



UNIVERSITY *of*  
RWANDA

# **Shade Tolerance and Increased Temperature Responses in Tropical Montane Trees in Rwanda**

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College of Science and Technology

School of Science

Master of Science in Biodiversity Conservation and Natural  
Resources Management

February, 2020



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# **Shade Tolerance and Increased Temperature Responses in Tropical Montane Trees in Rwanda**

By

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213000041

A Thesis submitted in fulfilment of the requirements for the degree of  
MASTER OF SCIENCE IN BIODIVERSITY CONSERVATION AND NATURAL  
RESOURCES MANAGEMENT

In the College of Science and Technology

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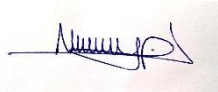
February, 2020

## Declaration

I declare that this Thesis is the result of my own work and has not been submitted for any other degree at the University of Rwanda or any other Institution.

Names: Elisée Bahati Ntawuhiganayo

Signature:



Date: 28<sup>th</sup> February 2020

I, Prof. Donat Nsabimana, main supervisor of this Thesis, approve its submission for examination.

Signature:

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Soli Deo Gloria!

## Abstract

Tropical montane forests have a large influence on the rate of ongoing climate change, but they remain relatively understudied. This study investigated physiological and structural traits of selected tree species aiming to understand plant traits that control shade tolerance in tropical montane forest tree species and their response to increased temperature. Three climax and three pioneer species were planted in pots under canopies of three different light transmittance (open, sparse, and dense) in the Arboretum of Ruhunde to study shade tolerance among species from March 2015 to May 2016, while other three climax and three pioneer species were planted in pots at Sigira (high-elevation), Rubona (mid-elevation), and Makera (low-elevation) from December 2017 to December 2018 to study effects of temperature changes on growth. Results indicated that climax species had higher fractional biomass in leaves and branches while pioneer species invested more into stems and these differences were greater under low radiation. The relative growth rate of the studied species, except *Harungana montana* increased with temperature from low- to mid-elevation but dropped at the high-elevation site. Leaf temperatures in open plots were markedly higher in climax species due to their low transpiration rates and large leaf sizes. Climax species demonstrated low thermal acclimation capacity with low growth in the low elevation site, thus confirming that they are less flexible in their responses to rising temperature. The results of this study confirm that tropical climax species are susceptible to global warming and suggest that interspecific variation in shade tolerance of tropical trees is controlled by species differences in whole-plant biomass allocation strategy.

*Keywords:* shade tolerance, climax species, pioneer species, leaf temperature, biomass allocation, photosynthetic capacity

## List of Publications

- I. Ntawuhiganayo Bahati, E., Uwizeye, K. F.; Zibera, E.; Dusenge, M. E.; Ziegler, C.; Ntirugulirwa, B.; Nsabimana, D.; Wallin, G.; and Uddling, J. 2019. Traits controlling shade tolerance in tropical montane trees. *Tree Physiology*. 00, 1-15.  
doi:10.1093/treephys/tpz119.
- II. Ntawuhiganayo Bahati, E., Mujawamariya M., Zibera E., Wittenmann M., Dusenge M.E., Nsabimana D, Wallin G, Uddling J (In prep). Physiological responses of tropical tree species to increased temperature along elevation gradient in Rwanda. *Manuscript*.
- III. Mujawamariya, M.; Manishimwe, A.; Ntirugulirwa, B.; Zibera, E.; Ganszky, D.; Ntawuhiganayo Bahati, E.; Nyirambangutse, B.; Nsabimana, D.; Wallin, G.; Uddling, J. 2018. Climate Sensitivity of Tropical Trees Along an Elevation Gradient in Rwanda. *Forests*, 9, 647

This thesis is based on the work contained in Paper I and II and contributed to Paper III.

## List of symbols and acronyms

$A_n$ : light-saturated net CO<sub>2</sub> assimilation

**AQY**: apparent quantum yield of photosynthesis

**DP**: dense canopy plot

$g_s$ : stomatal conductance

**iWUE**: intrinsic water use efficiency

$J_{max}$ : maximum rates of electron transport

**LCP**: light compensation point

**LMA**: leaf mass per unit leaf area

$N_a$ : leaf nitrogen content per unit area

$N_m$ : leaf nitrogen content per unit mass

**OP**: open sky plot

$P_a$ : leaf phosphorous content per unit area

$P_m$ : leaf phosphorous content per unit mass

$R_d$ : leaf dark respiration

**RGR**: relative growth rate

**SI**: shade intolerant species

**SP**: sparse canopy plot

**ST**: shade tolerant

**SPAD**: proxy for leaf chlorophyll content

$V_{cmax}$ : maximum rates of photosynthetic carboxylation

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# 1. Introduction

Tropical forests surpass other biomes in their role in hydrology, carbon uptake and controlling the rate of climate change (Lewis 2006; Stephens et al. 2007; Lewis et al. 2015). They host a high diversity of species and are highly productive ecosystems, accounting for about one third of global terrestrial gross primary production (Dirzo & Raven, 2003; Lewis, 2006; Beer et al., 2010; Malhi et al. 2013). An important reason for the high diversity and productivity of tropical forests is that they typically have complex canopies with multiple layers, pronounced gap dynamics and a broad range of tree species with varying levels of shade tolerance (Osunkoya et al. 1994; Poorter 1999; Markesteijn et al. 2011; Gommers et al. 2013). Shade tolerance has been attributed to many different plant traits that maximize carbon gain and/or survival rates under low light.

Two hypotheses on the suites of traits responsible for species shade tolerance have been proposed: the carbon gain hypothesis and the stress tolerance hypothesis. The stress tolerance hypothesis attributes shade tolerance to high investments in traits that maximize the resistance to biotic and abiotic stresses in the understory (Kitajima, 1994; Gommers *et al.*, 2013). Such traits include defense metabolites, high wood density and leaf mass per unit area (LMA). The carbon gain hypothesis states that shade tolerance is the consequence of high light use-efficiency resulting from traits maximizing carbon gain and minimizing carbon losses in a low-light environment. Such traits include high apparent quantum yield (AQY) of photosynthesis, low leaf dark respiration ( $R_d$ ) and light compensation point (LCP), low LMA and a high fraction of biomass invested in organs contributing to light interception in the understorey (i.e. leaves and branches) (Givnish 1988; Kitajima 1994; Lusk et al. 2002; Niinemets 2006; Baltzer & Thomas 2007; Luttge 2008; Valladares & Niinemets 2008).

The predictions of the carbon gain hypothesis have been challenged (Coste et al. 2005, Niinemets 2006, Mao et al. 2014). For example, contrary to the carbon gain hypothesis but in line with the stress tolerance hypothesis, some studies have indicated that ST plants invest more biomass into roots than SI species to escape carbohydrate loss and increase survival rate in the understory (Poorter et al., 2010; Mao et al., 2014). Therefore, more data on tropical montane forest species using a high number of traits and species with a broad range of shade tolerance, is

needed to clearly understand the most prevailing strategy (energy conservation vs energy capture) and involved trade-offs in these ecosystems.

Tropical rainforest tree species—adapted to temporary stable climatic conditions—have been suggested to have a more narrow optimum temperature range compared to tree species from more seasonal climates (Doughty and Goulden, 2008; Wright *et al.*, 2009). It has further been proposed that they operate near their thermal optimum, above which their growth and CO<sub>2</sub> assimilation may decline (Clark *et al.*, 2010; Way and Oren, 2010 ). Climax species were reported to be particularly vulnerable to the rise in temperature, indicating an urgent need for more research on thermal responses of tropical trees in order to better assess their sensitivity to rising temperature (Dusenge *et al.* 2015, Varhammar *et al.* 2015).

Tropical elevation gradients are natural laboratories for ecosystem ecology, global change research and assessing how changing climate can influence tropical forests (Malhi *et al.* 2010, Enquist *et al.* 2017). They offer the potential to study temperature responses of plants and ecosystems under ecologically realistic conditions (Körner 2007, Malhi *et al.* 2010) with ecologically different flora and unique species composition. Studying species along an elevation gradient provides a simulation of natural phenomena in tropical montane forests that are understudied. Often species that fail to acclimate or tolerate rising average temperatures migrate uphill along an elevation gradient where the temperature is cooler further increasing biodiversity and at the same time increasing competition among local species which often can lead to shifts in species composition as local species fail to keep up with new-coming species and are pushed out from their niche environment (Feeley *et al.* 2011, Corlett 2012).

In this thesis, three climax and three pioneer species were planted in pots under canopies of three different light transmittance (open, sparse, and dense) in the Arboretum of Ruhande, while other three climax and three pioneer species were planted in pots at Sigira (high-elevation), Rubona (mid-elevation), and Makera (low-elevation) with two objectives: 1) to explore which traits were most important in controlling species shade tolerance in tropical montane trees; and 2) to investigate effects of elevation and pronounced rise in temperature on growth rate of tropical montane tree species.

## 2. Materials and Methods

### 2.1. Study site and environmental measurements

The experiment on shade tolerance was conducted in the Arboretum of Ruhande (**Paper I**) (altitude: 1638-1737 m; lat. 2°36'S and long. 29°44'E). The plantation includes 216 tree species (169 exotic and 47 native) and covers an area of 200 ha that is subdivided into around 500 plots of 50 x 50 m. The tree species of the present study originate from seeds collected in Nyungwe National Park ('Nyungwe' in the following), a tropical montane rainforest that covers an area of approximately 1000 km<sup>2</sup> in southwestern Rwanda 50-100 km west of the Arboretum (2°17'–2°50'S, 29°07'–29°26'E, 1600–2950 m altitude). The experiment on growth temperature responses was conducted at three sites along an elevation gradient, Sigira, Rubona and Makera (**Paper II**). Sigira is a high-altitude site located at S 2° 30' 54"; E 29° 23' 44" in Nyamagabe district at the elevation of 2400 m. a. s. l. The average temperature at the site is 15.1°C while the maximum temperature gets to 23.8°C. Rubona is a mid-altitude site located at S 2° 28' 30"; E 29° 46' 49" in Huye district at 1600 m. a. s. l. elevation. The average temperature at the site is 20.0°C and the maximum temperature is 28.6°C. Makera is a low-altitude site located at S 2° 6'31"; E 30°51'16" E in Kirehe district at 1300 m. a. s. l. The site's maximum temperature is 31.4°C while the average is 20.5°C.

### 2.2. Plant materials and experimental design

Three climax species (*Carapa grandiflora*, *Entandrophragma excelsum*, and *Syzygium guineense*) and three pioneer species (*Croton megalocarpus*, *Dombeya goetzenii*, and *Polyscias fulva*) were first cultivated at the Arboretum's nursery, in pots containing clayey soil from the surrounding area. They were transplanted into 10 liters pots with the same type of soil. Plants were randomly distributed to nine different plots in the Arboretum, differing in overstorey leaf area index and canopy light transmittance. These nine plots were divided into three radiation regimes: three open plots, three plots with the overstorey consisting of rather sparse canopies of early-successional species, and three plots with the overstorey consisting of dense canopies of later-successional species. Each species had six replicate seedlings in each plot and the total

number of seedlings in the experiment was thus 324 (3 radiation regimes x 3 plots in each regime x 6 species x 6 replicates) (**Paper I**).

### *2.3 Gas exchange and growth measurements*

Leaf gas exchange was measured in three plants per species in each plot during February-April 2016, using a portable LI-6400 leaf gas exchange instrument (LI-COR, Lincoln, NE, USA). Two leaves per plant were measured: one for net photosynthesis ( $A_n$ ) and one for dark respiration ( $R_d$ ) (**Paper I**). On all leaves measured for gas exchange, leaf discs were sampled for LMA determination and subsequent nutrient and stable isotope analyses. Leaf samples were oven dried at 70 °C for at least 72 h. The selected leaves were at least two months old, fully developed and had comparatively healthy appearance. Leaf temperature was measured on all plants in all plots at 12:00-15:00 h on three sunny days, using three infrared thermometers (Model: BP 10, TROTEC, Germany) (**Paper I and II**). In the final harvest at the end of experiment, stem diameter and height were first measured, and leaf discs were taken for LMA determination and chemical analyses. A SPAD-502 meter (Konica Minolta Sensing, Inc., Ltd.: Osaka, Japan) was used to measure the SPAD value (a proxy of area-based leaf chlorophyll content) of one fully developed, healthy and at least two months old leaf per plant. The seedlings were then harvested and divided into roots, main stem, branches, petioles and leaves, which were oven-dried at 70 °C until constant mass (**Paper I and II**). In addition, eight plants per species which were not included in the main experiment were harvested in at the beginning of the experiment, to allow for RGR determination (**Paper I and II**).

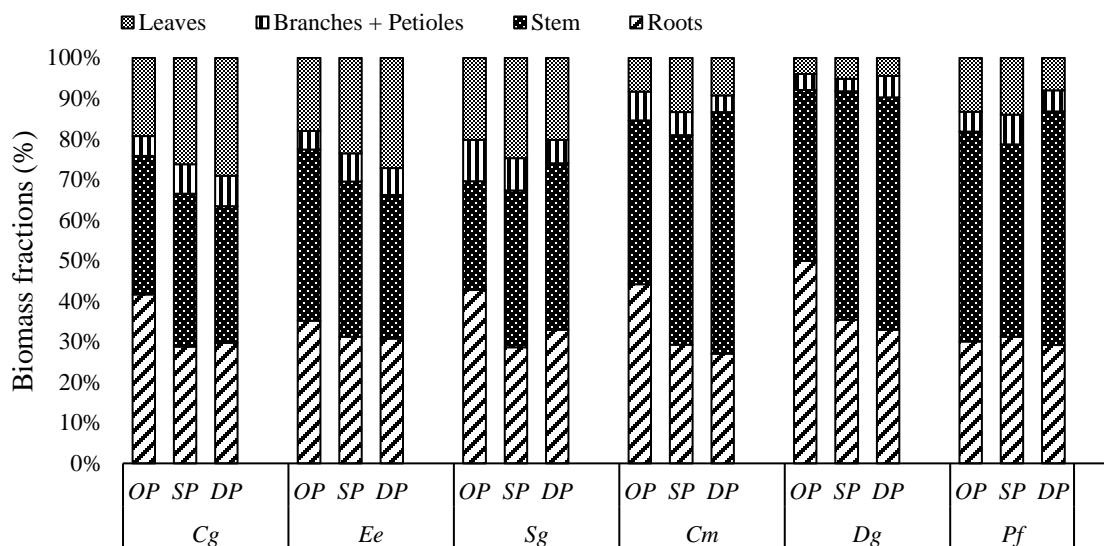
### *2.4 Statistical analyses*

Data were statistically analyzed using analysis of variance (ANOVA) with radiation regime as a fixed factor and plot and species as random factors. Plots were nested within radiation regimes and plant replication for each species in a plot was six. Significant species by radiation interactions were found for most variables and main effects of radiation were tested by simple 1-way ANOVA within each species individually. Effects were considered statistically significant at  $P \leq 0.05$ .

### 3. Results

#### 3.1 Growth and biomass allocation

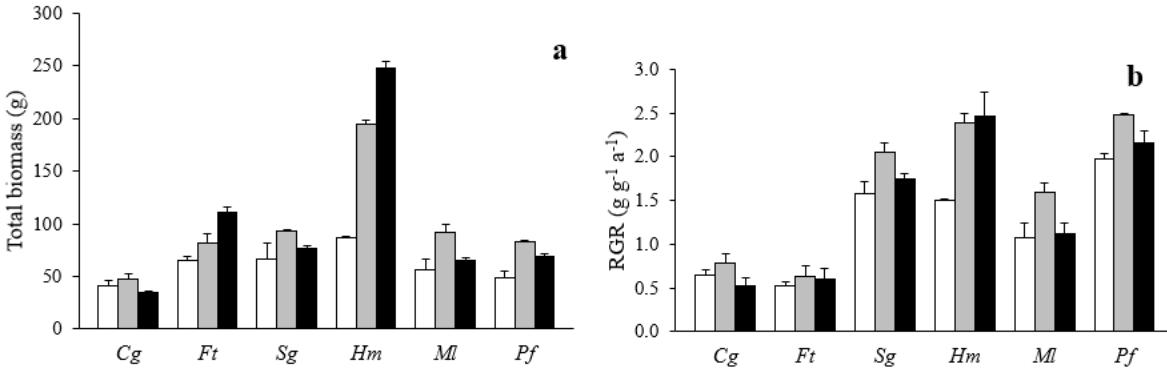
All tree biomass and structure parameters exhibited significant species by radiation interactions (**Paper I**). The pioneer species responded to shading by increasing their stem biomass fractions while this was not the case in climax species. The latter species instead responded to shading by increasing the fractions of leaves, branches and petioles; a strategy not observed in the other species. These differences were generally more pronounced in low radiation environments (Fig. 1).



**Fig. 1.** Biomass allocation (%) of *Carapa grandiflora* (Cg), *Entandrophragma excelsum* (Ee), *Syzygium guineense* (Sg), *Croton megalocarpus* (Cm), *Dombeya goetzenii* (Dg) and *Polyscias fulva* (Pf) planted in open (OP), sparse canopy (SP) and dense canopy (DP) plots. Species to the left (Cg, Ee) are shade tolerant and species to the right (Cm, Dg, Pf) shade intolerant; Sg was intermediate in Paper I. Leaf biomass data represent leaves attached at the time of harvest. The error bars represent standard errors ( $n = 3$ ). Overall statistics results are provided in Paper I.

Seedlings at high-elevation site Sigira had low total biomass and relative growth rate (Fig. 2a-b). The temperature significantly affected the variation in species' total biomass ( $p=0.0001$ ). RGR of species significantly depended on the elevation ( $p= 0.029$ ). The relative growth rate of all studied species, except *Hm*, increased with temperature from low- to mid-elevation site but dropped at the high-elevation site (**Paper II**). Across the entire dataset, *Hm* was the fastest growing species,

followed by *Pf* and *Sg*. *Cg* and *Ft* were the slowest growing species. Surprisingly, *Cg* couldn't grow well in its original conditions at Sigira whereas *Sg* thrived there with a growth rate that was nearly double its growth in Makera and Rubona.

