiment with soil from the highest-elevation site used at all sites. We studied the thermal acclimation of photosynthesis and its component processes in two species.

2.2 Studied traits

The traits that were studied overlap between the five papers, but with a different focus in almost all studies. In **paper I and II** similar traits were studied, but in different study systems (Table 2).

2.2.1 Photosynthesis, respiration and stomatal conductance

Leaf gas exchange was measured using LI-6400 portable photosynthetic systems (Li-Cor Biosciences, Lincoln, NE, USA). The instrument quantifies the leaf-air exchange of CO_2 and water vapor by comparing the gas concentrations in a chamber enclosing the studied leaf with reference air. From CO_2 fluxes, A_n rates can be determined, or R_{dark} , if there is no photosynthesis. For the broader studies, A_n was measured at ambient conditions, that is, at T_{growth} (block temperature was set to ambient T_{air}), saturating light intensity (photosynthetic photon flux density, PPFD, of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$) and an incoming CO_2 concentration of 410 or $415 \mu\text{mol mol}^{-1}$. For the studies focusing on component processes, A_n was measured at different temperatures and CO_2 concentrations to create temperature response curves of A_n and its biochemical capacities: V_{cmax} and J_{max} . The latter were determined by fitting curves to the instantaneous CO_2 response of A_n , where V_{cmax} and J_{max} limits A_n at low and high CO_2 , respectively, according to the Farquhar model (Farquhar et al. 1980). Photosynthetic CO_2 responses were measured at $5 \text{ }^\circ\text{C}$ steps in the temperature range $15\text{-}40 \text{ }^\circ\text{C}$. We set light conditions according to growth conditions in the two systems: a PPFD of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the chamber study, $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the field studies.

Temperature response data were fitted to second-order equations to determine T_{opt} of A_n , and peaked Arrhenius equations to determine T_{opt} of V_{cmax} and J_{max} .

Next to plant uptake or release of CO_2 , the LI-6400 measures also the release of water vapor, which allows for the determination of transpiration and g_s . The g_s was measured within a couple of minutes after clamping onto a leaf, with chamber temperature set at ambient air temperature in the field studies. This means that although chamber acclimation time was too short for stomata to respond to new environmental conditions (which can take up to 30 minutes), g_s was at least fully acclimated to the measurement temperature.

2.2.2 Photosynthetic heat tolerance and lipids

Chlorophyll fluorescence measurements were made on three species with differing successional strategies to quantify the upper thermal limits of photosynthesis. Leaves were collected pre-dawn, when stress levels on the photosynthetic apparatus are low. They were dark acclimated and the first measurement was made at ambient room temperature (20-25 °C), used as an unstressed control measurement. We then heated leaves to seven target temperatures between 30-50 °C using infrared lamps. T_{leaf} was followed closely using thermocouples (Testo, Lenzkirch, Germany) and chlorophyll fluorescence was measured using a Pocket-PEA fluorimeter (Hansatech Instruments, King's Lynn, UK).

As opposed to other approaches quantifying stress levels by measuring chlorophyll fluorescence, we heated leaves using infrared lamps instead of water baths, and whole leaves instead of small punched leaf discs (e.g. Feeley *et al.*, 2020; Perez and Feeley, 2020; Slot *et al.*, 2021; Tiwari *et al.*, 2021). Our approach is also different by measuring instantaneous heat effects rather than the more irreversible damage estimated after a recovery period (often 24 h) under unstressful conditions, as otherwise mostly done. It is easy to apply under field conditions and does not require access to multiple heat baths. We evaluated how the direct heat effect differs from longer-term effects (up to 5 days) in one of the species, *H. montana*.

Chlorophyll fluorescence is measured to quantify the function of PSII and thereby stress levels on the photosynthetic light reactions. To quantify interspecies differences in the temperatures at which photosynthesis starts to be impaired, we determined the critical temperature (T_{crit}) at which the minimum value of chlorophyll fluorescence rapidly increases and the temperature at which the quantum yield of PSII is reduced by 50 or 20% of its initial level (T_{50} and T_{20}).

Thylakoid lipid composition was studied to relate species differences and acclimation capacity in the chlorophyll fluorescence parameters to biochemical differences in the membranes that encompass PSII. We sampled the leaves of 16 species on which also gas exchange was measured in **paper III** and **V**. Discs were punched from the center of the leaf blade, and immediately frozen in a liquid nitrogen dry shipper (-196 °C).

For lipid extraction from the discs, we used phase separation between lipophilic chloroform and hydrophilic acetic acid (described in Kourtsenko *et al.* 2007). The extracted lipids were separated and their relative amounts quantified using liquid chromatography coupled to a mass spectrometer. We quantified four groups of thylakoid lipids, MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol), PG (phosphatidylglycerol) and SQDG (sulfoquinovosyldiacylglycerol) as well as their degree of saturation (quantified as the number of double bonds per lipid, DBI). Lipid saturation can be adjusted by plants to increase the thermal stability of thylakoid membranes in which the light-dependent reactions of photosynthesis take place.

2.2.3 Water-use traits

We measured four different water-use traits, in addition to g_s described above: leaf osmolality, g_{min} , K_{plant} , and net defoliation.

Leaf osmolality was measured on leaf discs of 16 species, collected on the same occasion as lipid samples. Since osmolality is influenced not only by solutes, but also by the water status of the leaf, we chose to collect leaves pre-dawn (between 5:00 and 6:00 hours) when leaves

could be expected to be fully hydrated. Measurements were conducted using a VAPRO vapor pressure osmometer (EliTechGroup, Puteau, France). Osmolality of each sample disc was measured in the osmometer every 2 minutes until values stabilized.

Measurements of g_{\min} were made on all 20 species using the bench drying method. Leaves were collected in the evening, stored in a cool and moist box and measured in the lab the following day. They were put on a bench with the abaxial side facing upward and weighed every hour during 10 hours. The rate of mass loss when stomata are closed as much as possible was recorded as the residual transpiration of the leaf.

K_{plant} was determined from daytime gas exchange measurements of transpiration and measurements of leaf water potential (ψ_{leaf}), taken both pre-dawn and at the same time as the transpiration measurement. The pre-dawn leaf water potential was assumed to be equal to soil water potential, ψ_{soil} , and the difference between ψ_{soil} and daytime ψ_{leaf} is the driving force for plant water transport. Leaves were cut at the petiole, and since ψ_{leaf} determines how far the water column will retract into the petiole/leaf, ψ_{leaf} could be measured by application of external air pressure until water exited the petiole again. This measurement was done using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). K_{plant} was determined as the transpiration divided by the water potential difference between leaves and soil.

Defoliation was measured as the reduction in the number of leaves on a branch after the dry season 2021 compared to the number of leaves on the same branch before the dry season. It was measured in all 20 species, but only at the lowest elevation site. These data thus serve to explore species differences in drought response strategies rather than to compare sites or treatments to determine acclimation potential.

2.2.4 Biomass and growth

In the climate chamber study, trees were harvested after measurements and their above- and belowground parts were separated, dried at 70 °C and measured for dry mass.

In the field, tree stem height and mortality were determined every 3-4 months from December 2017 to January 2021. We report here on growth relative to initial tree size during the period when trees at all sites were irrigated to avoid water scarcity (from March 2018 to June 2019) as well as after seasonal drought and water manipulation treatments started (from June 2019 to January 2021). Mortality data from the same two periods were used, normalized to the number of living trees at the start of each period. The split into two periods allowed for a separation of pure heat versus combined heat and drought sensitivity.

3 Findings and discussion

3.1 Leaf physiological responses

In **paper I-V**, we studied the warming responses of leaf physiology. Overall, we found lower acclimation capacity of photosynthesis than in temperate and boreal trees, while acclimation in respiration was surprisingly high.

3.1.1 Net photosynthesis rates

Responses of A_n to T_{growth} (A_{growth}) were demonstrated using different experimental approaches. They were studied in the climate chamber experiment (**paper I**), in the field with survey spot measurements during 8:00 – 15:00 hours (**paper III**), and during the warmest part of the day at 11:00 – 15:00 hours (**paper VI**). In the climate chamber study, we found contrasting responses of two species from high and low elevation, such that the high-elevation species (*Mla*) decreased in A_{growth} with increasing T_{growth} , while the low-elevation species (*Mlu*) increased (open boxes in Fig. 2).

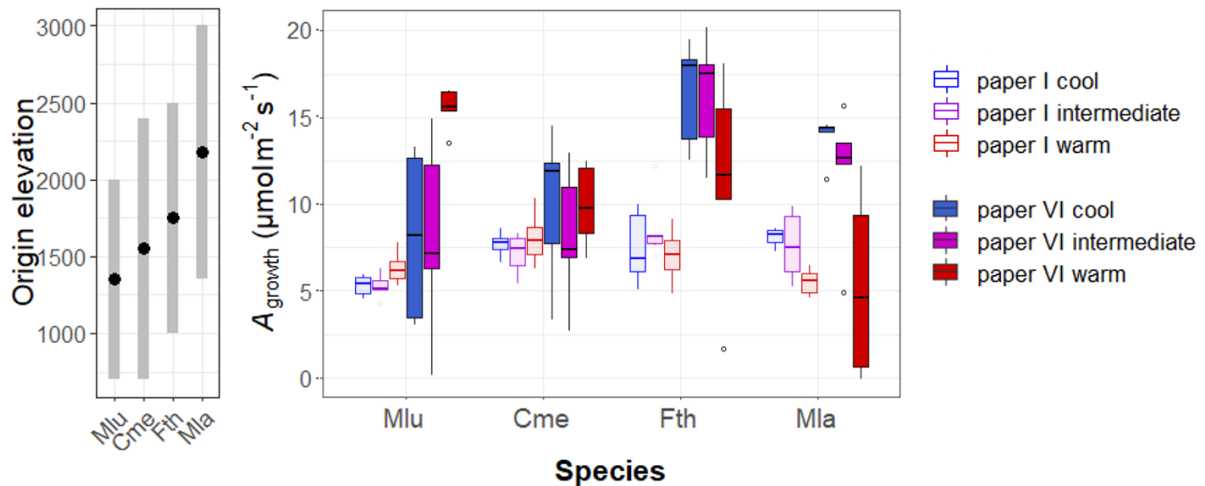


Fig. 2: Comparison of A_{growth} at different growth temperatures studied in climate chambers (**paper I**, open boxes) and in the field (**paper VI**, filled boxes) in four species with differing origin climates. Boxplots show the median, 25th and 75th percentiles (hinges) and values no further than 1.5 * the distance between the 25th and 75th percentiles (whiskers).