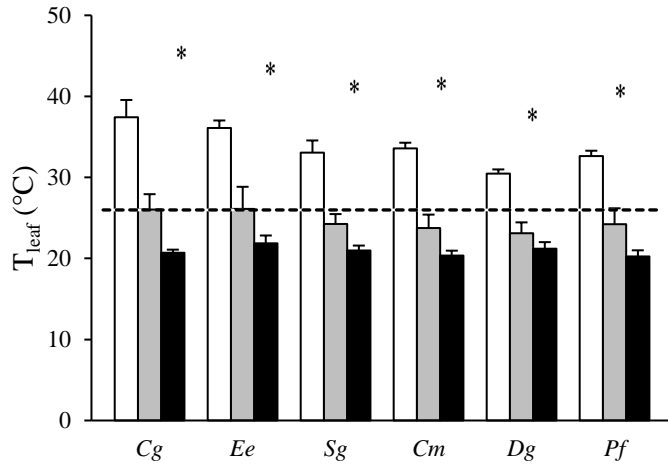


**Fig. 2** Total biomass (a) and relative growth rate (b) of six species: *Carapa grandiflora* (*Cg*), *Ficus thonningii* (*Ft*), *Syzygium guineense* (*Sg*), *Harungana montana* (*Hm*), *Maesa lanceolata* (*Ml*) and *Polyscias fulva* (*Pf*) grown in Sigira (white), Rubona (gray) and Makera (black). Species to the left (*Cg*, *Ft*, *Sg*) are climax and species to the right (*Hm*, *Ml*, *Pf*) are pioneers. Bars represent standard errors.

### 3.2 Leaf temperature

Plant species in open plots experienced higher leaf temperatures ( $T_{\text{leaf}}$ ) compared to their counterparts under sparse and dense canopies (Fig. 3). In open plots,  $T_{\text{leaf}}$  was always considerably higher than the prevailing air temperature measured at the local Arboretum weather station (Paper I). This exceedance was 10-12 °C in the ST species *Cg* and *Ee*, while it was 5-8 °C in the other species. In dense canopy plots,  $T_{\text{leaf}}$  was rather similar across species, around 5 °C below the air temperature recorded by the weather station.  $T_{\text{leaf}}$  was also similar at the mid- and low-elevation sites (Paper II). The species with the highest  $T_{\text{leaf}}$  in open plots, *Cg* and *Ee*, also had the largest leaf sizes (60 - 64 cm<sup>2</sup> compared to 10 - 42 cm<sup>2</sup> for the other species) and the lowest stomatal conductance (Paper I).





**Fig. 3.** Leaf temperature ( $T_{\text{leaf}}$ ) of six tropical tree species grown in open (white), sparse canopy (gray) and dense canopy (black) plots. The dashed line indicates the Arboretum weather station air temperature (25.9 °C) during the period of the leaf temperature measurements. Species to the left (*Cg*, *Ee*) are shade tolerant and species to the right (*Cm*, *Dg*, *Pf*) shade intolerant; *Sg* is intermediate in Paper I. The error bars represent standard errors ( $n = 3$ ). The symbol \* indicates significant variation among radiation regimes within a species. Species: *Carapa grandiflora* (*Cg*), *Entandrophragma excelsum* (*Ee*), *Syzygium guineense* (*Sg*), *Croton megalocarpus* (*Cm*), *Dombeya goetzenii* (*Dg*) and *Polyscias fulva* (*Pf*).

## 4. Discussion

### 4.1 Growth and biomass allocation

**Paper I** has revealed that climax species invested relatively more into plant organs maximizing light interception (i.e. leaves and branches) than pioneer species. A successful shade tolerant species thus seem to need both a whole-plant architecture that is favorable for light interception (in agreement with the carbon gain hypothesis) and leaves that are strong enough to tolerate biotic and abiotic stress in the understorey (in agreement with the stress tolerance hypothesis; Valladares and Niinemets, 2008; Valladares *et al.*, 2016). Shade intolerant species grown under dense overstorey canopies had high investments in stem biomass and exhibited low RGR (**Paper I**). This represents a strategy to try to escape from the shade of neighbors by rapid vertical growth (Montgomery, 2004; Grubb, 2015; Poorter *et al.*, 2018). However, unless stem elongation results in increased light interception the strategy will not be successful in the long run (Henry and Aarssen, 1997; Valladares *et al.*, 2016).

In **Paper II**, Climax species, *Cg* and *Ft*, invested more of their biomass in roots whereas pioneer species, *Hm* and *Ml*, invested in leaves. The results of **Paper II** supported the plant balanced growth hypothesis whereby biomass is allocated preferentially to the plant organ that harvests the limiting growth resource (Roa-Fuentes *et al.* 2012). A range of plant functions are impaired by low temperature (photosynthesis, nutrient uptake, growth), but reduced rates of water uptake are a probable cause of increased allocation to roots (Lambers *et al.* 2008, Fang 2018). The poor growth of climax species at Makera can be explained by the fact that they might be suffering intense sunlight which affects their physiological processes and therefore growth (Varhammar *et al.* 2015). There is therefore a need to understand environmental controls over photosynthesis to better understand how tree growth will respond in future environments (Ryan 2010). This is a particular need in tropical montane forest trees.

## 4.2 Leaf temperature

For **Paper II**, both climax and pioneer species have shown no difference in leaf temperature because they were grown in similar conditions. The results of **Paper II** present little evidence of the effect on rising temperature on biomass allocation of tropical montane tree species. The leaf temperatures under sunny conditions in open plots were highest in the climax species *Cg* and *Ee* (**Paper I**). In these species and under these conditions, leaf temperatures were on average 36 - 38 °C. These values clearly exceed the biochemical optimal  $T_{\text{leaf}}$  for light-saturated  $A_n$  (at a common intercellular  $\text{CO}_2$  concentration of  $272 \mu\text{mol mol}^{-1}$ ) of these species, which are at 25 - 30 °C (Vårhammar et al. 2015). There were indeed indications that climax trees growing in open plots suffered in our experiment. The climax species *Cg* and *Ee* had markedly lower values of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  than pioneer species in open plots but not in lower radiation regimes (Fig. 3), which may indicate negative heat effects on photosynthetic enzymes and the electron transport chain (Sage and Kubien 2007). These results are in agreement with the emerging poly-tolerance concept which suggests that shade tolerance should be evaluated together with plant tolerance to other stressors, such as drought and waterlogging (Grubb 2015, Kunstler et al. 2016, Laanisto and Niinemets 2015, Valladares et al. 2016). Our study indicates that species growing in the understorey are not only shade tolerant but may in fact also be sun intolerant at seedling stage due to negative effects of high leaf temperature on stomatal conductance and photosynthetic biochemistry.

## 5. Conclusions

The results of **Paper I** suggest that interspecific variation in shade tolerance of tropical montane trees is controlled by species differences in whole-plant biomass allocation strategy rather than by difference in physiological leaf traits determining leaf carbon balance at low radiation. Climax species invested relatively more into leaves and branches to maximize light interception in the understorey pioneer species invested more into stems trying to escape the deep shade through vertical stem growth. The growth rate of all studied species in **Paper II**, except *Harungana montana*, increased with temperature from low- to mid-elevation site but dropped at the high-elevation site. *Carapa grandiflora* and *Ficus thonningii* demonstrated low thermal acclimation capacity with a critically low growth in the low elevation site, thus confirming that they are less flexible in their responses to rising temperature than their pioneer counterparts. The results of this thesis suggest that climax species in tropical montane forests have low acclimation capacity with regards to climate change and will be more vulnerable to the rise in temperature.

# **Paper I**

## **Traits Controlling Shade Tolerance in Tropical Montane Trees**

## **Abstract**

Tropical canopies are complex, with multiple canopy layers and pronounced gap dynamics contributing to their high species diversity and productivity. An important reason for this complexity is the large variation in shade tolerance among different tree species. At present we lack a clear understanding of which plant traits control this variation, e.g. regarding the relative contributions of whole-plant versus leaf traits or structural versus physiological traits. We investigated a broad range of traits in six tropical montane rainforest tree species with different degrees of shade tolerance, grown under three different radiation regimes (under the open sky or beneath sparse or dense canopies). The two distinct shade tolerant species had higher fractional biomass in leaves and branches while shade intolerant species invested more into stems and these differences were greater under low radiation. Leaf respiration and photosynthetic light compensation point did not vary with species shade tolerance, regardless of radiation regime. Leaf temperatures in open plots were markedly higher in shade tolerant species due to their low transpiration rates and large leaf sizes. Our results suggest that interspecific variation in shade tolerance of tropical montane trees is controlled by species differences in whole-plant biomass allocation strategy rather than by difference in physiological leaf traits determining leaf carbon balance at low radiation.

*Keywords:* shade tolerance, shade intolerance, tropical montane forest, plant traits, biomass allocation, leaf temperature, Rwanda

# 1. Introduction

Tropical forests host a high diversity of species and are extremely productive ecosystems, accounting for about one-third of global terrestrial gross primary production (Dirzo and Raven 2003, Lewis 2006, Beer et al. 2010, Malhi et al. 2013). One reason for the high diversity and productivity of tropical forests is that they harbor tree species with strongly varying degrees of shade tolerance, forming complex canopies with multiple layers and pronounced gap dynamics (Osunkoya et al. 1994, Gommers et al. 2013, Valladares et al. 2016). Shade tolerance has been attributed to many different plant traits that maximize carbon gain and/or increase stress tolerance and survival rates under low light. At present, however, we lack a clear understanding of which traits are most important in controlling interspecific variation in shade tolerance (Valladares et al. 2016, Valladares and Niinemets 2008). Previous studies have often focused on either leaf physiological traits or whole-plant biomass allocation and structure (Valladares and Niinemets, 2008). Furthermore, it remains unclear if there is a difference in light acclimation capacity between shade tolerant (ST) and shade intolerant (SI) tropical tree species, with conflicting reports in the literature (Rozendaal et al. 2006, Coste et al. 2009, 2010, Houter and Pons 2014, Dusenge et al. 2015). This is partly because, in many previous studies, ST species are not grown at high light and SI species not at low light (Poorter et al. 2019). Moreover, traits change with age and size (Houter and Pons 2012), complicating the use of sun- and shade-leaf data from mature trees for predicting plasticity and shade-tolerance of juvenile trees. There is thus a need for new experimental studies exploring the contributions of different plant traits (i.e. leaf vs. whole-plant level, and physiological vs. structural) in controlling shade tolerance of tropical trees (Valladares et al. 2016, Valladares and Niinemets 2008, Poorter et al. 2019).

Two main hypotheses on the suites of traits responsible for species shade tolerance have been proposed: the carbon gain hypothesis and the stress tolerance hypothesis (Valladares and Niinemets 2008). The carbon gain hypothesis states that shade tolerance is the consequence of high light use-efficiency resulting from traits maximizing carbon gain and minimizing carbon losses in a low-light environment. Such traits include high quantum yield of photosynthesis (QY) and low leaf respiration leading to low photosynthetic light compensation point (LCP), low leaf mass per unit area (LMA), and a high fraction of biomass invested in organs contributing to light interception in the understorey (i.e. leaves and branches; Givnish 1988, Kitajima 1994, Lusk and

Pozo 2002, Niinemets 2006, Baltzer and Thomas 2007b, Luttge 2008, Valladares and Niinemets 2008). The stress tolerance hypothesis attributes shade tolerance to high investments in traits that maximize the resistance to biotic and abiotic stresses in the understorey (Kitajima 1994, Gommers et al. 2013). Such traits include high concentrations of defense metabolites and high wood density and LMA. The two hypotheses are not necessarily mutually exclusive and it is plausible that both contribute to explain shade tolerance.

The carbon gain hypothesis has been very influential for our understanding of shade tolerance, but is currently under challenge. It originally predicts that ST species should have higher relative growth rates (RGR) than SI species when growing in the shade (Givnish 1988), but in most studies lower RGR was observed in ST seedlings when growing in both high and low light conditions (Kitajima 1994, Walters and Reich 1999, Poorter et al. 2016). The carbon gain hypothesis also suggests lower LMA for ST compared to SI species to maximize light interception (Givnish 1988, Poorter et al. 2009, Valladares and Niinemets 2008), but some studies with tropical trees found the opposite (Coste et al. 2005, Kitajima 1994, Mao et al. 2014). This suggests that the conservation of carbon is more important than the efficiency of its capture (Reich et al. 2003, Lusk et al. 2011), which is more in line with the stress tolerance hypothesis. Some studies have indeed reported that plant survival in the understorey is poorly linked to whole plant carbon gain (Kitajima 1994, Reich et al. 2003). In addition, contrary to the carbon gain hypothesis but in agreement with the stress tolerance hypothesis, some studies have indicated that ST plants invest relatively more biomass into roots than SI species when growing in the understorey (Mao et al. 2014, Poorter et al. 2010).

Leaf physiological data also sometimes conflict with the carbon gain hypothesis. The hypothesis predicts higher QY and lower respiration and LCP for ST compared to SI species (Valladares and Niinemets 2008), but Walters and Reich (1999) could not find any significant differences in these traits between the two groups. Moreover, in a study on tropical montane rainforest tree species differing in shade tolerance, higher chlorophyll content per unit leaf area in ST compared to SI species did not translate into higher apparent QY (i.e. the initial slope of the light response of net photosynthesis; Dusenge et al. 2015). This suggests that the common observation of higher chlorophyll content in ST species (Niinemets 1997, Valladares and Niinemets 2008) does not necessarily contribute to increased leaf carbon gain. All these findings show that new and more



holistic studies—including side-by-side comparisons of different types of traits across species with a broad range of shade tolerance—are needed to explore which traits and strategies are most important in controlling tropical tree shade tolerance (Valladares et al. 2016).

Tropical rainforest tree species—adapted to temporally stable climatic conditions—have been suggested to have a more narrow optimum temperature range compared to tree species from more seasonal climates (Doughty and Goulden 2008, Wright et al. 2009). It has further been proposed that they operate near their thermal optimum, above which their CO<sub>2</sub> assimilation and growth may decline (Clark et al. 2010, Way and Oren 2010 ). Indeed, photosynthesis was negatively affected by warming in both branch chamber experiments (Doughty 2011) and indoor growth chamber experiments (Cheesman and Winter 2013a, Slot and Winter 2017). Moreover, daytime leaf temperatures greatly exceeded both air temperature and the optimal temperature for photosynthesis in a common garden experiment with tropical tree seedlings (Vårhammar et al. 2015). This exceedance was particularly high in sun exposed ST species with low transpiration rates and large leaf size. Controlled experiments also indicate that tropical ST species are more sensitive to warming than SI species (Doughty 2011, Cheesman and Winter 2013b). It is therefore possible, but hitherto unexplored, that high leaf temperatures result in physiological heat stress and lower competitiveness of ST species growing in high radiation environments.

The overall aim of this study was to explore which traits were most important in controlling species shade tolerance in tropical montane trees. The specific hypotheses were: (1) ST species have a whole-plant biomass allocation strategy that maximizes light interception when grown in the understorey, i.e. higher relative investment into branches and leaves. (2) ST species have physiological leaf traits that allow for a more favorable leaf carbon balance in a low-light environment, i.e. lower respiration and LCP. In addition, a third hypothesis related to high radiation intolerance was included to explore if ST species might suffer under open sky conditions: (3) ST species have leaf traits that cause high leaf temperature (i.e. low transpiration rates and large leaf size) and consequent physiological heat stress under high sun exposure. To address these hypotheses, we investigated a broad range of traits in seedlings of six tropical montane tree species with varying degrees of shade tolerance grown under three different radiation regimes: open sky, and beneath either sparse or dense overstorey canopies. Traits

measured include structural, chemical and physiological leaf traits as well as whole-plant growth and biomass allocation.

## 2. Materials and methods

### *2.1. Study site and environmental measurements*

The experiment was conducted in the Arboretum of Ruhande ('Arboretum' in the following), Rwanda (altitude: 1638-1737 m; lat. 2°36'S and long. 29°44'E). The Arboretum was established in 1933 after the relocation of the population that lived and farmed in that area (Nsabimana 2009). The plantation includes 216 tree species (169 exotic and 47 native) and covers an area of 200 ha that is subdivided into around 500 plots of 50 x 50 m, most of which being monospecific (Dusenge et al. 2015, Nsabimana et al. 2008). The plants of the present study originate from seeds collected in Nyungwe National Park ('Nyungwe' in the following), a tropical montane rainforest that covers an area of approximately 1000 km<sup>2</sup> and is located in southwestern Rwanda 50-100 km west of the Arboretum (2°17'–2°50'S, 29°07'–29°26'E, 1600–2950 m altitude). The Arboretum is within the altitudinal range of all six species studied (see below).

The long term climate at the Arboretum is characterized by small variations in monthly mean air temperature and a bimodal rain pattern, with one strong dry period from mid-June to mid-August and a less distinct dry period in January and February (Nsabimana et al. 2009). Mean daytime and 24 h air temperatures during 2013-2017 were 21.1 and 19.5 °C, respectively. The difference in mean temperature between the warmest and coldest month was 1.5 °C. Mean daytime vapor pressure deficit of the air (VPD) was 1.02 kPa, mean annual rainfall 979 mm, and mean daytime photosynthetic photon flux density (PPFD) 733  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the same period. At a meteorological station located at 2465 m altitude in Nyungwe, mean daytime and 24 h air temperatures were 15.6 and 14.3 °C, respectively, mean daytime VPD 0.39 kPa, mean annual rainfall 1879 mm, and mean daytime PPFD 633  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during 2007–2013. More

information on meteorological conditions at both Arboretum and Nyungwe were provided by Mujawamariya et al. (2018).

In addition, a weather station (VP-3, PYR and ECRN-100, Decagon Device, Inc, Pullman, WA, USA) recorded air temperature, relative humidity, solar radiation and precipitation at another open place by the Arboretum office during the last quarter of the study. These data were used for comparisons with leaf temperature measurements (see below).

Seven Tiny Tags (Model TinyTag Plus 2, Gemini data loggers Ltd., United Kingdom) recorded air temperature and relative humidity at 1.8 m in plots with different radiation regimes (six plots with different overstorey canopy transmission plus one open plot; see below) during 15 successive days in March 2016. To estimate relative radiation transmission of the overstorey in each plot, below-canopy radiation at the position of each seedling was measured with a portable quantum meter (MQ-500, Apogee Instruments, Inc., UT, USA) during 12:00-14:00 h on two sunny days, one in the wet and one in the dry season.

## 2.2. Plant materials and experimental design

The six montane tree species used in this species represent common ST and SI species in Nyungwe, as judged from their abundance in forest stands at different successional stages in the forest (Nyirambangutse et al. 2017 and references therein). The ST species are all among the 20 most common species in Nyungwe, together comprising 25% of the large trees (diameter at breast height > 30 cm) in the forest. The ST species, which are more abundant in late-successional stands, were: *Carapa grandiflora* (*Cg*; family: Meliaceae; altitudinal range in Rwanda: 1600-2500 m), *Entandrophragma excelsum* (*Ee*; family: Meliaceae; altitudinal range in Rwanda: 1500-2100 m) and *Syzygium guineense spp parvifolium* (*Sg*, family: Myrtaceae; altitudinal range in Rwanda: 1000-2100 m). The SI species, which are more abundant in early-successional stands, were: *Croton megalocarpus* (*Cm*; family: Euphorbiaceae; altitudinal range in Rwanda: 1600-2400 m), *Dombeya goetzenii* (*Dg*, family: Malvaceae; altitudinal range in Rwanda: 1300-3000 m) and *Polyscias fulva* (*Pf*; family: Araliaceae; altitudinal range in Rwanda: 1200-2400 m). Species' altitudinal range were taken from Bloech et al. (2009) or Fischer and Killmann (2008). Since shade tolerance varies along a continuum, classification of species into distinct successional groups is not straightforward. While *Cm* and *Dg* are typical short-lived

early-successional species, trees of *Pf* can grow quite large and may stay around longer after canopy closure. Although all three ST species are more abundant in late-successional stands, *Sg* is also quite common in early-successional stand. We therefore did not apply successional group identity (ST versus SI) in the statistical analysis (see *Statistical analyses* section below).

The plants, grown from seeds collected in Nyungwe, were first cultivated for 3.5 months at the Arboretum's nursery, in small pots which were irrigated daily and contained <1 liter clayey soil from the surrounding and added organic fertilizer. In March-April 2015 they were transplanted into 10 liters pots with local clayey soil. Plants were randomly distributed to nine different plots in the Arboretum, differing in overstorey leaf area index and canopy light transmittance (Table 1). These nine plots were divided into three radiation regimes: three open plots, three plots with the overstorey consisting of rather sparse canopies of early-successional species (*Dg*, *Cm* and *Prunus africana*), and three plots with the overstorey consisting of dense canopies of later-successional species (*Cg*, *Sg* and *Magnistipula butayei*). Each species had six replicate seedlings in each plot and the total number of seedlings in the experiment was thus 324 (3 radiation regimes x 3 plots in each regime x 6 species x 6 replicates).

Pots were placed 0.7 m apart to minimize shading among seedlings. Plants were regularly irrigated to assure high soil water availability throughout the study. During the three-month dry season, seedlings in open plots were watered daily while those in sparse and dense canopy plots were watered three times per week. During the rest of the year, plants were watered regularly and as needed depending on rainfall. No fertilizer was added during the shade tolerance experiment. This may have caused increased nutrient constraints in fast-growing plants in open and sparse canopy plots. However, such constraints were probably not unrealistically large since our findings of decreased mass-based but increased area-based leaf nitrogen (N) content of plants in high radiation regimes (see below) is the typical observation also in freely rooted plants (Poorter et al. 2019).

Monthly health inspections were conducted from May 2015 to April 2016. In total, 21 out of the 324 seedlings died during the experiment. All plants were harvested in May 2016.

### *2.3. Gas exchange measurements and calculations*

Leaf gas exchange was measured in three plants per species in each plot during February-April 2016, using a portable LI-6400 leaf gas exchange instrument (LI-COR, Lincoln, NE, USA). Two leaves per plant were measured: one for net photosynthesis ( $A_n$ ) and one for dark respiration ( $R_d$ ). The selected leaves were at least two months old, fully developed and had comparatively healthy appearance. The  $A_n$  measurements to determine photosynthetic capacity (i.e.  $V_{cmax}$ : maximum rate of Rubisco carboxylation, and  $J_{max}$ : maximum rate of photosynthetic electron transport) were done at a PPFD of  $1800 \mu\text{mol m}^{-2}\text{s}^{-1}$ , an air flow of  $400 \mu\text{mol s}^{-1}$ , and the block temperature of the instrument at  $25 \text{ }^\circ\text{C}$ . In two out of three plants per species and plot, measurements were done at three different  $\text{CO}_2$  concentrations of the air entering the leaf chamber:  $410$ ,  $200$  and  $2000 \mu\text{mol mol}^{-1}$ . For the third plant, photosynthesis was only measured at  $410 \mu\text{mol mol}^{-1}$ . Measurements were conducted within a few minutes after clamping onto the leaf or changing  $\text{CO}_2$  concentration, without waiting for stomatal conductance to respond to altered environmental conditions. Data of  $A_n$  in shaded plots (and the derived  $V_{cmax}$  and  $J_{max}$  values) are thus representative for photosynthesis during sunflecks and do not reflect values where both photosynthesis and stomata have fully responded to the high measurement radiation. The stomatal conductance data point reported is that measured at  $410 \mu\text{mol mol}^{-1} \text{CO}_2$  concentration. The photosynthesis model by Farquhar et al. (1980), with modifications of photosynthetic temperature dependencies by Bernacchi et al. (2001), was parameterized using the one-point method (De Kauwe et al. 2016). The measurements at  $200 \mu\text{mol mol}^{-1}$  and  $2000 \mu\text{mol mol}^{-1} \text{CO}_2$  concentrations were used to determine  $V_{cmax}$  and  $J_{max}$ , respectively, and tree-specific  $R_d$  data described below (i.e. dark respiration) were used in both cases. The measurements at  $410 \mu\text{mol mol}^{-1} \text{CO}_2$  concentration were used only for determination of light-saturated  $A_n$ , not for  $V_{cmax}$  or  $J_{max}$ .

On the same leaf measured for  $A_n$  at different  $\text{CO}_2$  concentrations, the initial light response of  $A_n$  was also measured at PPFD of  $25$  and  $75 \mu\text{mol m}^{-2}\text{s}^{-1}$ , the  $\text{CO}_2$  concentration at  $410 \mu\text{mol mol}^{-1}$  and the block temperature of the instrument at  $25 \text{ }^\circ\text{C}$ . These data were originally planned to be used for the determination of apparent QY and LCP. However, subsequent tests with measurements at  $25$ ,  $50$  and  $75 \mu\text{mol m}^{-2}\text{s}^{-1}$  showed that the light response was not linear in the entire PPFD range of  $25$ - $75 \mu\text{mol m}^{-2}\text{s}^{-1}$ . We therefore do not report any QY results, while LCP was instead calculated by linear interpolation between the  $A_n$  measurement at a PPFD of  $25 \mu\text{mol m}^{-2}\text{s}^{-1}$  and  $R_d$  measured on a neighboring leaf on the same plant (see below). The method does

not account for the Kok effect (Kok et al. 1948), which is difficult to determine for large numbers of plants in the field since it requires several data points around the LCP. Although not ideal, this use of two data points from two neighboring leaves to derive LCP is not likely to cause significant systematic bias with respect to species or radiation treatments.

Before  $R_d$  measurements, leaves were dark acclimated by covering them with tinfoil for at least 30 minutes. This measurement was done with an air flow of  $250 \mu\text{mol s}^{-1}$ ,  $\text{CO}_2$  concentration of  $410 \mu\text{mol m}^{-2} \text{s}^{-1}$  and leaf chamber block temperature of at  $25 \text{ }^\circ\text{C}$ . After the measurement of each plant, an empty chamber measurement was done to allow for corrections of possible leaks and gasket diffusion.

#### 2.4. Other leaf trait measurements

Leaf temperature was measured on all plants in all plots at 12:00-15:00 h on three sunny days, using three infrared thermometers (Model: BP 10, TROTEC, Germany). Three people measured simultaneously, one in each radiation regime (OP, SP, DP) and then rotating such that each person measured one plot of each regime. The infrared thermometer had a 6:1 ratio of the distance to the surface compared to the diameter of the surface area being measured. It was held about 6 cm from the leaf surface and pointed towards the leaf without shading it, aiming at the central position on one of the leaf halves for large leaves and at the middle for small leaves. The leaf was horizontally positioned ( $= 0^\circ$ ) and the angle of the infrared thermometer was held at  $45^\circ$  relative to the leaf.

On all leaves measured for gas exchange, leaf discs were sampled (three discs with 18 mm diameter for large leaves or five discs with 10 mm diameter for smaller leaves) for LMA determination and subsequent nutrient and stable isotope analyses. Leaf samples were oven dried at  $70 \text{ }^\circ\text{C}$  for at least 72 h. After determining LMA, leaf discs and remaining leaf material was ground into a fine powder with a ball mill grinder (MM 301). In total, 160 samples were sent for analyses of leaf N and stable carbon isotope composition using a continuous flow isotope ratio mass spectrometer (UC Davis Stable Isotope Facility, CA, USA). Carbon isotopes were used to estimate plant intrinsic WUE (iWUE). Analysis of 37 essential non-N elements was done for one combined sample per species (except *Dg* where there was too little leaf sample material) and plot, using inductively coupled plasma mass spectrometry (ACME Analytical Laboratories,

Canada). Out of these non-N elements, only data for phosphorous are reported. The iWUE determined based on carbon isotope data was calculated as the difference between the ambient CO<sub>2</sub> concentration and intracellular CO<sub>2</sub> concentration times 1.6 to account for the difference in stomatal conductance of water vapor compared to CO<sub>2</sub>. Ambient CO<sub>2</sub> was assumed to be 402 μmol mol<sup>-1</sup> and intercellular CO<sub>2</sub> was calculated using equations in Farquhar et al. (1989).

### *2.5. Biomass harvest*

In the harvest at the end of experiment, we first measured stem diameter and height and counted the number of attached leaves in each living plant. Leaf angles of five leaves per plant at randomly selected canopy positions were also measured and leaf discs were taken from the same leaves for LMA determination and chemical analyses. A SPAD-502 meter (Konica Minolta Sensing, Inc., Ltd.: Osaka, Japan) was used to measure the SPAD value (a proxy of area-based leaf chlorophyll content) of one fully developed, healthy and at least two months old leaf per plant. Stem base diameter and height were measured using a ruler and digital caliper, respectively. The seedlings were then harvested and divided into roots, main stem, branches, petioles and leaves. Plant parts were oven-dried at 70 °C until constant mass. Wood density of dry wood was determined based on measurements of mass and volume on a piece of a dry stem without bark from each plant.

In addition, eight plants per species which were not included in the main experiment were harvested in April 2015 to determine the initial biomass of different plant parts at the beginning of the experiment. The mean total initial dry mass ranged from 5 g to 14 g in the six species (Supplementary material, Table S1).

### *2.6. Statistical analyses*

Data were statistically analyzed using analysis of variance (ANOVA) with radiation regime as a fixed factor and plot and species as random factors. The linear model of the experiment was the following:

$$y = \mu + R_i + S_j + P(R)_{k(i)} + RS_{ij} + SP(R)_{jk(i)} + e_{l(ijk)}$$

where  $y$  is the response variable,  $\mu$  is the intercept,  $R$  is radiation regime,  $S$  is species identity,  $P$  is plot,  $e$  is individual random variation, and the subscripts indicate the levels of each factor. Plots were nested within radiation regimes and plant replication for each species in a plot was six. With the experimental design used, F-ratios for the effects of  $S_j$ ,  $P(R)_{k(i)}$  and  $RS_{ij}$  were calculated using  $SP(R)_{jk(i)}$  as the error term, while the F-ratio for  $SP(R)_{jk(i)}$  was calculated using  $e_{l(ijk)}$  as the error term (Underwood 1997). Significant species by radiation interactions were found for most variables and main effects of radiation were tested by simple 1-way ANOVA within each species individually. Effects were considered statistically significant at  $P \leq 0.05$ .

### 3. Results

#### 3.1. Environmental conditions

Daytime temperatures at 1.8 m above ground were 2 - 3 °C lower in both sparse and dense canopy plots compared to open plots (Table 1). Nighttime temperatures were on average 1.1 - 1.3 °C higher in dense canopy plots compared to open or sparse canopy plots. Nighttime temperature was lowest in two of the sparse canopy plots as a result of their lower position in the Arboretum area, just at the end of a downhill slope. On average across two measurement days, dense canopy plots had 10% midday canopy transmission while sparse canopy plots had 30%. Daytime measurements made during the dry season in August showed marked differences in canopy transmission between all sparse versus dense canopy plots. In the wetter season in January, however, one of the sparse canopy plots (with *D. goetzenii* as overstorey species) had canopy transmission comparable to that in dense canopy plots. This difference between August and January was a result of high seasonality in both leaf shedding and leaf production in *D. goetzenii*. On average across the two measurement days, however, the *D. goetzenii* sparse canopy plot had higher canopy transmission than those in all three dense canopy plots.



**Table 1.** Comparison of environmental conditions of the nine plots of the study. Temperature values are averages over 15 days with simultaneous measurements in all plots. Data on PPFD were recorded between 12:00 and 14:00 h on two sunny days, one in August (dry season) and one in January (wet season). Light transmission is the plot radiation divided by the open sky radiation at a large open area 50 m outside the Arboretum of Ruhande.

Treatment	Plot**	$T_{\text{air}}$ day (°C)	$T_{\text{air}}$ night (°C)	PPFD, dry season, mid- day ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	PPFD, wet season, mid- day ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	Light transmission (%), on dry / wet season days
Open field	Open 1	24.2*	16.1*	1970	2059	100/98
	Open 2	24.2*	16.1*	1806	2044	95/97
	Open 3	24.2*	16.1*	1990	1979	98/95
Sparse canopy	<i>Cm</i>	21.2	18.0	853	789	42/38
	<i>Dg</i>	21.3	15.5	670	138	31/7
	<i>Pa</i>	22.1	15.2	932	409	43/19
Dense canopy	<i>Cg</i>	21.6	17.8	258	179	13/9
	<i>Mb</i>	21.6	17.7	216	278	11/13
	<i>Sg</i>	21.4	16.6	233	117	11/6

\* Data from the same weather sensor.

\*\* Open 1, 2 and 3 are the open plots; *Cm*, *Dg*, and *Pa* are the sparse canopy plots with overstorey consisting of *Croton megalocarpus*, *Dombeya goetzenii* and *Prunus africana*; *Cg*, *Mb*, and *Sg* are the dense canopy plots with overstorey consisting of *Carapa grandiflora*, *Magnistipula butayei* and *Syzygium guineense*.

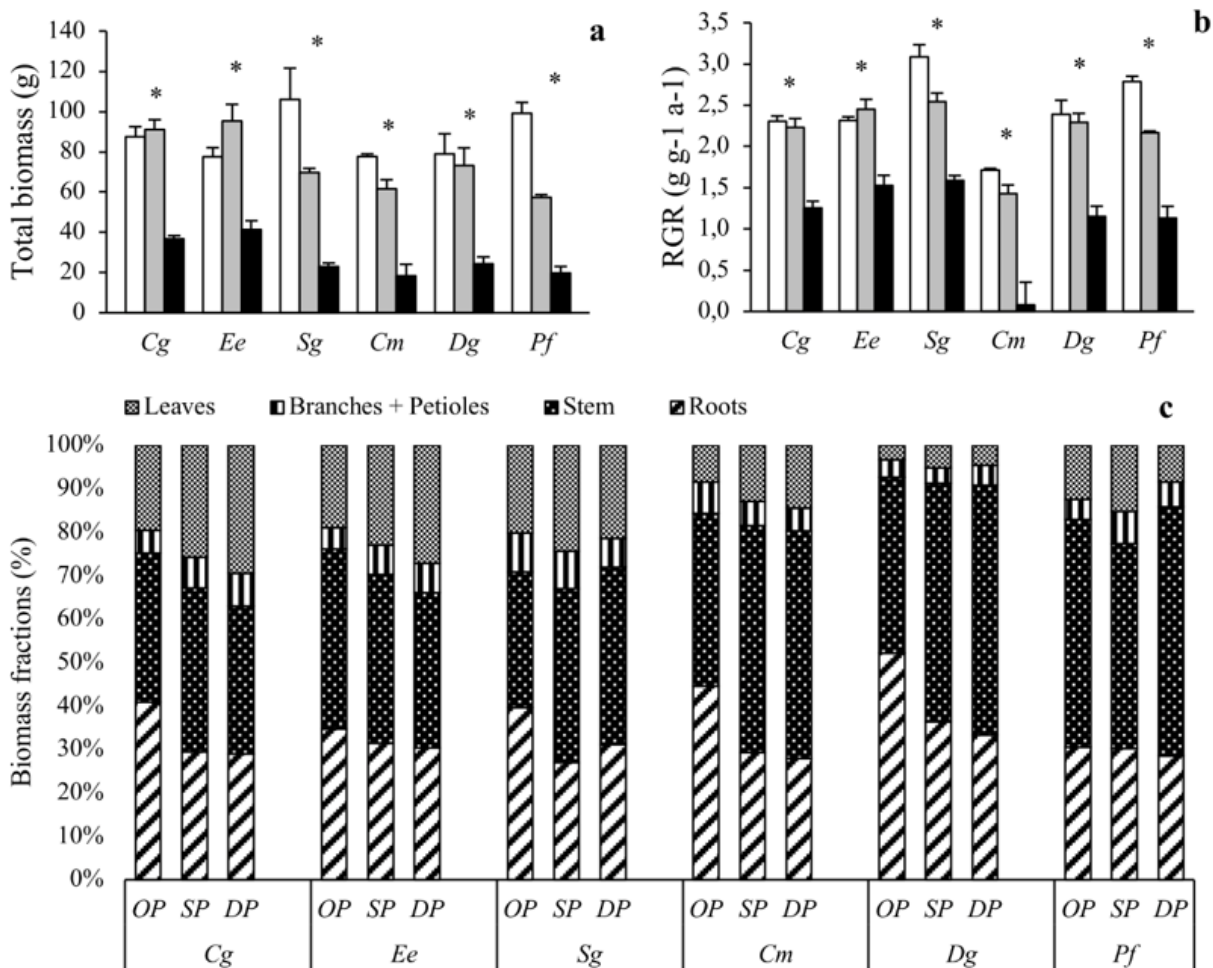
### 3.2. Biomass, allocation and health inspections

All tree biomass and structure parameters exhibited significant (i.e.  $P \leq 0.05$ ) or, in two cases, near significant ( $0.050 < P \leq 0.068$ ) species by radiation interactions (Table 2), meaning that effects of shade were species dependent. Radiation regime had a significant influence on total tree biomass at harvest (Fig. 1a) and RGR (Fig. 1b) in all species. However, the reduction in RGR in dense canopy compared to open plots was smallest in the ST species *Ee* (34%) and greatest in the SI species *Cm* (95%). In the other four species, the RGR reductions were

intermediate but somewhat larger in the SI species *Pf* (59%) and *Dg* (52%) than in the ST species *Sg* (49%) and *Cg* (46%).