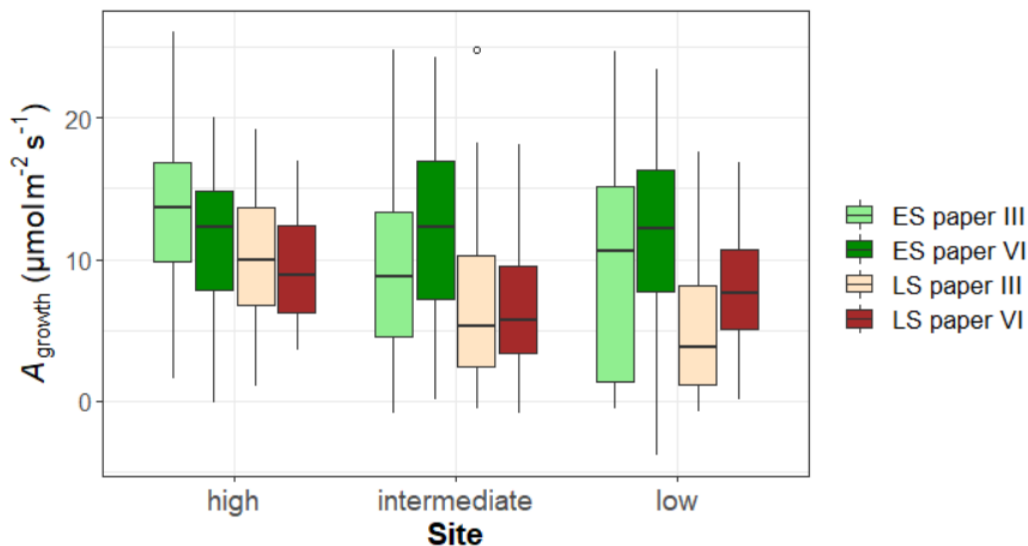


Fig. 3: Comparison of A_{growth} at differing site elevation (high, intermediate, low) in ES and LS species in two field studies (**paper III** and **VI**). ES species in green tones, LS in brown. **Paper III** and **VI** in brighter and darker shades, respectively. Boxplots show the median, 25th and 75th percentiles (hinges) and values no further than 1.5 * the distance between the 25th and 75th percentiles (whiskers).

This was caused by a decline in g_s at T_{growth} with temperature in *Mla* and an increase in *Mlu*. The two intermediate elevation species (*Fth* and *Cme*) had similar values of A_{growth} at all temperatures.

A_{growth} response patterns were similar in the field study (**paper VI**), although the magnitude of values in the climate chambers were lower in the field (filled boxes in Fig. 2). Also in the field, *Mla* exhibited declining A_{growth} at warmer sites, while the opposite was found in *Mlu*. This fits well with observations of thermophilization of tree communities along elevational gradients (Duque, Stevenson and Feeley, 2015; Fadrique *et al.*, 2018): warm-affiliated trees gain an advantage compared to cold-affiliated species in a warming climate.

In both field studies (**paper III** and **VI**), we found downregulation in A_{growth} at warmer sites in LS but not in ES species (Fig. 3). One possible mechanism to keep high A_{growth} at increasing T_{growth} would be leaf cooling via transpiration, thus avoiding high leaf temperatures at which A_n declines (Fig. 1). The higher g_s of ES compared to LS species may thus be the reason why A_{growth} does not decline at warmer sites in the former group. The result is also in accordance with suggestions that ES species are in general more plastic than LS species (Valladares *et al.*, 2000). The stable rate of carbon fixation implied by a constant A_{growth} in ES species will be of advantage in a warming climate.

3.1.2 Acclimation in T_{opt}

On average across the four species studied in climate chambers (**paper I**), T_{opt} was found to shift by 0.41, 0.24 and 0.33 °C per °C warming in A_n , V_{cmax} and J , respectively ('African CC' data in Fig. 4). In potted plants in the field (**paper II**), the corresponding values were lower (0.25, 0.29 and 0.06 °C per °C) and generally statistically non-significant ('African field' data in Fig. 4). A recent climate chamber study on tropical trees from Australia (Choury *et al.*, 2022) found similar acclimation capacity in T_{opt} of A_n but higher acclimation capacity in V_{cmax} and J_{max} compared to our studies (0.34, 0.55 and 0.49 °C per °C, respectively; values for the tropical species of that study).

To the best of my knowledge, these three are the only studies on T_{opt} acclimation of photosynthesis and its underlying biochemical capacities in tropical trees published to date. The average acclimation capacity in the T_{opt} of A_n in these three studies (0.36 °C per °C warming) was similar to that reported across biomes (0.34 - 0.38 °C per °C, Yamori, Hikosaka and Way, 2014; Kumarathunge *et al.*, 2019, Crous *et al.*, 2022). Acclimation in the African field study, however, was lower (0.25 °C per °C) and not statistically significant. The average acclimation capacity in the T_{opt} of V_{cmax} and J_{max} across the three mentioned studies on tropical trees (0.36 and 0.29 per °C) was also strikingly similar to that reported for a seasonal dataset across biomes (0.36 and 0.31 °C per °C, respectively; Kumarathunge *et al.*, 2019). However, acclimation was lower in the African field study, especially in J_{max} (0.06 °C per °C).