The ST species all exhibited lower stem biomass fractions but higher fractions of leaves and branches plus petioles compared to the SI species (Fig. 1c). The ST species *Cg* and *Ee* responded to shading by increasing the fractions of leaves, branches and petioles; a strategy not observed in the other species. The SI species plus *Sg* instead responded to shading by increasing their stem biomass fractions. Both ST and SI species had similar fractional investments into roots and all except *Ee* and *Pf* responded to shading by decreasing this fraction.

Monthly health inspections showed that some seedlings suffered from different kinds of health problems at some point during the experiment, including insect infection (4 plants) and herbivory (8 plants), partial dehydration (22 plants) and mammal herbivory (41 plants). Insect infection or herbivory caused no significant reduction in total biomass at harvest. Partially dehydrated plants either recovered well or subsequently died and were excluded from the biomass analysis. Seedlings affected by mammal herbivory had 21% lower total biomass at the final harvest and these were therefore removed from the biomass analysis. A total number of 21 out of 324 trees died throughout the experiment. Twelve of these trees were in the dense canopy plots and 14 belonged to SI species (i.e. *Cm*, *Dg*, *Pf*), but differences among species or treatments were not statistically significant.



**Fig. 1.** (a) Total biomass (g), (b) relative growth rate (RGR) and (c) biomass allocation (%) of *Carapa grandiflora* (Cg), *Entandrophragma excelsum* (Ee), *Syzygium guineense* (Sg), *Croton megalocarpus* (Cm), *Dombeya goetzenii* (Dg) and *Polyscias fulva* (Pf) planted in open (white in a-b; OP in c), sparse canopy (gray in a-b; SP in c) and dense canopy (black in a-b; DP in c) plots. Species to the left (Cg, Ee) are shade tolerant and species to the right (Cm, Dg, Pf) shade intolerant; Sg is intermediate in this study. Leaf biomass data represent leaves attached at the time of harvest. The error bars represent standard errors ( $n = 3$ ). The symbol \* in (a) and (b) indicates significant variation among radiation regimes within a species. Overall statistics results are provided in Table 2.

**Table 2.** *P* values for effects on different plant traits according to the ANOVA. Brackets indicate that the preceding factor is nested inside the factor within brackets; x indicate interacting effects of the preceding and following factors.

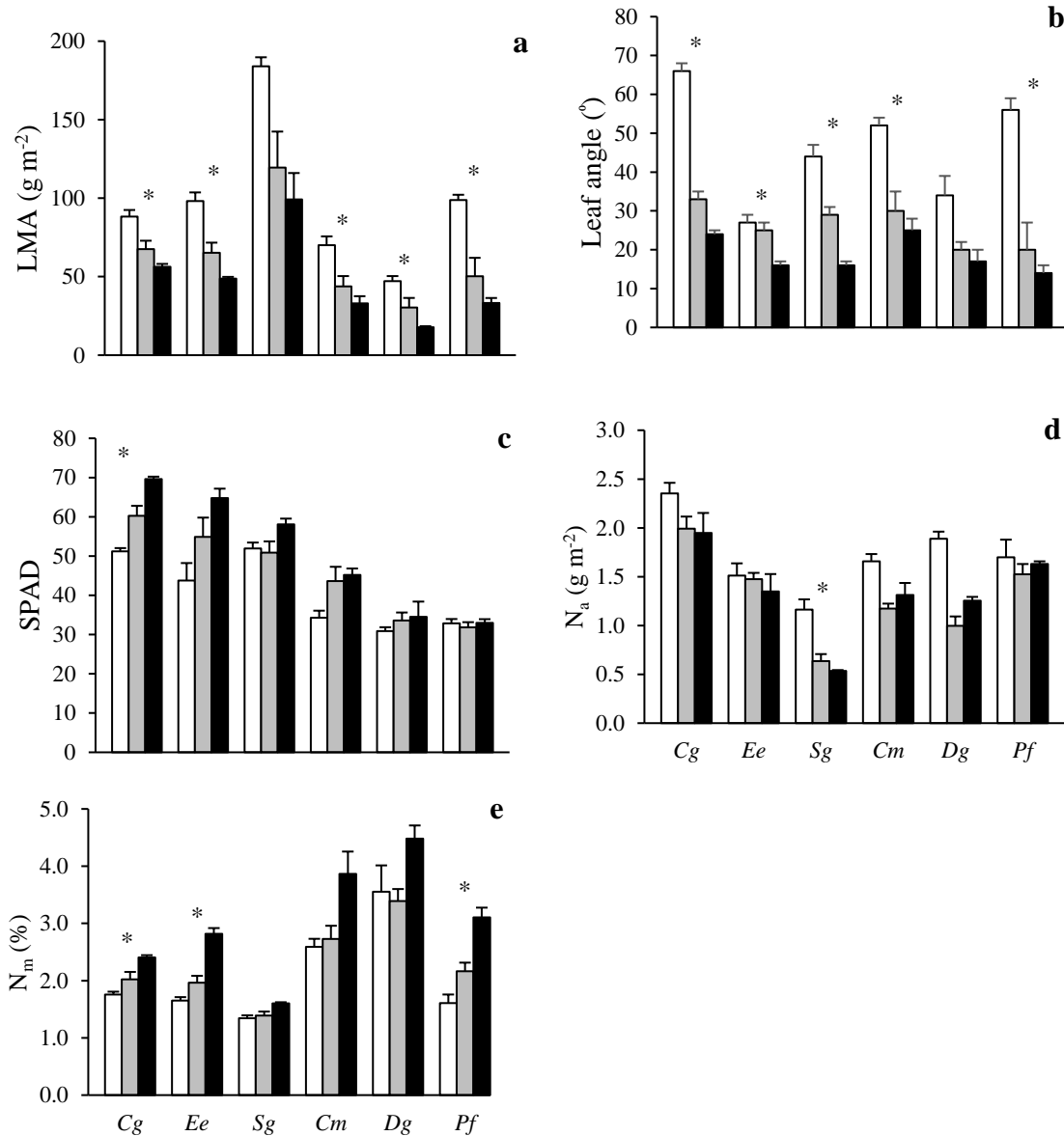
Plant trait	P values for different sources of variation			
	Species	Plot (Radiation)	Species x Radiation	Species x Plot (Radiation)
<i>Tree size and structure</i>				
Total	<b>0.001</b>	0.199	<b>0.008</b>	<b>0.0001</b>
Leaves	<b>&lt;.0001</b>	<b>0.008</b>	<b>0.002</b>	<b>0.001</b>
Branches + Petiols	<b>&lt;.001</b>	0.232	<b>0.002</b>	0.253
Stem	<b>0.268</b>	0.489	<b>0.006</b>	<b>0.001</b>
Roots	<b>0.023</b>	<b>0.005</b>	<b>0.015</b>	<b>&lt;.0001</b>
RGR	<b>&lt;.0001</b>	0.183	0.052	<b>&lt;.0001</b>
% Leaves	<b>&lt;.0001</b>	<b>0.004</b>	0.068	<b>&lt;.0001</b>
% Branches + Petiols	0.076	<b>0.014</b>	<b>0.029</b>	0.127
% Stem	<b>&lt;.0001</b>	0.355	<b>0.003</b>	<b>&lt;.0001</b>
% Roots	<b>0.002</b>	<b>0.0001</b>	<b>0.007</b>	<b>0.029</b>
Root:Shoot ratio	<b>0.002</b>	<b>0.001</b>	<b>0.012</b>	<b>0.014</b>
Stem diameter	<b>&lt;.0001</b>	0.158	<b>0.030</b>	<b>0.014</b>
Stem height	<b>&lt;.0001</b>	0.087	<b>0.020</b>	<b>0.0002</b>
Stem height:diameter ratio	<b>&lt;.0001</b>	0.084	<b>0.048</b>	<b>&lt;.0001</b>
Height increment	<b>&lt;.0001</b>	<b>0.036</b>	<b>0.001</b>	<b>0.001</b>
Canopy leaf number	<b>&lt;.001</b>	<b>0.006</b>	<b>0.0001</b>	<b>0.002</b>
Wood density	<b>&lt;.0001</b>	0.251	<b>0.003</b>	0.351
<i>Leaf structure</i>				
LMA	<b>&lt;.001</b>	<b>&lt;.001</b>	<b>&lt;.001</b>	<b>0.007</b>
Leaf mass	<b>&lt;.001</b>	0.140	<b>0.0004</b>	0.174
Leaf area	<b>&lt;.001</b>	<b>0.0004</b>	<b>0.0002</b>	<b>0.006</b>
Leaf length	<b>&lt;.001</b>	<b>0.032</b>	0.176	0.082
Leaf width	<b>&lt;.001</b>	0.317	<b>0.003</b>	0.447
Leaf thickness	<b>&lt;.001</b>	<b>&lt;.001</b>	0.503	0.599
Leaf angle	<b>&lt;.001</b>	0.063	<b>0.002</b>	<b>0.050</b>
<i>Leaf physiology</i>				
$A_n$ , light-saturated	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>0.002</b>	0.872
$V_{cmax}$	<b>0.006</b>	0.086	<b>0.006</b>	0.720
$J_{max}$	0.198	0.616	0.075	<b>0.037</b>
$J_{max} \cdot V_{cmax}$ ratio	<b>0.004</b>	<b>0.010</b>	0.262	0.165
$R_d$ , area-based	0.400	<b>0.003</b>	0.795	0.115
$R_d$ , mass-based	<b>&lt;.0001</b>	<b>0.006</b>	0.750	0.142
LCP	0.593	<b>0.041</b>	0.704	0.374
$g_s$	<b>&lt;.0001</b>	<b>&lt;.0001</b>	0.115	0.490
iWUE	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>0.015</b>	0.082
<i>Leaf chemistry</i>				
$N_a$	<b>&lt;.001</b>	0.784	0.332	<b>0.022</b>
$N_m$	<b>&lt;.001</b>	0.090	0.551	<b>0.005</b>
$P_a$	<b>0.0002</b>	0.163	0.070	<b>&lt;.001</b>
$P_m$	<b>0.0003</b>	0.101	<b>0.040</b>	<b>&lt;.001</b>
SPAD	<b>&lt;.001</b>	<b>0.002</b>	<b>0.005</b>	0.105

\* Figures in bold indicate significant P values.

### 3.3. Leaf structural, chemical and physiological traits

Values of LMA were highest in *Sg*, lowest in *Cm* and *Dg*, and quite similar in the remaining three species (Fig. 2a). It declined with shading in all species. Mean leaf angles (horizontal = 0 °) were below or about 30 ° in all plants growing beneath sparse or dense canopies (Fig. 2b). They were considerably steeper in open plots in all species and the highest values were found for *Cg* (66 °) followed by *Pf* (56 °) and *Cm* (52 °). Neither mean leaf angles nor their sun-shade plasticity differed in a systematic way between ST and SI species. SPAD values were higher in the ST compared to the SI species (Fig. 2c). Moreover, there was a significant species by radiation interaction on SPAD (Table 2), with all species except the SI species *Dg* and *Pf* exhibiting increases in SPAD under low radiation conditions. The ST species thus had both intrinsically high chlorophyll content and an ability to increase these levels further upon shading.

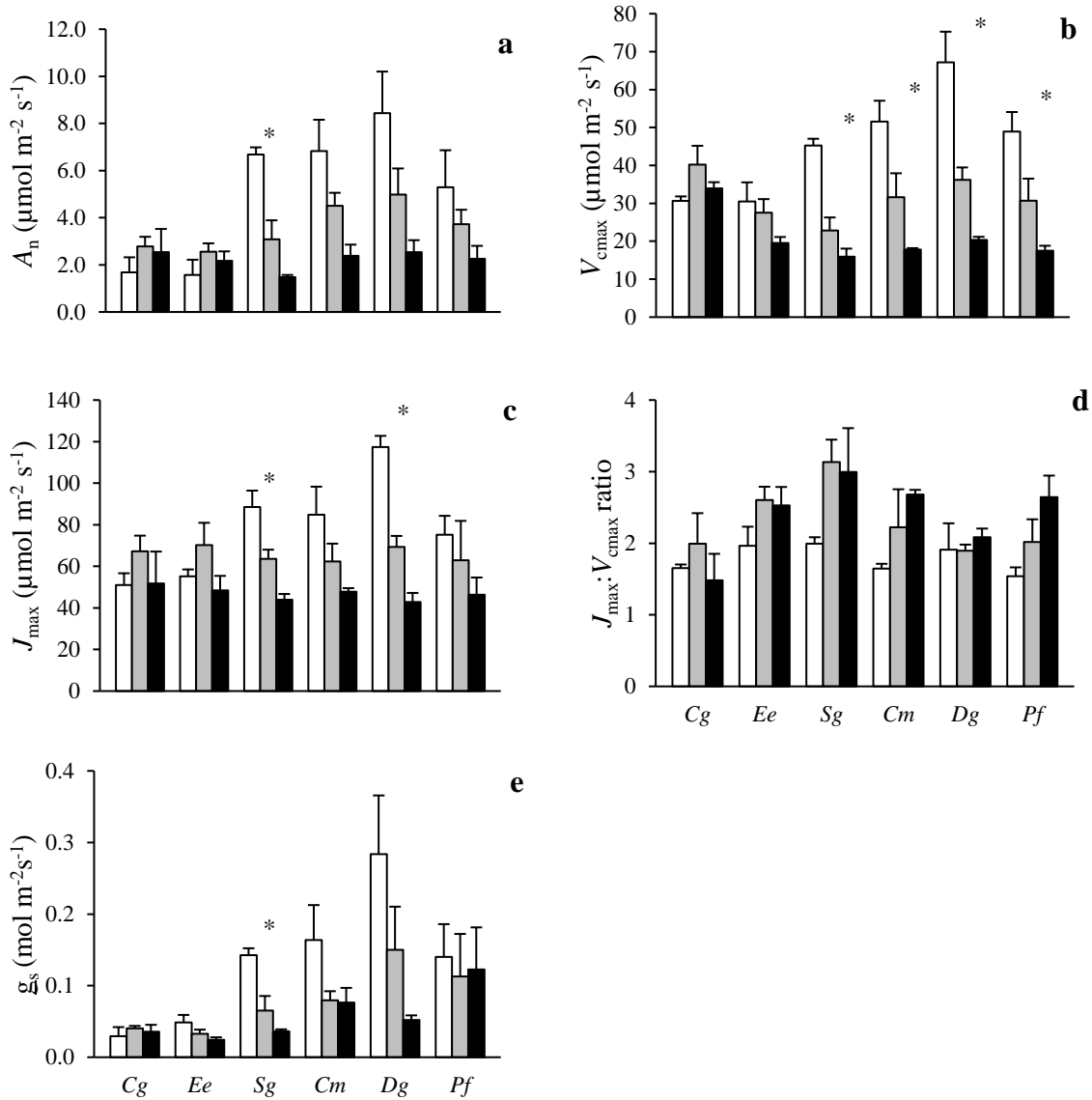
Mass-based leaf N concentration was generally higher under shading while the opposite was sometimes true for area-based leaf N content (Fig. 2d-e). There were no species by radiation interaction on area- or mass-based leaf N (Table 2). Area-based leaf N was weakly positively correlated with both photosynthetic capacity parameters:  $V_{\text{cmax}}$  and  $J_{\text{max}}$ . For leaf P, mass-based concentration was highest in *Ee* trees growing in sparse canopy plots and area-based P content was generally lowest in dense canopy plots. Species by radiation interactions had *P* values of 0.040 and 0.070 for mass- and area-based leaf P, respectively (Table 2).



**Fig. 2.** The (a) leaf mass per unit leaf area (LMA), (b) leaf angle, (c) SPAD, (d) nitrogen content per unit leaf area ( $N_a$ ) and (e) nitrogen content per unit leaf mass ( $N_m$ ) of six tropical tree species grown in open (white), sparse canopy (gray) and dense canopy (black) plots. Species to the left (Cg, Ee, Sg) are shade tolerant and species to the right (Cm, Dg, Pf) shade intolerant. The error bars represent standard errors ( $n = 3$ ). The symbol \* indicates significant variation among radiation regimes within a species. Species: *Carapa grandiflora* (Cg), *Entandrophragma excelsum* (Ee), *Syzygium guineense* (Sg), *Croton megalocarpus* (Cm), *Dombeya goetzenii* (Dg) and *Polyscias fulva* (Pf).

In open plots, light-saturated  $A_n$ ,  $V_{cmax}$  and  $J_{max}$  were higher in the three SI species (i.e. Cm, Dg, Pf) and Sg than in Cg and Ee (Fig. 3a-c). In dense canopy plots, however, values were mostly

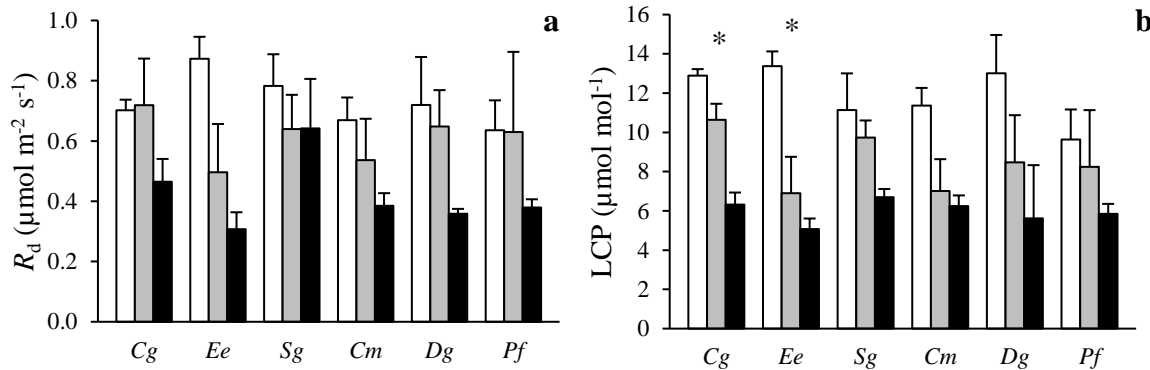
similar among species. In the ST species *Cg* and *Ee*, photosynthetic parameters were mostly equal in the three radiation regimes while in the other species they decreased with shading. These interspecific differences in shading responses resulted in significant ( $A_n$ ,  $V_{cmax}$ ) or nearly significant ( $J_{max}$ ,  $P = 0.075$ ) species by radiation interactions (Table 2). The  $J_{max}:V_{cmax}$  ratios also differed among species but not in a pattern consistent with species shade tolerance (Fig. 3d). It was generally higher under shading and there was no significant difference in radiation responses among species ( $P = 0.26$ ; Table 2). Stomatal conductance exhibited similar patterns as  $A_n$  except that SI species had higher values than ST species also in the dense canopy plots (Fig. 3e).