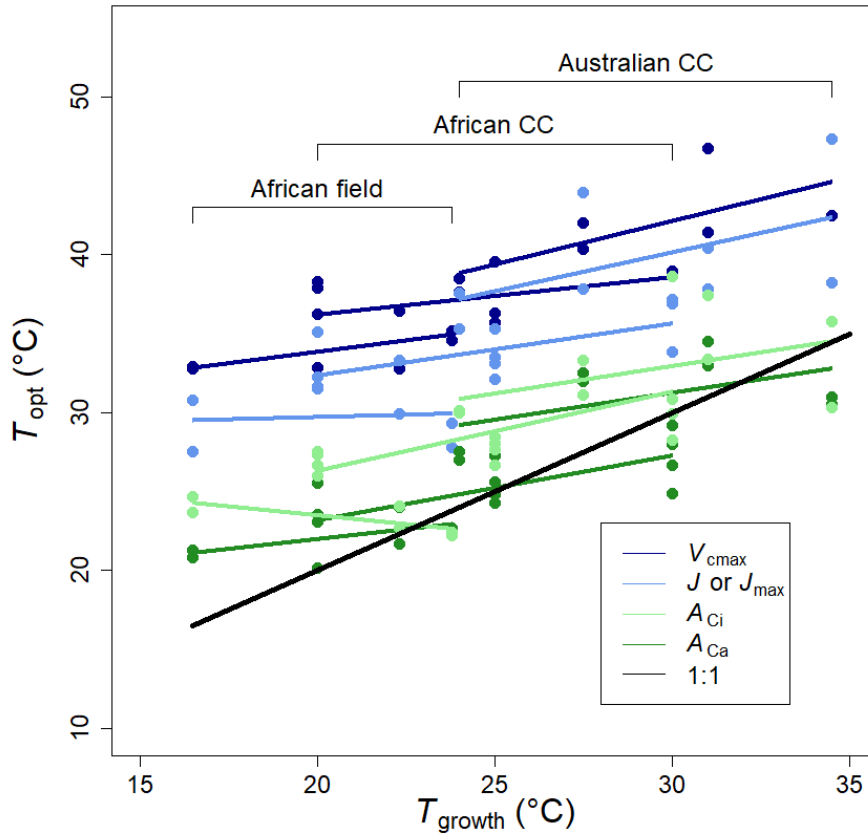


Fig. 4: T_{opt} of A_n and its underlying biochemical capacities at different mean daytime T_{growth} in **paper I** (African CC, climate chamber study), **paper II** (African field study) and Choury *et al.*, 2022 (Australian CC, climate chamber study). Regression lines for T_{opt} of V_{cmax} , J , J_{max} , A_{Ci} (A_n at a fixed C_i of 290 in **paper I**, 287 in **paper II** and 300 in Choury) and A_{Ca} (A_n at ambient C of 415 in **paper I** and Choury *et al.*, 2022, and 400 in **paper II**) are shown for the three different studies separately.

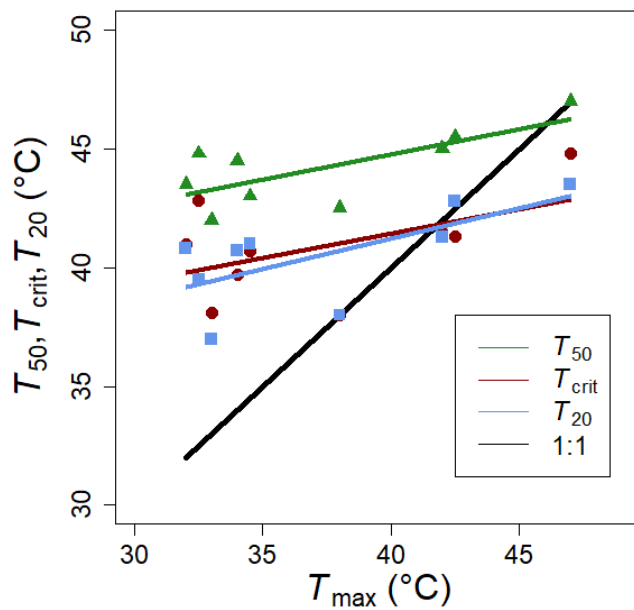


Fig. 5: Increase in upper thermal limits to photosynthesis (T_{50} , T_{crit} and T_{20}) with the maximum temperature experienced by the leaves (T_{max}) of three species grown at three sites in **paper IV**.

While tree species adapted to a certain climate (African/Australian) show a different magnitude of T_{opt} , they are equally close to their thermal limits, shown by T_{growth} surpassing T_{opt} of A_n in all studies at the highest T_{growth} (Fig. 4). This finding is in line with the suggested proximity of tropical species to their thermal limits due to their stable thermal environment (Way and Oren, 2010). Interestingly, there is also a difference in magnitude of T_{opt} (at comparable T_{growth}) between the African field and climate chamber studies. This might reflect that the climate chamber study (higher T_{opt}) included three transitional rainforest species and only one higher-elevation montane rainforest species while the field study (lower T_{opt}) was on two montane tree species.

To sum up, we demonstrated that transitional and montane African tropical tree species are capable of acclimation in T_{opt} of A_n and its component processes, but field-grown trees not to the extent of boreal and temperate species.

3.1.3 Acclimation in upper heat tolerance

We found acclimation in the upper thermal limits to photosynthesis in all three studied species at the field sites. Only one of the three species (*Eex*, the LS species with lowest g_s and largest leaf size) acclimated in T_{crit} , but all three species acclimated in T_{50} . The rate of acclimation was 0.21 °C per °C of warming in T_{50} , 0.26 °C in T_{20} and 0.21 °C in T_{crit} (Fig. 5). These values are similar to those of the acclimation capacity in T_{opt} of A_n in the field study (0.25 °C), but lower than in the chamber study (0.41 °C). Note that in **paper IV**, this is expressed per °C change in maximum T_{leaf} (T_{max}) not in T_{growth} as in **paper I** and **II**.

We conclude that both the optimal temperatures and the upper temperature limits of photosynthesis show approximately the same rate of acclimation, and that the temperature range in which photosynthesis can be maintained therefore does not get narrower (see Fig. 1). Similarly to T_{opt} of A_n , where T_{growth} surpasses T_{opt} in trees that are grown at warmer temperatures (Fig. 4), T_{crit} approaches the T_{max} that trees experience at the warmer sites (Fig. 5), with thermal safety margins being exceeded at least in one species at the warmer sites.

Along with an acclimation in T_{50} , we found adjustments in the thylakoid lipid membranes. Trees grown at the lowest elevation (highest temperature) site had a significantly lower DBI (averaged across the thylakoid lipid classes MGDG, DGDG and PG) than trees at the highest elevation site (Fig. 6a). Trees at the lowest site were also significantly higher in T_{leaf} and T_{50} compared to trees at the highest site (Fig. 6b).

With increasing temperature, membranes get more fluid which can lead to leakiness and structural disintegration. The thylakoid membrane is the location of the light reactions of photosynthesis, and one vital part of these reactions is to build up a proton gradient across the membrane for the generation of ATP. A leaky membrane would fail at this task. Without the generation of ATP, PSII would be less active, because electrons cannot pass on to proteins downstream. Increased fluidity in the membranes may also lead to physical separation of the protein complexes that are part of the electron transport chain, including PSII. Therefore, plants acclimate by decreasing the amount of double bonds in thylakoid lipids (Zhu *et al.*, 2018). Saturated acids have a straight 3-dimensional structure which causes less strain on intermolecular bonds and will make the membranes stable even at higher temperatures, whereas double bonds in *cis* configuration induce “bends” in the acyl chains and thus cause decreased order as well as lowered freezing point.

3.1.4 Acclimation in respiration rates

The thermal acclimation capacity of R_{dark} was quantified in two studies, one in climate chambers (**paper I**) and one in the field (**paper V**). Results were similar, showing complete acclimation to homeostasis (same R_{dark} at different T_{growth}) in the four species grown in chambers and complete or even over-compensatory acclimation in the 16 species studied in the field. In temperate and boreal forests, tree acclimation in R_{dark} is usually partial, homeostasis is rarely reached (Slot and Kitajima, 2015; Vanderwel *et al.*, 2015; Smith and Dukes, 2017).

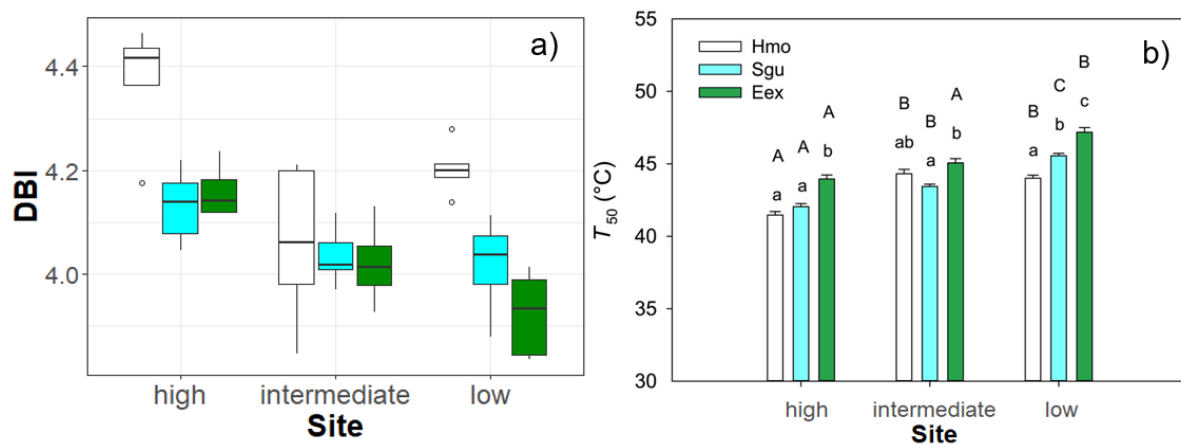


Fig. 6: Average fatty acid desaturation (DBI, a) and T_{50} (b) as related to site temperature in three different species in **paper IV**. Boxplots in a) show the median, 25th and 75th percentiles (hinges) and values no further than 1.5 * the distance between the 25th and 75th percentiles (whiskers). Mean and SE in b).

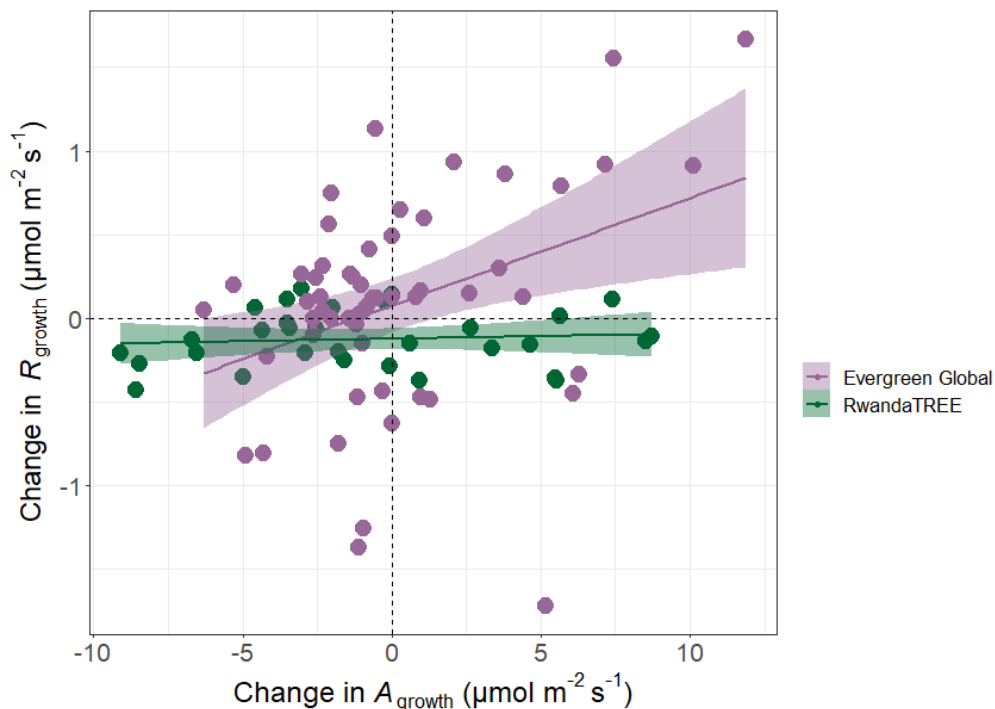


Fig. 7: The relationship between changes in A_{growth} and R_{growth} with growth temperature in 16 species in **paper VI** and **V** (green) and in evergreen woody species from 23 different studies across different biomes, extracted from Dusenge, Duarte and Way (2019, violet). Lines are regressions, shading represents the 95% confidence intervals.