**Fig. 3.** The (a) light-saturated net CO<sub>2</sub> assimilation ( $A_n$ ), (b) maximum rates of photosynthetic carboxylation ( $V_{cmax}$ ) and (c) electron transport ( $J_{max}$ ), (d)  $J_{max}:V_{cmax}$  ratio, and (e) stomatal conductance ( $g_s$ ) of six tropical tree species grown in open (white), sparse canopy (gray) and dense canopy (black) plots. Species to the left (*Cg*, *Ee*) are shade tolerant and species to the right (*Cm*, *Dg*, *Pf*) shade intolerant; *Sg* is intermediate in this study. The error bars represent standard errors ( $n = 3$ ). The symbol \* indicates significant variation among radiation regimes within a species. Species: *Carapa grandiflora* (*Cg*), *Entandrophragma excelsum* (*Ee*), *Syzygium guineense* (*Sg*), *Croton megalocarpus* (*Cm*), *Dombeya goetzenii* (*Dg*) and *Polyscias fulva* (*Pf*).

Dark respiration did not vary much among species and was generally lower under shading (Fig. 4a). There was no significant species by radiation interaction on  $R_d$  ( $P = 0.80$ ; Table 2). The LCP was lower under shading (Fig. 4b). There was no overall significant species by radiation interaction for this trait ( $P = 0.70$ ) and LCP was 5-7  $\mu\text{mol mol}^{-1}$  for all species in dense canopy plots.

Intrinsic WUE was considerably higher in open plots and in dense canopy plots it was highest in the ST species *Cg* and *Ee*. There was a significant species by radiation interaction, reflecting a smaller shade-induced reduction in iWUE in ST compared to SI species ( $P = 0.015$ ; Table 2).



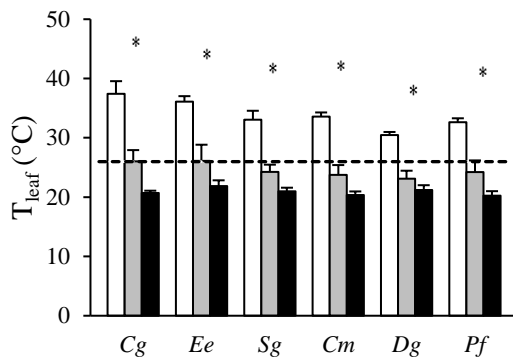
**Fig. 4.** The (a) leaf dark respiration ( $R_d$ ) and (b) photosynthetic light compensation point (LCP) of six tropical tree species grown in open (white), sparse canopy (gray) and dense canopy (black) plots. Species to the left (*Cg*, *Ee*) are shade tolerant and species to the right (*Cm*, *Dg*, *Pf*) shade intolerant; *Sg* is intermediate in this study. The error bars represent standard errors ( $n = 3$ ). The symbol \* indicates significant variation among radiation regimes within a species. Species: *Carapa grandiflora* (*Cg*), *Entandrophragma excelsum* (*Ee*), *Syzygium guineense* (*Sg*), *Croton megalocarpus* (*Cm*), *Dombeya goetzenii* (*Dg*) and *Polyscias fulva* (*Pf*).



### 3.4. Leaf temperature

Plant species in open plots experienced higher leaf temperatures ( $T_{\text{leaf}}$ ) compared to their counterparts under sparse and dense canopies (Fig. 5). In open plots,  $T_{\text{leaf}}$  was always considerably higher than the prevailing air temperature measured at the local Arboretum weather station. This exceedance was 10-12 °C in the ST species *Cg* and *Ee*, while it was 5-8 °C in the other species. In dense canopy plots,  $T_{\text{leaf}}$  was rather similar across species, around 5 °C below the air temperature recorded by the weather station. These low  $T_{\text{leaf}}$  values likely resulted from a combination of low radiation, lower daytime air temperature under dense canopies compared to open plots (Table 1) and transpiratory cooling.

The species with the highest  $T_{\text{leaf}}$  in open plots, *Cg* and *Ee*, also had the largest leaf sizes (60 - 64 cm<sup>2</sup> compared to 10 - 42 cm<sup>2</sup> for the other species; Appendix, Table 1) and the lowest stomatal conductance (Fig. 3e). Their large air temperature exceedances can thus be explained by a combination of poor heat dissipation (i.e. low leaf boundary layer conductance) and low transpiratory cooling.



**Fig. 5.** Leaf temperature ( $T_{\text{leaf}}$ ) of six tropical tree species grown in open (white), sparse canopy (gray) and dense canopy (black) plots. The dashed line indicates the Arboretum weather station air temperature (25.9 °C) during the period of the leaf temperature measurements. Species to the left (*Cg*, *Ee*) are shade tolerant and species to the right (*Cm*, *Dg*, *Pf*) shade intolerant; *Sg* is intermediate in this study. The error bars represent standard errors ( $n = 3$ ). The symbol \* indicates significant variation among radiation regimes within a species. Species: *Carapa grandiflora* (*Cg*), *Entandrophragma excelsum* (*Ee*), *Syzygium guineense* (*Sg*), *Croton megalocarpus* (*Cm*), *Dombeya goetzenii* (*Dg*) and *Polyscias fulva* (*Pf*).

## 4. Discussion

The overall aim of this study—to explore which traits that were most important in controlling species shade tolerance in tropical montane trees—could be fulfilled by taking a controlled experimental approach in which we investigated a broad range of structural, chemical and physiological traits in species with different successional strategies grown under three different radiation regimes. The dense canopy plots transmitted more radiation (Table 1; 10% at noon, corresponding to about 7-8% for the daylight period if assuming 75% direct radiation and 25% diffuse radiation and spherical leaf angle distribution) than multi-layered canopies of many lowland rainforests (1-2%, Montgomery and Chazdon, 2001). However, this radiation regime is representative for many late-successional stands of the highly topographical and steeply sloped montane forest from which our species originate and obviously dense enough to induce strong structural (Fig 1) and physiological (Fig 3-5) responses to shading. Our results suggest that interspecific variation in shade tolerance of tropical montane trees is controlled by species differences in whole-plant biomass allocation strategy (Fig. 1) rather than by difference in physiological leaf traits determining leaf carbon balance at low radiation (Fig. 4). In the shade, two of the ST species (*Cg* and *Ee*) had high fractional biomass investments in laterally light-intercepting organs (i.e. leaves, petioles and branches) and comparatively small RGR reductions below dense canopies (Fig. 1). At the other end of the shade tolerance spectrum, the SI species (*Cm*, *Dg* and *Pf*) had high fractional investments in stems and larger growth reductions when shaded. The sixth species (*Sg*), which is most common in late-successional stands but also abundant in early-successional stands in Nyungwe (Nyirambangutse et al. 2017), was intermediate with respect to both RGR reductions and biomass allocation. Species with contrasting shade tolerance did not differ in leaf physiological traits controlling leaf carbon balance at low radiation (i.e.  $R_d$  and LCP; Fig. 4).

### 4.1. Growth and biomass allocation

Biomass allocation results were in line with our first hypothesis and provided partial support for both the carbon gain and stress tolerance hypotheses. The ST species invested relatively more into plant organs maximizing light interception (i.e. leaves and branches) than SI species (Fig. 1c). In the two typical ST species (*Cg* and *Ee*), this difference was strongest under low radiation,

showing that it is the result of both species adaptations and acclimation responses. The allocation strategy of these two ST species was successful with respect to total growth, as judged by the smaller growth declines under low radiation in ST compared to SI species (Fig. 1a-b). Leaf morphology, however, did not vary in agreement with the carbon gain hypothesis since ST species had higher LMA than SI species in the shade (Fig. 2a), in line with several previous studies on tropical tree species (Coste et al. 2005, Kitajima 1994, Mao et al. 2014). A successful ST species thus seem to need both a whole-plant architecture that is favorable for light interception (in agreement with the carbon gain hypothesis) and leaves that are strong enough to endure biotic and abiotic stress in the understorey (in agreement with the stress tolerance hypothesis; Valladares and Niinemets 2008, Valladares et al. 2016). Even though high LMA does not maximize short-term light interception and carbon gain, it might increase the carbon gain over the entire leaf lifespan thanks to better physical protection against herbivores and mechanical stresses (Kitajima and Poorter 2010, Mao et al. 2014) and longer leaf lifespan (Coste et al. 2011, Gommers et al. 2013, Reich 2014).

Shade intolerant species grown under dense canopies had high investments in stem biomass and exhibited low RGR (Fig. 1). This represents a strategy to try to escape from the shade of neighbors by rapid vertical growth (Grubb 2015, Montgomery 2004, Poorter et al. 2018). However, unless stem elongation results in increased light interception this strategy will not be successful in the long run (Henry and Aarssen 1997, Valladares et al. 2016). In this one-year study, SI seedlings prioritizing stem growth were not awarded by reaching higher radiation since overstorey canopies were several meters above. There was an indication that the shade intolerant growth strategy was linked to increased mortality since seedlings that died were predominantly SI species growing below dense canopies. However, differences in mortality rates between species or radiation regimes were not statistically significant and the total number of trees that died during the experiment was rather low (21 out of 324 trees).

#### *4.2. Leaf physiology*

Contrary to our second hypothesis, seedlings of ST and SI species did not significantly differ in  $R_d$  or LCP, two key physiological traits controlling leaf carbon balance at low radiation (Fig. 4). However, light-saturated  $A_n$ , photosynthetic capacity (i.e.  $V_{cmax}$  and  $J_{max}$ ) and stomatal conductance were generally lower in ST compared to SI species grown in open plots (Fig. 3).

These results indicate that light-use efficiency under low radiation is not higher in ST compared to SI species while carbon gain in open plots is clearly superior in SI species. The higher leaf-level iWUE in the ST species *Cg* and *Ee* in dense canopy plots (Fig 5) is not necessarily indicating better drought tolerance since these species also had relatively more transpiring leaf biomass and less root biomass compared to the other species (Fig. 1c).

Both  $R_d$  and LCP were markedly lower in dense canopy plots, demonstrating typical sun-shade acclimation of leaf physiology (Fig. 4). However, neither the degree of radiation acclimation nor the magnitude of  $R_d$  or LCP in dense canopy plots differed among species, indicating that these traits are poor predictors of species shade tolerance in tropical montane forests. This study therefore gives little support to the carbon gain hypothesis at the leaf physiological level. A previous study with tropical seedlings indicated leaf  $R_d$  to be the best predictor of shade tolerance; better than photosynthetic capacity or leaf N content (Baltzer and Thomas 2007a). However, that study as well as many previous investigations (Valladares et al. 2008, Coste et al. 2010, Cheng et al. 2013, Grubb 2015) have measured leaf gas exchange of understory plants where they naturally grow, with the obvious risk of confounding effects of acclimation and adaptation. The design of the present study allowed for the separation of acclimation and adaptation. Additional experimental studies are needed to determine if our results are valid across a broader range of species and conditions.

For  $R_d$  and LCP, acclimation to different radiation regimes was similar in ST and SI species (Fig. 4) while the responses of photosynthetic capacity (i.e.  $V_{\text{cmax}}$  and  $J_{\text{max}}$ ) to growth radiation differed markedly between the two groups (Fig. 3b-c). These findings thus only partially support previous studies on tropical trees which have indicated that sun–shade leaf plasticity is similar in ST and SI species (Rozendaal et al. 2006, Coste et al. 2009, 2010, Dusenge et al. 2015). They are rather in line with another study showing that ST species are more plastic in some traits while SI species are more plastic in others (Houter and Pons 2014). Our results indicate that interspecific variation in sun–shade leaf plasticity may markedly differ among physiological traits, but that shade tolerance is not the consequence of higher acclimation capacity of traits linked to leaf carbon balance at low radiation (i.e.  $R_d$  and LCP).

Photosynthetic capacity was lower in ST compared to SI species in open plots (Fig. 3) and SPAD values (a proxy for chlorophyll content; Fig. 2c) higher in ST species generally. This reflects a

shift from investments in structures and compounds maximizing photosynthetic capacity towards higher investments for light harvesting in ST compared to SI species (Anderson et al. 1995, Dusenge et al. 2015). However, the link between area-based leaf N content and photosynthetic capacity appears weak since the latter but not the former differed markedly between ST and SI species (Fig. 2 and 3). This result adds to a growing number of studies on tropical tree species challenging the view that leaf nutrient content is a good predictor of interspecific variation in photosynthesis (Bahar et al. 2016, Coste et al. 2005, Dusenge et al. 2015, Hasper et al. 2017, Houter and Pons 2014, van de Weg et al. 2012).

#### 4.3. Leaf temperature

The leaf temperatures under sunny conditions in open plots were highest in the ST species *Cg* and *Ee*, in line with our third hypothesis (Fig. 6). In these species and under these conditions, leaf temperatures were on average 36 - 38 °C. These values clearly exceed the biochemical optimal  $T_{\text{leaf}}$  for light-saturated  $A_n$  (at a common intercellular  $\text{CO}_2$  concentration of 272  $\mu\text{mol mol}^{-1}$ ) of these species, which are at 25 - 30 °C (Vårhammar et al. 2015). There were indeed indications that ST trees growing in open plots suffered in our experiment. The ST species *Cg* and *Ee* had markedly lower values of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  than SI species in open plots but not in lower radiation regimes (Fig. 3), which may indicate negative heat effects on photosynthetic enzymes and the electron transport chain (Sage and Kubien 2007).

It should be noted, however, that the standardized measurement of  $T_{\text{leaf}}$  (around noon in leaves held in horizontal position) may have caused some overestimation for leaves with steep leaf angles in open plots. The  $T_{\text{leaf}}$  of a horizontal leaf at noon may be comparable to that of a sun-facing leaf at 45 ° at 15:00, since 14:00-15:00 is the hottest hour of the day at the Arboretum and solar radiation is then still high at an angle perpendicular to the sun. However, *Cg* in open plots had a mean leaf angle of 66 ° and the  $T_{\text{leaf}}$  measured for this species in open plots is thus an overestimation for most leaves at their natural angles (but may occur in some individual leaves with angles at or below 45 or even 30 °, which also occurred).

The magnitude of leaf to air temperatures exceedance among species was linked to interspecific variation in leaf size and stomatal conductance, as also found in a previous common garden experiment with tropical ST tree seedlings (Vårhammar et al. 2015). Species with large leaf size

and low stomatal conductance have poor heat dissipation and low transpiratory cooling capacity, leading to high leaf temperatures. Large leaf size and low stomatal conductance are common traits of ST species (Valladares and Niinemets 2008) and may offer partial explanation of why ST species are more negatively affected by warming than SI species in controlled experiments (Doughty 2011, Cheesman and Winter 2013).

These results are in agreement with the emerging poly-tolerance concept which suggests that shade tolerance should be evaluated together with plant tolerance to other stressors, such as drought and waterlogging (Grubb 2015, Kunstler et al. 2016, Laanisto and Niinemets 2015, Valladares et al. 2016). Our study indicates that species growing in the understory are not only shade tolerant but may in fact also be sun intolerant at seedling stage due to negative effects of high leaf temperature on stomatal conductance and photosynthetic biochemistry.

## 5. Conclusions

Results from this study suggest that the large interspecific variation in shade tolerance among tropical montane trees is controlled by whole-plant biomass allocation strategy rather than by variation in physiological leaf traits determining leaf carbon balance at low radiation. Species with varying shade tolerance had distinctly different biomass allocation patterns (Fig. 1): the two distinct ST species (*Cg* and *Ee*) invested relatively more into plant organs maximizing light interception in the understorey (i.e. leaves and branches) while SI species invested more into stems. These differences in fractional biomass investments were more pronounced under dense canopies. This shows that ST and SI species in our tropical montane forest have two fundamentally different strategies to deal with low-light conditions: ST species maximize light-use efficiency in the shade while SI species try to escape the deep shade through vertical stem growth. Contrary to our expectations, however, ST and SI species did not differ in leaf  $R_d$  or LCP, i.e. traits controlling leaf carbon balance at low radiation (Fig. 4).

Our results highlight the importance of a whole-plant perspective for understanding tree shade tolerance. Based on leaf level physiological data only, we found little support for the carbon gain hypothesis (stating that shade tolerance is the consequence of high light use-efficiency resulting from traits maximizing carbon gain and minimizing carbon losses in a low-light environment). However, based on both leaf physiological and whole-plant structural data we can conclude that the carbon gain hypothesis is relevant, but that carbon gain of our two distinct ST species is increased by biomass allocation strategy rather than by leaf level physiological traits.