The unusually high downregulation in R_{growth} could potentially be due to substrate limitation by declining photosynthesis, as suggested by Dusenge, Duarte and Way (2019). They found that a change in R_{growth} with warming was related to a concurrent change in A_{growth} across 23 studies on evergreen woody species. However, using A_{growth} data from **paper VI**, it is apparent that the changes in A_{growth} and R_{growth} across the 16 studied tropical tree species are not related (Fig. 7). This is caused by a consistent downregulation in R_{growth} with temperature, even though species vary greatly in their responses of A_{growth} . Moreover, R_{dark} acclimation in **paper V** was not accompanied by a downregulation in V_{cmax} .

3.2 Acclimation in water-use traits

Concerning acclimation in water-use related traits, we found that the tropical tree species included in **paper VI** showed a certain level of flexibility in most traits, apart from osmolality, which was a more fixed trait.

The g_{min} decreased on average by 26 and 43% towards the intermediate and the lowest site, respectively. This level of acclimation is roughly half of what has been shown in a global meta-analysis on drought responses (Duursma *et al.*, 2019), but still remarkably high, given that we only studied warming responses of g_{min} , which may be less crucial than responses to drought. The g_{s} at growth temperatures was constant across sites (**paper III** and **VI**). A few species, however, showed significant down- or upregulation in g_{s} with temperature, as did also some of the species in the chamber and the potted field study. The interspecific variation in g_{s} responses to warming explained the differences in temperature response of A_{n} (Fig. 2) and growth (not shown here) in *Mla* and *Mlu*. It may also explain why previous studies on a limited number of tropical tree species reported different warming effects on g_{s} (Slot and Winter, 2017; Fauset *et al.*, 2019; Li *et al.*, 2020). K_{plant} acclimated towards lower levels with increased growth temperature (**paper VI**). Previous research on the acclimation of K_{plant} to drought in tropical trees had contrasting results: one study found a decrease (Schuldt *et al.*, 2011) and another study no change with drought (Bittencourt *et al.*, 2020). The K_{plant} acclimation in Rwanda TREE was partly due to higher water fluidity with increasing temperatures, but probably mostly caused by the formation of more narrow xylem vessels that decrease the risk of cavitation.

Leaf osmolality was found to be a fixed, species-specific trait that did not change with temperature. This contrasts with a global meta-analysis that found a limited, but clear upregulation in leaf solutes with drought (Bartlett *et al.*, 2014). We conclude that osmolality in general is not a highly plastic trait (as also shown in the meta-analysis), and that warming alone does not induce a significant change in solute levels.

3.3 Differences between groups of species

The choice of species for our studies was made to represent species belonging to different groups concerning successional strategy and origin elevation. If differences are found in the groups, results may be generalized to other species belonging to the same groups. This approach could be used for modelling of plant responses under future climate change scenarios. In the present studies, we found large differences between successional groups (**paper III, IV** and **VI**), but only limited evidence for differences in species from differing origin elevation (**paper I** and **VI**).

3.3.1 Successional strategies

In accordance with our expectations, we found that ES species were higher than LS species in g_s (**paper II, III, IV and VI**), K_{plant} (**paper VI**), A_n (**paper III, VI**) and growth (**paper II, VI**, Fig. 8). The combination of these traits reflects the high-growth strategy of ES species which is successful at the early stages of succession.

Fast-growing ES species do not invest as much in their leaves as LS species do. This is in accordance with our findings of lower leaf osmolality in ES species (Fig. 8a). They also show higher g_{min} (Fig. 8b), likely because of a higher density and size of stomata (which is needed for a high g_s). This higher g_{min} will lead to higher water losses under extreme drought conditions, a risk that ES species counteract with defoliation when pressure in the xylem drops too low.

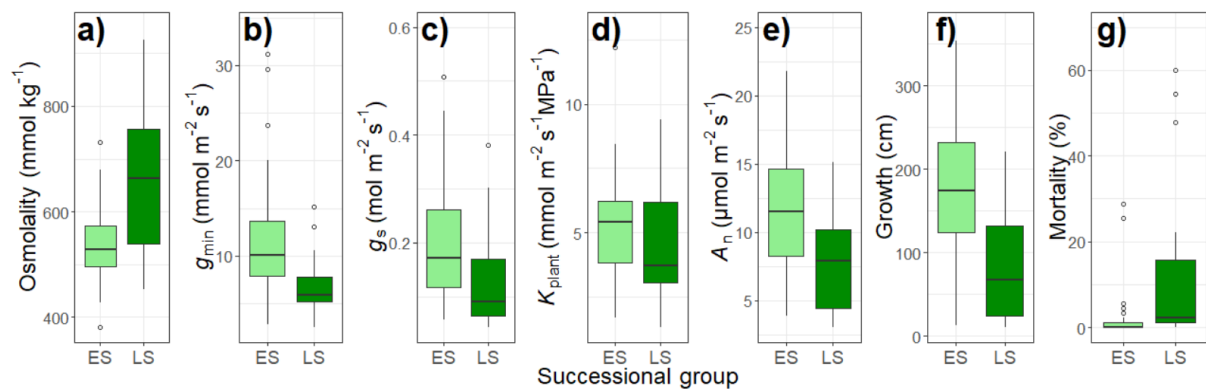


Fig 8: Differences between ES and LS species in leaf osmolality, leaf minimum conductance (g_{min}), stomatal conductance (g_s), plant hydraulic conductance (K_{plant}), net photosynthesis (A_n), growth as the change in tree height and mortality in **paper VI**. Growth and mortality data are from the first period of the experiment, before drought occurred. Boxplots show the median, 25th and 75th percentiles (hinges) and values no further than 1.5 * the distance between the 25th and 75th percentiles (whiskers).