## 6. References

- Anderson JM, Chow WS, Park YI (1995) The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research* 46:129–139.
- Bahar NHA, Ishida FY, Weerasinghe LK, Guerrieri R, Sullivan OSO, Bloomfield KJ, Atkin OK (2016) Leaf-level photosynthetic capacity in lowland Amazonian and high-elevation Andean tropical moist forests of Peru. *New Phytologist* 214:1002 – 1018.
- Baltzer JL, Thomas SC (2007a) Physiological and morphological correlates of whole-plant light compensation point in temperate deciduous tree seedlings. *Oecologia*, 153:209–223.
- Baltzer JL, Thomas SC (2007b) Determinants of whole-plant light requirements in Bornean rain forest tree saplings. *Journal of Ecology* 95:1208–1221.
- Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Dario P (2010) Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. *Science* 329:834-838.
- Bloesch U, Troupin G, Derungs N (2009) Les plantes ligneuses du Rwanda Flore, ecologie et usages Shaker, Aachen
- Cheesman AW, Winter K (2013a) Elevated night-time temperatures increase growth in seedlings of two tropical pioneer tree species. *New Phytologist* 197:1185–1192.
- Cheesman AW, Winter K (2013b) Growth response and acclimation of CO<sub>2</sub> exchange characteristics to elevated temperatures in tropical tree seedlings. *Journal of Experimental Botany* 64: 3817–3828
- Cheng AX, Yu M, Wang GG, Wu T, Zhang C (2013) Growth, morphology and biomass allocation in response to light gradient in five subtropical evergreen broadleaved tree seedlings. *Journal of Tropical Forest Science* 25:537–546.
- Clark DB, Clark DA, Oberbauer SF (2010) Annual wood production in a tropical rain forest in NE Costa Rica linked to climatic variation but not to increasing CO<sub>2</sub>. *Global Change Biology* 16:747–759.
- Coste S, Roggy JC, Imbert P, Born C, Bonal D, Dreyer E (2005) Leaf photosynthetic traits of 14 tropical rain forest species in relation to leaf nitrogen concentration and shade tolerance. *Tree Physiology* 25:1127–1137.



- Coste S, Roggy JC, Garraud L, Heuret P, Nicolini E, Dreyer E (2009) Does ontogeny modulate irradiance-elicited plasticity of leaf traits in saplings of rain-forest tree species? A test with *Dicorynia guianensis* and *Tachigali melinonii* (Fabaceae, Caesalpinioideae). *Annals Of Forest Science* 66:709-709.
- Coste S, Roggy JC, Schimann H, Epron D, Dreyer E (2011) A cost-benefit analysis of acclimation to low irradiance in tropical rainforest tree seedlings: Leaf life span and payback time for leaf deployment. *Journal of Experimental Botany* 62:3941–3955.
- Coste S, Roggy JC, Sonnier G, Dreyer E (2010) Similar irradiance-elicited plasticity of leaf traits in saplings of 12 tropical rainforest tree species with highly different leaf mass to area ratio. *Functional Plant Biology* 37:342–355.
- De Kauwe MG, Lin YS, Wright IJ, Medlyn BE, Crous KY, Ellsworth DS, Domingues TF (2016) A test of the “one-point method” for estimating maximum carboxylation capacity from field-measured, light-saturated photosynthesis. *New Phytologist* 210:1130–1144.
- Dirzo R, Raven PH (2003) Global state of biodiversity and loss. *Annu. Rev. Environ. Resour* 28:137–167.
- Doughty CE(2011) An In Situ leaf and branch warming experiment in the Amazon. *Biotropica* 43:658–665.
- Doughty CE, Goulden ML (2008) Are tropical forests near a high temperature threshold? *Journal of Geophysical Research* 113:1–12.
- Dusenage ME, Göran W, Gårdesten J, Niyonzima F, Adolfsson L, Nsabimana D, Uddling J (2015) Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature. *Oecologia* 177:1183–1194.
- Farquhar GD, Caemmerer SV, Berry JA(1980) A biochemical model of photosynthetic CO<sub>2</sub> assimilation. *Planta* 90:78–90.
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.
- Fischer E, Killmann D (2008) Illustrated field guide to the plants of Nyungwe National Park Rwanda. Koblenz Geographical Colloquia Series Biogeographical Monographs 1Koblenz, Koenigstein
- Givnish TJ (1988) Adaptation to Sun and Shade : A whole-plant perspective *Australian. Journal*

- of *Plant Physiology* 15:63–92.
- Gommers CMM, Visser EJW, Onge KRS, Voeselek LAC, Pierik R (2013) Shade tolerance: when growing tall is not an option. *Trends in Plant Science* 18:65–71.
- Grubb PJ (2015) Trade-offs in interspecific comparisons in plant ecology and how plants overcome proposed constraints. *Plant Ecology and Diversity* 9:3–33.
- Hasper TB, Dusenge ME, Breuer F, Uwizeye FK, Wallin G, Uddling J (2017) Stomatal CO<sub>2</sub> responsiveness and photosynthetic capacity of tropical woody species in relation to taxonomy and functional traits. *Oecologia* 184:43–57.
- Henry HAL, Aarssen LW (1997) On the Relationship between Shade Tolerance and Shade Avoidance Strategies in Woodland. *Oikos* 80:575–582.
- Houter NC, Pons TL (2012) Ontogenetic changes in leaf traits of tropical rainforest trees differing in juvenile light requirement. *Oecologia* 169:33–45.
- Houter NC, Pons TL (2014) Gap effects on leaf traits of tropical rainforest trees differing in juvenile light requirement. *Oecologia* 175:37–50.
- Kitajima K (1994) Relative importance of Photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–428.
- Kitajima K, Poorter L (2010) Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist* 186:708–721.
- Kok B (1948) A critical consideration of the quantum yield of *Chlorella*-Photosynthesis. *Enzymologia* 13:1–56.
- Kunstler G, Falster D, Coomes DA, Hui F, Kooyman RM, Laughlin DC, Westoby M (2016) Plant functional traits have globally consistent effects on competition. *Nature* 529:204–207.
- Laanisto L, Niinemets Ü (2015) Polytolerance to abiotic stresses: How universal is the shade-drought tolerance trade-off in woody species? *Global Ecology and Biogeography* 24: 571–580.
- Lewis SL (2006) Tropical forests and the changing earth system. *Phil. Trans. R. Soc. B* 261:195–210
- Lusk CH, Pozo AD (2002) Survival and growth of seedlings of 12 Chilean rainforest trees in two light environments: Gas exchange and biomass distribution correlates. *Austral Ecology* 27:173–182.

- Lusk CH, Saldan A, Piper FI, Matı M (2011) Ontogeny, understorey light interception and simulated carbon gain of juvenile rainforest evergreens differing in shade tolerance. *Annals of Botany* 108:419–428.
- Luttge U (2008) *Physiological ecology of tropical plants*. Springer-Verlag Berlin Heidelberg.
- Malhi Y, Adu-bredu S, Asare RA, Lewis SL, Mayaux P (2013) African rainforests: past, present and future. *Phil. Trans. R. Soc. B.* 368:1-10
- Mao P, Zang R, Shao H, Yu J (2014) Functional Trait Trade-Offs for the Tropical Montane Rain Forest Species Responding to Light from Simulating Experiments. *The Scientific World Journal* 2014:1–9.
- Montgomery R (2004) Relative importance of photosynthetic physiology and biomass allocation for tree seedling growth across a broad light gradient. *Tree Physiology* 24:155–167.
- Montgomery RA, Chazdon RL (2001) Forest structure, canopy architecture, and light transmittance in tropical wet forests. *Ecology* 82:2707–2718.
- Mujawamariya M, Manishimwe A, Ntirugulirwa B, Bahati EN, Nyirambangutse B, Nsabimana D, Wallin G, Uddling J (2018) Climate sensitivity of tropical trees along an elevation gradient in Rwanda. *Forests* 647:1-19.
- Niinemets Ü (1997) Role of foliar nitrogen in light harvesting and shade tolerance of four temperate deciduous woody species. *Functional Ecology* 11:518–531.
- Niinemets Ü (2006) The controversy over traits conferring shade-tolerance in trees : ontogenetic changes revisited. *Journal of Ecology* 94:464–470.
- Nsabimana D, Klemedtson L, Kaplin BA, Wallin G (2008) Soil carbon and nutrient accumulation under forest plantations in southern Rwanda. *African Journal of Environmental Science and Technology* 2:142–149.
- Nsabimana D (2009) Carbon stock and fluxes in Nyungwe forest and Ruhande Arboretum in Rwanda. PhD thesis, Gothenburg University
- Nyirambangutse B, Zibera E, Uwizeye FK, Nsabimana D, Bizuru E, Pleijel H (2017) Carbon stocks and dynamics at different successional stages in an Afromontane tropical forest. *Biogeosciences* 14:1285–1303.
- Osunkoya OO, Ash JE, Hopkins MS, Graham AW, Ash E, Hopkins MS (1994) Influence of seed size and seedling ecological attributes on shade-tolerance of rain-forest tree species in northern Queensland. *Journal of Ecology* 82:149–163.

- Poorter L, Kitajima K, Mercado P, Chubina J, Melgar I, Prins, HHT (2010) Resprouting as a persistence strategy of tropical forest trees: relations with carbohydrate storage and shade tolerance. *Ecology* 91:2613–2627.
- Poorter L, Castilho CV, Schiatti J, Oliveira RS, Costa FRC (2018) Can traits predict individual growth performance? A test in a hyperdiverse tropical forest. *New Phytologist* 219: 109–121.
- Poorter H, Niinemets Ü, Ntagkas N, Siebenkäs A, Mäenpää M, Matsubara S, Pons TL(2019) A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist* 223:1073–1105.
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R, Niinemets U, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* 182:565–588.
- Reich PB, Wright IJ, Bares JC, Craine JM, Oleksyn J, Westoby M (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164:143–164.
- Reich PB (2014) The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102:275–301.
- Rozendaal DM, Hurtado VH, Poorter L (2006) Plasticity in leaf traits of 38 tropical tree species in response to light. *Functional Ecology* 20:207–216.
- Sage RF, Kubien DS (2007) The temperature response of C<sub>3</sub> and C<sub>4</sub> photosynthesis. *Plant, Cell and Environment*. 30:1086–1106.
- Slot, M, & Winter, K(2017) Photosynthetic acclimation to warming in tropical forest tree seedlings *Journal of Experimental Botany*, 68(9), 2275–2284
- Underwood AJ (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press
- Valladares F, Laanisto L, Niinemets Ü, Zavala MA (2016) Shedding light on shade: ecological perspectives of understorey plant life. *Plant Ecology and Diversity* 9:237–251.
- Valladares F, Niinemets Ü (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. *Annual Review of Ecology, Evolution, and Systematics* 39:237–257.
- Van de Weg MJ, Meir P, Grace J, Ramos GD (2012) Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia* 168:23–

34.

- Vårhammar A, Mclean CM, Dusenge ME, Medlyn BE, Hasper TB, Nsabimana D, Uddling J (2015) Photosynthetic temperature responses of tree species in Rwanda : evidence of pronounced negative effects of high temperature in montane rainforest climax species. *New Phytologist* 206:1000-1012.
- Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: Do winter deciduous and broad-leaved evergreen species differ? *New Phytologist* 143:143–154.
- Way DA, Oren R (2010) Differential responses to changes in growth temperature between trees from different functional groups and biomes : a review and synthesis of data. *Tree Physiology* 30:669–688.
- Wright SJ, Muller-Landau HC, Schipper J (2009) The future of tropical species on a warmer planet. *Conservation Biology* 23:1418–1426.

## **Paper II**

### **Effects of temperature on growth of six tropical montane tree species planted in pots along an elevation gradient in Rwanda**

## Abstract

An elevation gradient in tropical montane forests offers the potential to study temperature responses of plants and ecosystems under ecologically realistic conditions. In order to understand effects of elevation and pronounced rise in temperature on growth rate of tropical montane tree species, the growth of three climax species (*Carapa grandiflora*, *Syzygium guineense* and *Ficus thonningii*) and three pioneer species (*Harungana montana*, *Maesa lanceolata* and *Polyscias fulva*) was investigated on potted trees. 144 seedlings were grown in pots at the high-, mid- and low-elevation sites, with the soil from the high-elevation site, in Rwanda from December 2017. Five growth monitoring measurement campaigns were conducted, and seedlings were harvested in December 2018. The relative growth rate of all studied species, except *Harungana montana*, increased with temperature from low- to mid-elevation site but dropped at the high-elevation site. *Carapa grandiflora* and *Ficus thonningii* demonstrated low thermal acclimation capacity with a critically low growth in the low elevation site, thus confirming that they are less flexible in their responses to rising temperature than their pioneer counterparts. Seedlings have allocated relatively more biomass in roots to address the challenge of reduced rates of water uptake. The results of this study present little evidence on the effect of rising temperature on biomass allocation of tropical montane tree species. Since climate change will surely affect above ground (CO<sub>2</sub>) and below ground resources (water, nutrients), growth and biomass allocation are likely to be complex and require further investigation to predict the acclimation capacity of tropical montane forest tree species.

Keywords: tropical montane forest, pioneer species, climax species, leaf temperature, relative growth rate, biomass allocation

# 1. Introduction

Tropical forests cover only ca 10% of the Earth's land surface (Malhi and Grace 2000) but have a major contribution in controlling the rate of ongoing climate change (Lewis 2006). They have a large influence on global patterns of biodiversity, ecosystem ecology, productivity and biogeochemical cycles, but they remain relatively understudied (Malhi et al. 2010, 2013; Schippers et al. 2015) For instance, carbon storage and sequestration in tropical montane forests and their dependence on elevation and temperature are not well understood (Moser et al. 2011). The majority of studies assessing carbon pools and carbon sequestration in tropical moist forests have been conducted in lowland forests (e.g. Clark et al. 2003; Feeley et al. 2007) while the carbon stores of tropical montane forests are less well known. Moreover, there is still large uncertainty regarding the direct effects of high temperature to tropical forests due to insufficient in situ warming experiments.

It has been hypothesized that many tropical species have a narrow thermal niche and operate close to a thermal limit above which they experience declines in function or fitness (Janzen 1967; Doughty and Goulден 2008). The rise in temperature negatively affects their photosynthesis and growth (Way and Oren 2010; Liang et al. 2013). The threat of warming to montane tree species will be particularly severe for species with a high sensitivity and limited ability to acclimate warming, climax species mostly. The stronger negative effect of high temperature has been reported in tropical climax species compared to pioneer species (Doughty 2011; Cheesman and Winter 2013; Vårhammar et al. 2015) The results of Dusenge et al. (2015) and Varhammar et al. (2015) suggest that montane rainforest climax species may be very sensitive to future global warming and highlight the urgent need for more research on thermal responses of tropical trees in order to better assess their sensitivity to rising temperature.

Tropical elevation gradients are natural laboratories for ecosystem ecology, global change research and assessing how changing climate can influence tropical forests (Malhi et al. 2010; Enquist et al. 2017). They offer the potential to study temperature responses of plants and ecosystems under ecologically realistic conditions (Körner 2007; Malhi et al. 2010) with ecologically different flora and unique species composition. Studying species along an elevation gradient provides a simulation of natural phenomena in tropical montane forests that are



understudied. Often species that fail to acclimate or tolerate rising average temperatures migrate uphill along an elevation gradient where the temperature is cooler further increasing biodiversity and at the same time increasing competition among local species which often can lead to shifts in species composition as local species fail to keep up with new-coming species and are pushed out from their niche environment (Feeley et al. 2011; Corlett and Westcott 2013; Ganszky 2018).

Increased temperature generally increases tree growth, except for tropical trees (Way and Oren 2010). That is probably because temperate and boreal trees currently operate below their temperature optimum, while tropical trees are at theirs (Ryan 2010). On the other hand, low temperatures decrease the fraction of stems and leaves and increase root mass fraction (Poorter 2011). A range of plant functions are impaired by low temperature (photosynthesis, nutrient uptake, growth), but reduced rates of water uptake are a probable cause of increased allocation to roots (Lambers et al. 2008). In a recent study, pioneer species attained taller heights at comparatively smaller diameters as juveniles (Cano et al. 2019). The plant growth is also explained by the allocation of above- and below-ground biomass which reflects its survival strategy (Xu et al. 2016). Biomass allocation at any given time is a strong driver of the capacity of plants to take up Carbon, water and nutrients for future use (Evans, 1972). As such, it plays an important role in the growth of plants (Poorter 2011). Forest biomass allocation patterns are important for understanding global carbon cycling and climate change, which might change with environmental conditions and forest characteristics (Fang et al. 2018). However, the effects of climate and forest characteristics on biomass allocation fractions remains unknown, particularly in tropical montane forests (Reich et al. 2014; Fang et al. 2018).

In the present study, three climax and three pioneer species were grown in three sites along an elevation gradient. They grew under varying temperature in the soil of their origin habitat to examine climate responses that are not confounded by possible effects of differences in soil properties. The main objective was to explore effects of elevation and pronounced rise in temperature on growth rate of tropical montane tree species. The specific objectives of the study were: i) to assess the relationship between temperature and the growth of six tropical montane tree species grown in similar soil; and ii) to investigate biomass allocation of six tropical montane tree species along the elevation gradient.

## 2. Materials and methods

### 2.1 Study site and environmental data

The experiment was conducted at three sites along an elevation gradient, Sigira, Rubona and Makera. Sigira is a high-altitude site located at S 2° 30' 54"; E 29° 23' 44" in Nyamagabe district. The site is adjacent to Nyungwe National Park and characterized as a montane rainforest at the elevation of 2400 m. a. s. l. The average temperature at the site is 15.1°C while the maximum temperature gets to 23.8°C. In the weather station that was set at the site in 2018-2019 the precipitation was 2100 mm. Rubona is a mid-altitude site located at S 2° 28' 30"; E 29° 46' 49" in Huye district. The site is a transitional rainforest at 1600 m. a. s. l. elevation. The average temperature at the site is 20.0°C and the maximum temperature is 28.6°C. The weather station has recorded 1576 mm precipitation at the site in 2018-2019. Makera is a low-altitude site located at S 2° 6'31"; E 30°51'16" E in Kirehe district at 1300 m. a. s. l. The site is characterized by evergreen and semi-evergreen bushland and thicket, near Ibanda Makera forest gallery. The site's maximum temperature is 31.4°C while the average is 20.5°C. During 2018-2019, the site's average precipitation was 1046 mm.

### 2.2 Plant materials and experimental design

The investigated six montane tree species were selected from the two highest vegetation zones: Afromontane rainforest and the transitional rainforest. The species include climax species: *Carapa grandiflora* (Cg), *Ficus thonningii* (Ft), *Syzygium guineense* (Sg); and pioneer species: *Harungana montana* (Hm), *Maesa lanceolata* (Ml) and *Polyscias fulva* (Pf). Their planting materials were propagated in Rubona nursery in 2016. In December 2017, 24 plants per species were planted into 10 litre pots at Sigira using soil from that site. This was done to avoid soil confounding factors. Potted seedlings were randomized and transported to two other sites. They were placed at the sites in a randomized order spaced at 1.5 x 1.5 m in a 6 x 8 plants square. There were 48 potted plants per site; 6 species x 8 replicates, i.e. 144 plants in total.

Plants were regularly watered twice a week the beginning for establishment. In the dry season, plants at Makera were watered three times a week, each plant was watered with 2 litres once per

day. Plants in Rubona were watered twice a week and in Sigira once a week. At both sites, 2 litres per plant were used twice a day.

### *2.3 Growth and temperature measurements*

Tree parameters were measured before planting trees: tree height was measured to the nearest cm with a measuring tape and the stem diameter was measured at 5 cm to the nearest 0.1 mm with a caliper. Tree growth monitoring data were collected in March, May, July and December 2018 and the plants were harvested in December 2018. Leaf temperature was measured on all plants in all plots at 12:00-15:00 h on sunny days, using three infrared thermometers (Model: BP 10, TROTEC, Germany). The infrared thermometer had a 6:1 ratio of the distance to the surface compared to the diameter of the surface area being measured. It was held about 6 cm from the leaf surface and pointed towards the leaf without shading it, aiming at the central position on one of the leaf halves for large leaves and at the middle for small leaves. The leaf was horizontally positioned, and the angle of the infrared thermometer was held at 45° relative to the leaf.

### *2.4 Leaf and chemical traits*

Leaf length and width were measured to the nearest mm with a ruler. Leaf thickness was measured at three locations of each leaf, to the nearest 0.01mm using an electronic caliper, avoiding major veins. Leaf discs were sampled (three discs with 18 mm diameter for large leaves or five discs with 10 mm diameter for smaller leaves) for LMA determination. Leaf samples were oven dried at 70 °C for at least 72 h. After determining LMA, leaf discs and remaining leaf material were grinded into a fine powder with a ball mill grinder (MM 301). In total, 133 samples were sent to Canada for analyses of leaf nitrogen (N) and stable carbon isotope composition.

### *2.5 Biomass harvest*

In December 2017, eight plants per species were harvested from Rubona nursery to determine initial biomass of seedlings and measure LMA and RGR consequently. The final harvest took place in December 2018. Before harvesting, a few parameters were measured. The seedling height and base diameter were measured using a measuring tape and digital caliper. SPAD-502 meter (Konica Minolta Sensing, Inc., Ltd.: Osaka, Japan) was used to measure the SPAD value

(a proxy of area-based leaf chlorophyll content) of one fully developed, healthy and at least two months old leaf per plant. The seedlings were then harvested and divided into roots, main stem, branches, petioles and leaves. Plant parts were oven-dried at 70 °C until constant mass. Wood density of dry wood was determined based on measurements of mass and volume on a piece of a dry stem without bark from each plant.

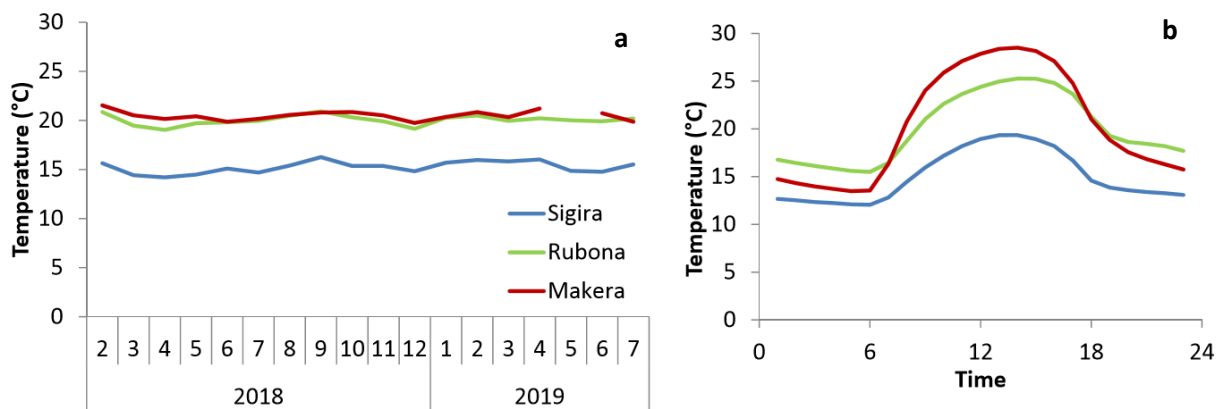
### *2.6 Data analysis*

Data were compiled and analyzed in SPSS. The sources of variation were analyzed between and within groups with sites and species as the main factors.  $R^2$  were shown and effects were considered statistically significant at  $P \leq 0.05$ .

### 3. Results

#### 3.1 Climate

Air temperature was nearly 6 °C lower in Sigira compared to Makera site (Fig. 1). There was no bigger difference between air temperature at 50 cm and 180 cm above ground. Plants at Rubona and Makera were experiencing almost the same air temperature (Fig. 1a). This was not surprising because, initially, the average temperature at Makera and Rubona is 20.5 and 20.0°C. The diurnal temperature variation increased with decreasing elevation (Fig. 1b). February was the hottest month during the experiment time. Maximum air temperature was 33, 30 and 23 °C in Makera, Rubona and Sigira, respectively. During this month and the rest of the dry season, seedlings were watered adequately and did not experience any drought stress. In this study, seedlings were not nutrient limited, nor water limited because they were provided with ample water and the Nitrogen percentage in plants' biomass varied from 40-50% for all species. The used soil from Sigira had 3.9 % organic carbon and 0.2 meq/100g of K.

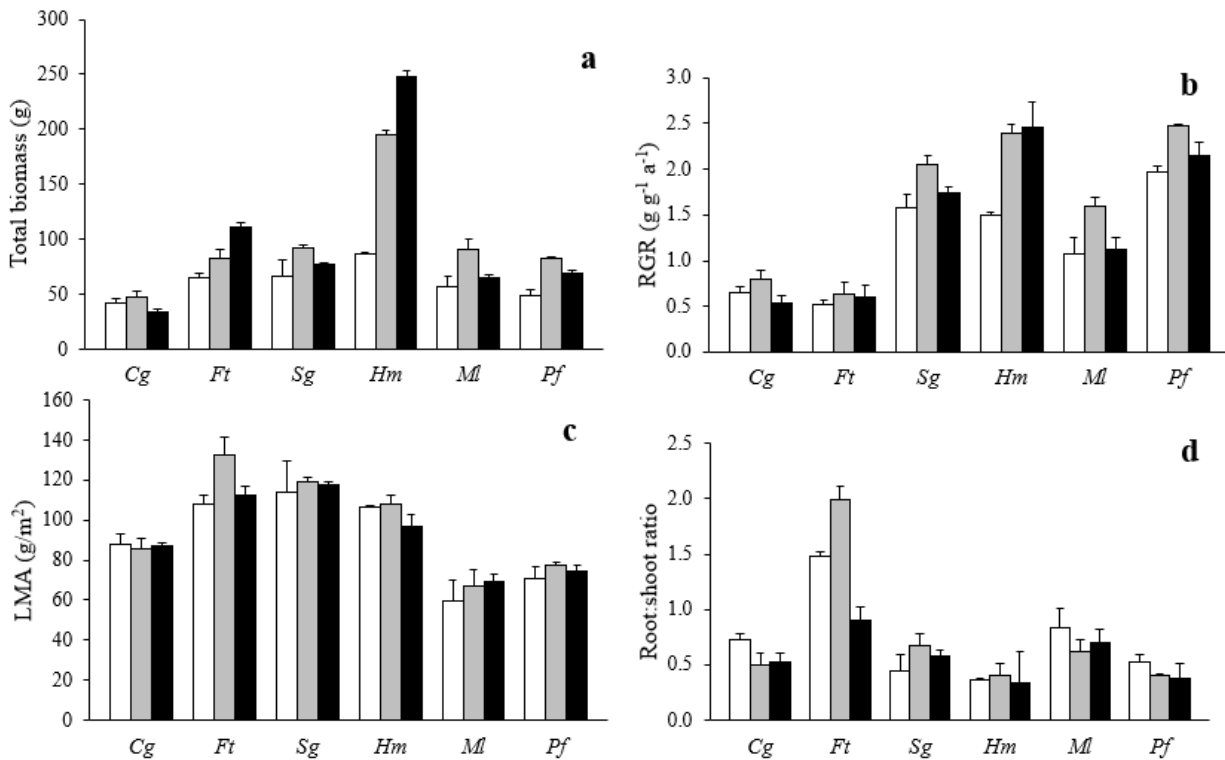


**Fig. 1** Monthly average air temperature (a) and diurnal variation (b) recorded at 180 cm above ground at the high-elevation site (Sigira), mid-elevation site (Rubona) and low-elevation site (Makera). The sensors recorded data every 30 minutes from February 2018 to July 2019.

#### 3.2 Growth and biomass allocation

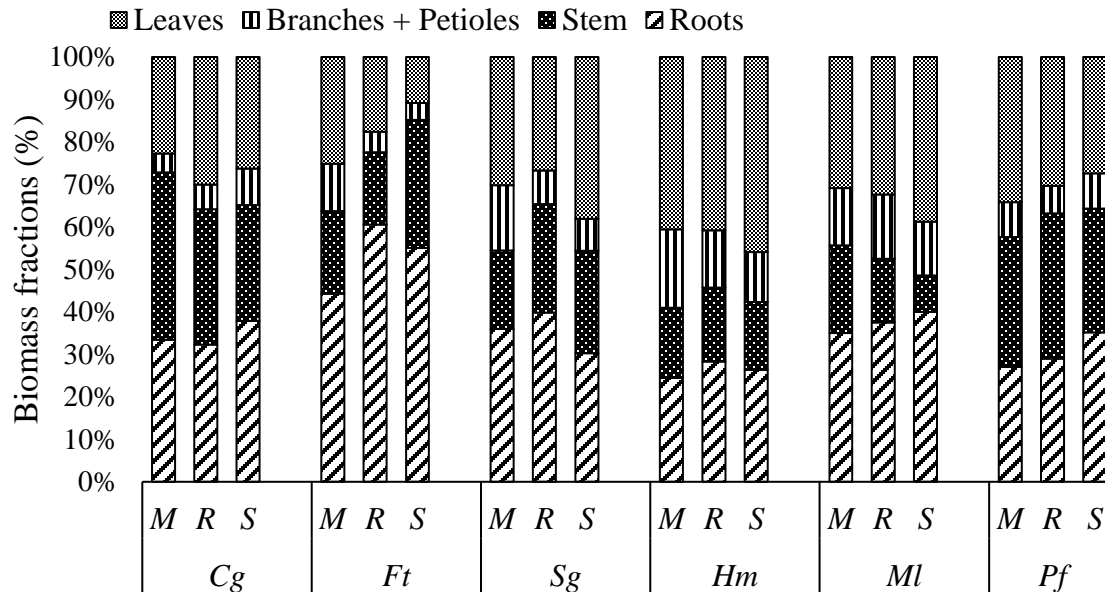
Seedlings at high-elevation site Sigira had low total biomass and relative growth rate (Fig. 2a-b). The temperature significantly affected the variation in species' total biomass ( $p = 0.0001$ ). RGR

of species significantly depended on the elevation ( $p = 0.029$ ). Across the entire dataset, *Hm* was the fastest growing species, followed by *Pf* and *Sg*. *Cg* and *Ft* were the slowest growing species. Surprisingly, *Cg* couldn't grow well in its original conditions at Sigira whereas *Sg* thrived there with a growth rate that was nearly double its growth in Makera and Rubona.



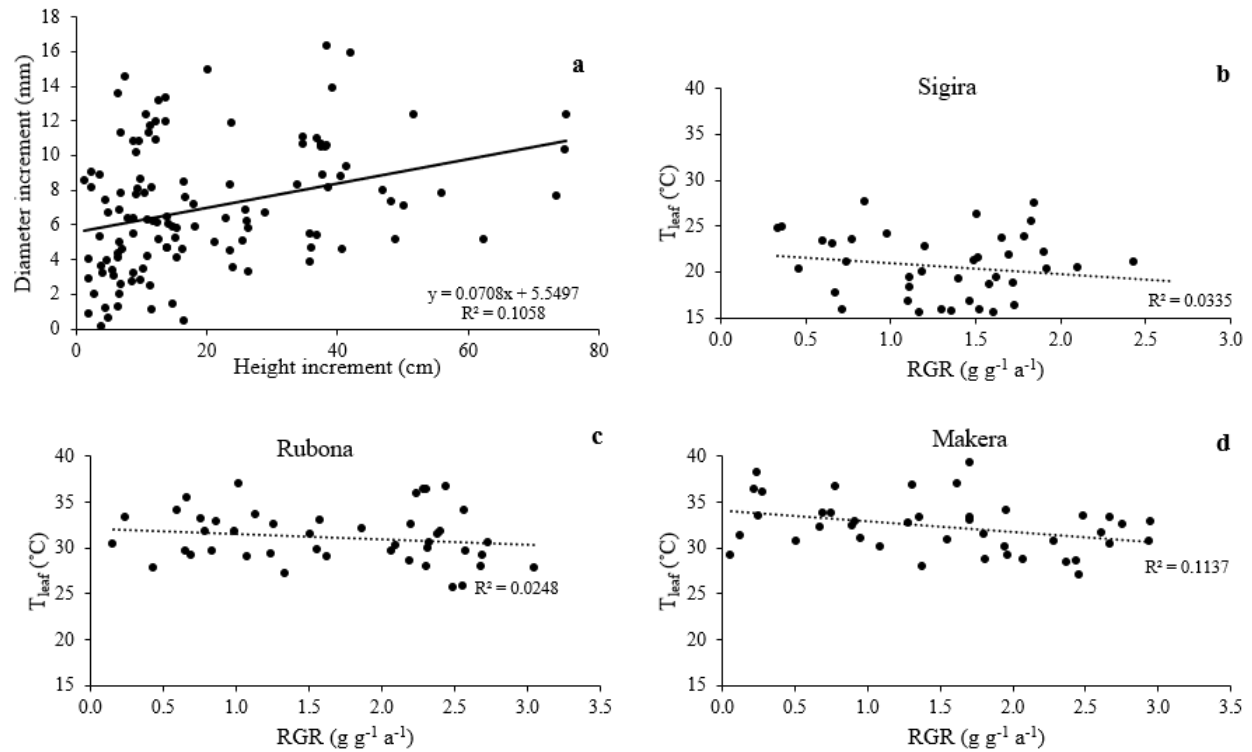
**Fig. 2** Total biomass (a), relative growth rate (b), leaf mass per area (c), and root to shoot ratio (d) of six species: *Carapa grandiflora* (*Cg*), *Ficus thonningii* (*Ft*), *Syzygium guineense* (*Sg*), *Harungana montana* (*Hm*), *Maesa lanceolata* (*Ml*) and *Polyscias fulva* (*Pf*) grown in Sigira (white), Rubona (gray) and Makera (black). Species to the left (*Cg*, *Ft*, *Sg*) are climax and species to the right (*Hm*, *Ml*, *Pf*) are pioneers. Bars represent standard errors.

Climax species, *Cg* and *Ft* in particular, invested more of their biomass in roots whereas pioneer species, *Hm* and *Ml* in particular, invested in leaves (Fig. 3). The difference in biomass allocation was clearly demonstrated at the high elevation site, Sigira. E.g. At Sigira, the climax species *Ft* has allocated 55% biomass into roots and only 10% in leaves while the pioneer species *Hm* allocated 46% biomass into leaves and only 26% into roots. Pioneer species did such an investment because they had little resources in leaves, as evidenced by their low LMA (Fig. 2b).



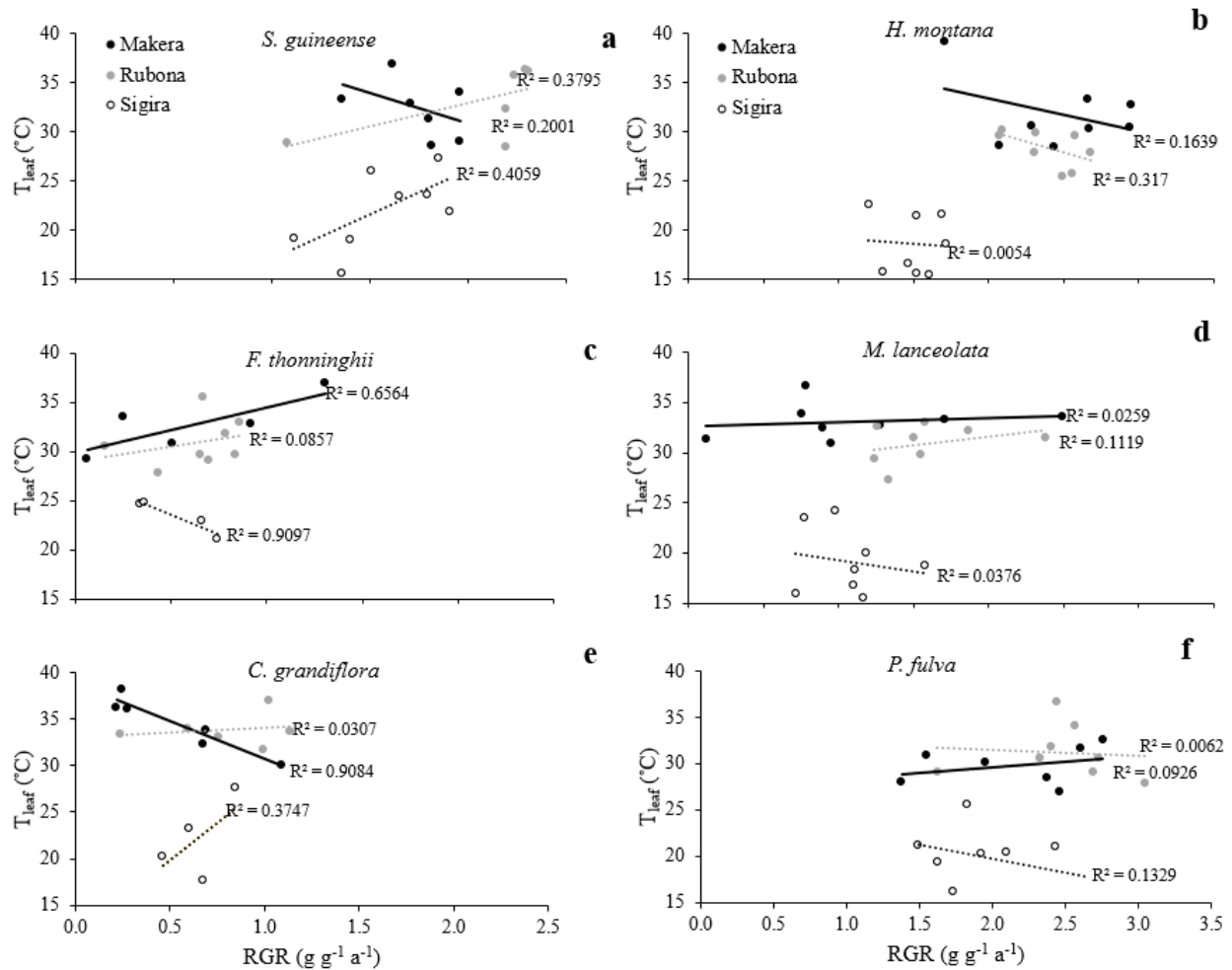
**Fig. 3** Biomass allocation to plant parts of six species: *Carapa grandiflora* (*Cg*), *Ficus thonningii* (*Ft*), *Syzygium guineense* (*Sg*), *Harungana montana* (*Hm*), *Maesa lanceolata* (*Ml*) and *Polyscias fulva* (*Pf*) grown at the low-elevation site Makera (M), mid-elevation site Rubona (R) and high-elevation site Sigira (S). Species to the left (*Cg*, *Ft*, *Sg*) are climax and species to the right (*Hm*, *Ml*, *Pf*) are pioneers.