LS species show the opposite syndrome of trait expression: low g_s , K_{plant} and A_n , resulting in the low growth typical for these species. We also found higher osmolyte concentrations and lower g_{min} in LS species, both traits improving drought resistance. High osmolyte concentrations simplify the extraction of water from the soil to keep up with water loss through transpiration. They also add to protein heat stability (Hüve *et al.*, 2006). Low values of g_{min} reduce leaf water loss under severe drought. This combination of water-saving trait expression coincided with, and likely caused, low defoliation (**paper VI**).

A drawback of low g_s is that leaves heat up more due to lower transpiratory cooling. The cooling effect of transpiration is therefore lower in LS compared to ES species. We found higher T_{leaf} in low- g_s LS than in high- g_s ES species (**paper IV**). The higher T_{leaf} in LS species was counteracted with a higher photosynthetic heat tolerance and high plasticity in the trait, but still safety margins were lower than in the ES species. T_{leaf} in the LS species were regularly close to or above the safety margins at the hottest site (Fig. 5). This may lead to carbon starvation and may also explain the higher mortality found in LS species due to heat alone (Fig. 8g).

The LS species in RwandaTREE grew at least for the first year of the experiment in full sunlight, which is not typical for trees of this strategy and may have caused excessive heat

stress. However, LS trees growing at the highest elevation site did not surpass critical leaf temperatures and showed high growth rates. This means that it is not radiation *per se* that causes high mortality rates, but rather increased temperature. The effect of radiation is thus only indirect, to the extent that it increases leaf temperatures.

3.3.2 Origin elevation

We found surprisingly little differences between origin elevation groups across all six studies. There were indications of a difference between g_s acclimation in a high- and a low-elevation species in **paper I**, but this was not confirmed across the two groups in **paper VI**. None of the hydraulic traits studied in **paper VI** differed between the two groups, though K_{plant} was near-significantly higher in species of higher elevation origin.

This lack of trait differences is surprising considering that other studies along elevational gradients found that high-elevation species were at a disadvantage against low-elevation species with climate warming (Duque, Stevenson and Feeley, 2015; Fadrique *et al.*, 2018). In these studies, species native to higher elevations showed higher mortality in their natural habitat than low-elevation species, leading to an increasing relative abundance of warmer-affiliated species over time. Even in Rwanda TREE, growth responses have been found to differ between high- and lower-elevation species. Especially ES, but also LS species originating from lower elevation showed increased relative abundance at the warmer sites (Ntirugulirwa *et al.*, 2022).

Possibly, we did not find trait differences between origin elevation groups because of high variability within the groups and large elevation overlaps of the species ranges. It may also be a combination of traits that can explain growth differences between these groups, or other traits not studied here.

3.4 Mechanisms and processes leading to mortality under climate change

One central goal of the work presented in this thesis was to explore the mechanisms and processes leading to decreased plant performance and increased mortality due to climate change. We found indications of both carbon starvation and hydraulic failure playing important roles. In **paper VI**, we found indications that high-growth species with high A_n and g_s were more sensitive to drought, likely due to their higher water loss. We found higher growth stimulation by increased temperature in species with high A_n only during the first part of the experiment. When seasonal drought and drought treatments started, this correlation was lost (Fig. 9a), showing that higher photosynthetically active species were relatively more affected by drought in terms of growth.

Mortality at the warmer sites was, even after drought treatments started, lower in high A_n species during the entire period studied here (Fig. 9c). Since then, however, it has been found to increase in some ES species as well (unpublished data). Possibly, high defoliation and partial shoot desiccation (unpublished observation) may have led to a progressive loss of competitive advantage, eventually resulting in mortality. Furthermore, when trees become larger with time and crown area increases, they also become more water demanding. The combination of lower competitive strength and higher hydraulic risk may thus lead to an increase in mortality in ES species over time.

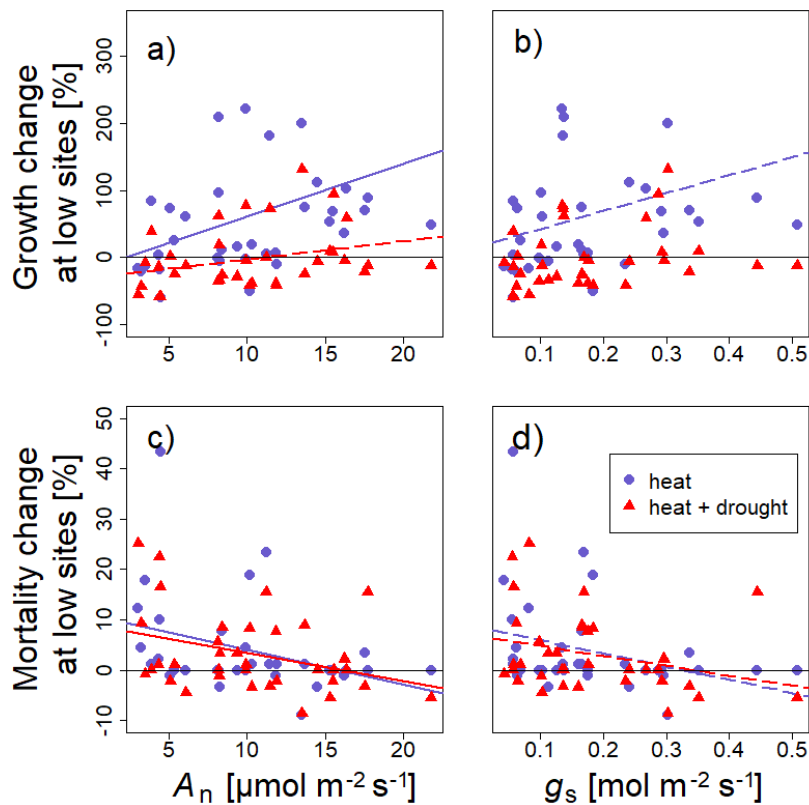


Fig. 9: Dependence of climate-induced changes in growth (a-b) and mortality (c-d) at the warmer sites compared to the coolest site before (blue) and after drought treatment (red) on A_n (a and c) and g_s (b and d). Solid lines represent significant relationships ($P \leq 0.05$) and dashed lines marginally significant relationships ($0.05 < P \leq 0.1$).

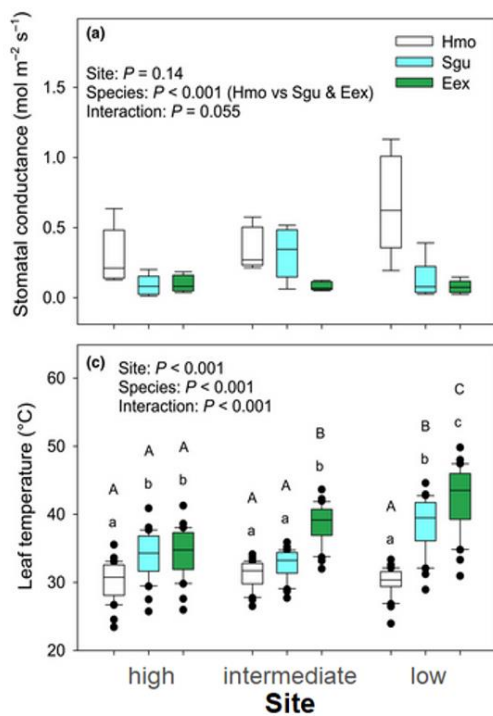


Fig. 10: Response of stomatal conductance and leaf temperature to increasing growth temperature at the three different sites in **paper IV** for three tree species. Central lines in the boxplots show the median, hinges the 25th and 75th percentile and whiskers the 10th and the 90th percentile.

Slow growing species, on the other hand, with lower A_n and g_s , showed higher mortality at the warmer sites, regardless of drought (Fig. 9c). The generally higher mortality in LS species is likely related to lower transpiration rates causing higher leaf temperatures and increased heat stress, as shown for some LS species (Fig. 10). In summary, the results suggest that hydraulic failure may be important especially for high-growth ES species with higher g_s and g_{min} , while carbon starvation may have been more prominent in LS species due to overheating and long-term reduction in C fixation.

4 Conclusions

The studies presented in this thesis show that:

Thermal acclimation in tropical tree species occurred in both leaf physiological and water-use traits. The acclimation in T_{opt} of A_n and its biochemical component processes (i.e. V_{cmax} and J_{max}) was similar to temperate and boreal species in a controlled chamber experiment. However, thermal acclimation was lower and not even significant in the field study, which is the only one existing on tropical trees so far. Acclimation in some water-use traits was also lower than in drought studies from other biomes (g_{min} and osmolality). Leaf respiration, however, acclimated more strongly than in other biomes, leading to homeostasis in respiration rates across sites with varying growth temperature.

Successional groups showed highly contrasting physiological strategies, with ES species reaching high A_n , g_s and K_{plant} to facilitate fast growth compared to LS species that are low in all these traits. LS species showed a low-growth and water-saving strategy, with low g_{min} and high osmolality, decreasing the risk of desiccation during drought. Accordingly, these species also showed a lower rate of net defoliation of their more costly leaves during drought.

Mortality due to heat was higher in the low growth LS species, indicating that low transpiration leads to overheating, strong heat stress and declines in photosynthesis. Carbon starvation may thus be an important factor predisposing trees to heat-induced mortality in these species. ES species showed higher sensitivity to drought, with drought reducing growth stimulation at the warmer sites. Hydraulic stress may thus be more prevalent in these species.

5 Outlook

With the work included in this thesis, a significant improvement has been made in our knowledge about thermal plasticity of tropical trees and their susceptibility to heat and drought. Our results will be valuable for vegetation modelling to assess climate change impacts on tropical forests, showing that acclimation capacity is often lower in tropical trees than in trees of other biomes. This is not represented in models so far (Crous, Uddling and De Kauwe, 2022).

We also found differing susceptibility to heat and drought in ES and LS species, supporting the inclusion of these groups in vegetation modelling to improve accuracy. The higher susceptibility of LS species to heat has important implications for both carbon storage and biodiversity. A disproportionately large amount of carbon is stored in taller trees in tropical forests (Gora and Esquivel-Muelbert, 2021), and these are mostly found in LS species. They usually also invest more in seed and fruit production (Foster and Janson, 1985), supporting a multitude of frugivore animal species.

The set-up of the studies in this thesis made it possible to compare seedlings at the same stage of stand development. This is important for exploring interspecific variation in climatic responses without confounding influences of varying microclimatic conditions. However, application to natural systems has to be done carefully. In our studies, LS species grew under full sunlight at least during the first year of the experiment, which is not typical for these species. Planting seedlings under the now developed canopies at the three sites may provide better insight into the climatic responses during the early stages of LS species development under more realistic conditions. Furthermore, an evaluation of our results against measurements in older, taller trees would make it possible to investigate differences in the expression of especially hydraulic traits.

Some key knowledge gaps regarding the mechanisms leading to mortality could not be included in this thesis, such as the rooting depth of the different species, tissue carbohydrate storage, and osmolality changes during the day due to photosynthate production. The temperature dependency of g_{\min} , and bark transpiration (i.e. the residual transpiration after leaves have been shed) have not been studied either. Moreover, it would have been interesting to include drought resistance traits, like embolism resistance. These traits may be key to further evaluate whether desiccation or carbon starvation leads to mortality after stomata have been closed.

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