Both height and diameter increment were significantly affected by the elevation and temperature,  $p = 0.004$  and  $p = 0.032$  respectively (Fig. 4a). Leaf temperature has also influenced the total biomass at three sites ( $p = 0.005$ ). Even though there was a significant interaction between leaf temperature and relative growth rate at all sites ( $p = 0.007$ ); and leaf temperature had a significant effect on RGR ( $p = 0.014$ ), the correlation between leaf temperature and RGR at each site didn't turn out positive or strong (Fig. 4b-d). There was a positive correlation between diameter and height increment at three sites ( $r^2 = 0.105$ ) (Fig. 4a), but there was no discernible pattern for both climax and pioneer species in height and basal diameter increment along the elevation gradient. The leaf temperature and relative growth rate did not show a clear pattern from the low to high elevation site, either (Fig. 5).



**Fig. 4** The relationship between (a) diameter and height increment; and (b-d) leaf temperature ( $T_{\text{leaf}}$ ) and relative growth rate (RGR) of six species at the low-elevation site Makera, mid-elevation site Rubona and high-elevation site Sigira. Leaf temperature values represent average leaf temperature data that was collected in the second, fifth and twelfth month of the experiment.





**Fig. 5** The correlation between leaf temperature ( $T_{\text{leaf}}$ ) and relative growth rate (RGR) of six species: *Syzygium guineense* (a), *Harungana montana* (b), *Ficus thonningii* (c), *Maesa lanceolata* (d), *Carapa grandiflora* (e), and *Polyscias fulva* (f) grown in Makera, Rubona and Sigira. Species to the left (*Cg*, *Ft*, *Sg*) are climax and species to the right (*Hm*, *Ml*, *Pf*) are pioneers. The leaf temperature is an average value ( $n = 3$ ) for the data were recorded in three seasons.

## 4. Discussion

Revisiting the objective of this study, the effect of elevation and increased temperature on the growth of six tropical montane species (*Carapa grandiflora*, *Syzygium guineense*, *Ficus thonningii*, *Harungana montana*, *Maesa lanceolata*, and *Polyscias fulva*) was thoroughly investigated. Three sites along an elevation gradient have provided a unique opportunity to simulate possible future warming scenarios, where a step down the gradient was meant to represent the future rise in temperature. When temperature is studied as the main driver of variation in plant growth along the gradient, there should be measures to minimize confounding influences of differences in soil fertility. The confounding effect of soil on the growth of these species was minimized by transplanting them in pots filled with similar soil that was taken from the high-elevation site. The soil was acidic though, with an average pH of 3. Regardless of the temperature effect, the growth of investigated species was relatively low compared to the growth of similar species grown in the soil found at the field at the mid- and low-elevation sites, Rubona and Makera. The biomass allocation strategy of climax and pioneer species has revealed their limiting growth resource (Roa-Fuentes et al. 2012) but the effect of temperature on biomass allocation was not clearly shown by the results of this study.

### 4.1. Growth

The poor growth of climax species at Makera (low-elevation site with high temperature) can be partly explained by the fact that they are adapted to little temperature variability within and among years. Negative effects of high temperature on tree growth are more pronounced in climax compared to pioneer tree species (Doughty and Goulden 2008; Way and Oren 2010; Liang et al. 2013; Dusenge et al. 2015). Montane climax species are particularly threatened by global warming. In a temperature experiment with tropical seedlings, increased temperature had a stronger negative effect on growth in climax species than in pioneer species (Cheesman and Winter 2013).

Another reason for poor growth of climax species in high-temperature site could be that they might be suffering intense sunlight which affects their physiological processes and therefore growth. Two recent studies demonstrated larger negative effects of high temperature on photosynthesis in climax compared to pioneer species (Doughty 2011; Vårhammar et al. 2015).

The higher photosynthetic heat stress was caused by low stomatal conductance and a large leaf size, leading to higher leaf temperature (Vårhammar et al. 2015). Schippers et al. (2015) have demonstrated that maximum temperature had a strong negative effect on the stem growth by increasing respiration, reducing stomatal conductance and thus mitigating a higher transpiration demand, and directly reducing photosynthesis. The effect of temperature on growth variables support the results of a tropical study that found such growth caused by plants getting closer to their photosynthetic optimum (Toledo et al. 2011). There is therefore a need to understand environmental controls over photosynthesis to better understand how tree growth will respond to future environmental changes (Ryan 2010). This is a particularly needed in tropical montane forest trees.

Long-term monitoring studies at specific sites in Central American and Asian tropical forests suggest that subtle increases in average temperatures negatively affect tree growth rates (Clark et al. 2003; Feeley et al. 2007). Few other studies, however, suggest the opposite for Amazon and tropical Africa (Phillips et al. 2004; Lewis et al. 2009) but the driver of this change is still a subject of future research (Ryan 2010). The speed of climate warming is generally higher than the potential speed of montane tree species migration to a higher altitude to maintain their thermal niches (Corlett and Westcott 2013). This will compromise the growth and survival of tropical trees unless they will thermally acclimate to warmer conditions (Mujawamariya et al. 2018).

#### *4.2. Biomass allocation*

Biomass allocation has an important role in acquisition of resources (light, nutrients and water) that often limit forest productivity (Litton et al. 2007). It is therefore essential to understand the effects of climate change on carbon allocation in tropical trees to predict how tropical forest ecosystem will be affected. Generally, biomass allocation to shoots is favored by shortage in aboveground resources while carbon allocation to roots is favored by shortage in belowground resources (Ericsson et al. 1996; Poorter and Nagel 2000; Lambers et al. 2008; Poorter et al. 2012). The results of this study support plant balanced growth hypothesis whereby biomass is allocated preferentially to the plant organ that harvests the limiting growth resource (Roa-Fuentes et al. 2012). Pioneer species in this study invested more biomass in leaves because they had little resources in leaves, as evidenced by their low LMA. Plants also opt to have shorter

branches to reduce hydraulic transport and embolism risk (Lines et al. 2012). Climax species did so in this study by investing less in branches. In this study, plants allocated more biomass to roots at the low temperature site. This concurs well with Poorter et al. (2012) who explained that low temperatures decrease the fraction of stems and leaves and increase root mass fraction. A range of plant functions are impaired by low temperature (photosynthesis, nutrient uptake, growth), but reduced rates of water uptake are a probable cause of increased allocation to roots (Lambers et al. 2008; Fang et al. 2018). Other literature report little or unclear effect of temperature on tropical tree biomass allocation (Raich et al. 2006; Reed et al. 2012). Since climate change will surely affect above ground (CO<sub>2</sub>) and below ground resources (water, nutrients), biomass allocation is likely to be complex and requires further investigation to predict the acclimation capacity of tropical forest species.

## 5. Conclusions

Tree seedlings in this study were grown along an elevation gradient to represent possible future warming scenarios. The growth rate of all studied species, except *Harungana montana*, increased with temperature from low- to mid-elevation site but dropped at the high-elevation site. *Carapa grandiflora* and *Ficus thonningii* demonstrated low thermal acclimation capacity with a critically low growth in the low elevation site, thus confirming that tropical montane climax species are less flexible in their responses to rising temperature than their pioneer counterparts. Seedlings have allocated relatively more biomass in roots to address the challenge of reduced rates of water uptake. The results of this study present little evidence of the effect of rising temperature on biomass allocation of tropical montane tree species.

## 6. References

- Cano, I. M., Muller-Landau, H. C., Joseph Wright, S., Bohlman, S. A., & Pacala, S. W. (2019). Tropical tree height and crown allometries for the Barro Colorado Nature Monument, Panama: A comparison of alternative hierarchical models incorporating interspecific variation in relation to life history traits. *Biogeosciences*, *16*(4), 847–862.
- Cheesman, A. W., & Winter, K. (2013). Growth response and acclimation of CO<sub>2</sub> exchange characteristics to elevated temperatures in tropical tree seedlings. *Journal of Experimental Botany*, *64*(12), 3817–3828.
- Clark, D. A., Piper, S. C., Keeling, C. D., & Clark, D. B. (2003). Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(10), 5852–5857.
- Corlett, R. T., & Westcott, D. A. (2013). Will plant movements keep up with climate change? *Trends in Ecology and Evolution*, *28*(8), 482–488.
- Doughty, C. E. (2011). An In Situ leaf and branch warming experiment in the Amazon. *Biotropica*, *43*(6), 658–665.
- Doughty, C. E., & Goulden, M. L. (2008). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research*, *113*, 1–12.
- Dusenge, M. E., Göran, W., Gårdesten, J., Niyonzima, F., Adolfsson, L., Nsabimana, D., & Uddling, J. (2015). Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature. *Oecologia*, *177*, 1183–1194.
- Ericsson, T., Rytter, L. & Vapaavuori, E. 1996. Physiology of carbon allocation in trees. *Biomass and Bioenergy*, *11*:115–127.
- Enquist, B. J., Bentley, L. P., Shenkin, A., Maitner, B., Savage, V., Michaletz, S., ... Malhi, Y. (2017). Assessing trait-based scaling theory in tropical forests spanning a broad temperature gradient. *Global Ecology and Biogeography*, *26*(12), 1357–1373.
- Evans, G.C. (1972) *The Quantitative Analysis of Plant Growth*. Blackwell Scientific

Publications, Oxford.

- Fang, Y., Zou, X., Lie, Z., & Xue, L. (2018). Variation in organ biomass with changing climate and forest characteristics across Chinese Forests. *Forests*, 9(9), 1–11.
- Feeley, K. J., Joseph Wright, S., Nur Supardi, M. N., Kassim, A. R., & Davies, S. J. (2007). Decelerating growth in tropical forest trees. *Ecology Letters*, 10(6), 461–469.
- Feeley, K. J., Silman, M. R., Bush, M. B., Farfan, W., Cabrera, K. G., Malhi, Y., ... Saatchi, S. (2011). Upslope migration of Andean trees. *Journal of Biogeography*, 38(4), 783–791.
- Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Society of Naturalists*, 101(919), 233–249.
- Körner, C. (2007). The use of “altitude” in ecological research. *Trends in Ecology and Evolution*, 22(11), 569–574.
- Lambers, H., Chapin III, F. S., & Pons, T. L. (2008). Plant physiological ecology. Second edition. Springer
- Lewis, S. L. (2006). Tropical forests and the changing earth system. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 361(1465), 195–210.
- Lewis, S. L., Lopez-Gonzalez, G., Sonké, B., Affum-Baffoe, K., Baker, T. R., Ojo, L. O., ... Wöll, H. (2009). Increasing carbon storage in intact African tropical forests. *Nature*, 457(7232), 1003–1006.
- Liang, J., Xia, J., Liu, L., & Wan, S. (2013). Global patterns of the responses of leaf-level photosynthesis and respiration in terrestrial plants to experimental warming. *Journal of Plant Ecology*, 6(6), 437–447.
- Lines, E. R., Zavala, M. A., Purves, D. W., & Coomes, D. A. (2012). Predictable changes in aboveground allometry of trees along gradients of temperature, aridity and competition. *Global Ecology and Biogeography*, 21(10), 1017–1028.
- Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P., & Saatchi, S. (2010). Introduction : Elevation gradients in the tropics : laboratories for ecosystem ecology and global change research. *Global Change Biology*, 16, 3171–3175.

- Malhi, Yadvinder, Adu-bredu, S., Asare, R. A., Lewis, S. L., Mayaux, P., B, P. T. R. S., ...  
 Mayaux, P. (2013). African rainforests : past , present and future African rainforests : past ,  
 present and future. *Phil. Trans. R. Soc. B*, 368.
- Malhi, Yadvinder, & Grace, J. (2000). Tropical forests and atmospheric carbon dioxide. *Trends  
 in Ecology and Evolution*, 15(8), 332–337.
- Moser, G., Leuschner, C., Hertel, D., Graefe, S., Soethe, N., & Iost, S. (2011). Elevation effects  
 on the carbon budget of tropical mountain forests (S Ecuador): The role of the belowground  
 compartment. *Global Change Biology*, 17(6), 2211–2226.
- Mujawamariya, M., Manishimwe, A., Ntirugulirwa, B., Zibera, E., Ganszky, D., Bahati, E. N.,  
 ... Uddling, J. (2018). Climate sensitivity of tropical trees along an elevation gradient in  
 rwanda. *Forests*, 9(10).
- Phillips, O. L., Baker, T. R., Arroyo, L., Higuchi, N., Killeen, T. J., Laurance, W. F., ... Vinceti,  
 B. (2004). Pattern and process in Amazon tree turnover, 1976-2001. *Philosophical  
 Transactions of the Royal Society B: Biological Sciences*, 359(1443), 381–407.
- Poorter, H., & Nagel, O. (2000). *The role of biomass allocation in the growth response of plants  
 to different levels of light , CO 2 , nutrients and water : a quantitative review.* (1971), 595–  
 607.
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass  
 allocation to leaves, stems and roots: Meta-analyses of interspecific variation and  
 environmental control. *New Phytologist*, 193(1), 30–50.
- Poorter, L. (2011). Hydraulics and life history of tropical dry forest tree species : coordination of  
 species ' drought and shade tolerance. *New Phytologist*, 191(2), 480–495.
- Raich, J. W., Russell, A. E., Kitayama, K., Parton, W. J., & Vitousek, P. M. (2006). Temperature  
 influences carbon accumulation in moist tropical forests. *Ecology*, 87(1), 76–87.
- Reed, S. C., Wood, T. E., & Cavaleri, M. A. (2012). Tropical forests in a warming world. *New  
 Phytologist*, 193(1), 27–29.
- Reich, P. B., Luo, Y., Bradford, J. B., Poorter, H., Perry, C. H., & Oleksyn, J. (2014).

- Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. *Proceedings of the National Academy of Sciences of the United States of America*, 111(38), 13721–13726.
- Roa-Fuentes, L. L., Campo, J., & Parra-Tabla, V. (2012). Plant Biomass Allocation across a Precipitation Gradient: An Approach to Seasonally Dry Tropical Forest at Yucatán, Mexico. *Ecosystems*, 15(8), 1234–1244.
- Ryan, M. G. (2010). Editorial: Temperature and tree growth. *Tree Physiology*, 30(6), 667–668.
- Schippers, P., Sterck, F., Vlam, M., & Zuidema, P. A. (2015). Tree growth variation in the tropical forest: understanding effects of temperature, rainfall and CO<sub>2</sub>. *Global Change Biology*, 21(7), 2749–2761.
- Toledo, M., Poorter, L., Peña-Claros, M., Alarcón, A., Balcázar, J., Leño, C., ... Bongers, F. (2011). Climate is a stronger driver of tree and forest growth rates than soil and disturbance. *Journal of Ecology*, 99(1), 254–264.
- Vårhammar, A., Mclean, C. M., Dusenge, M. E., Medlyn, B. E., Hasper, T. B., Nsabimana, D., & Uddling, J. (2015). Photosynthetic temperature responses of tree species in Rwanda : evidence of pronounced negative effects of high temperature in montane rainforest climax species. *New Phytologist*.
- Way, D. A., & Oren, R. A. M. (2010a). Differential responses to changes in growth temperature between trees from different functional groups and biomes : a review and synthesis of data. *Tree Physiology*, 30.
- Way, D. A., & Oren, R. A. M. (2010b). Differential responses to changes in growth temperature between trees from different functional groups and biomes : a review and synthesis of data. *Tree Physiology*, 30, 669–688.
- Xu, M., Liu, M., Xue, X., & Zhai, D. (2016). Warming effects on plant biomass allocation and correlations with the soil environment in an alpine meadow, China. *Journal of Arid Land*, 8(5), 773–786.



## Appendix Paper I

**Table 1.** Entire database, including physiological, chemical and structural leaf traits as well as tree biomass and its allocation in open sky plots (OP), sparse canopy plots (SP) and dense canopy plots (DP). Values are mean  $\pm$  SE.

Leaf traits		Plant species						
		<i>C. grandiflora</i>	<i>E. excelsum</i>	<i>S. guineense</i>	<i>C. megalocarpus</i>	<i>D. goetzenii</i>	<i>P. fulva</i>	<i>All</i>
$V_{\text{cmax}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	OP	30.5 $\pm$ 1.2	30.5 $\pm$ 5.0	45.2 $\pm$ 1.8	51.6 $\pm$ 5.5	62.8 $\pm$ 8.5	48.9 $\pm$ 5.1	44.9 $\pm$ 1.8
	SP	41.1 $\pm$ 5.0	27.5 $\pm$ 3.6	22.8 $\pm$ 3.4	31.6 $\pm$ 6.3	36.2 $\pm$ 3.3	30.7 $\pm$ 3.4	31.7 $\pm$ 3.0
	DP	34.2 $\pm$ 1.6	19.0 $\pm$ 1.6	14.9 $\pm$ 2.1	17.9 $\pm$ 0.4	20.9 $\pm$ 1.7	17.5 $\pm$ 1.1	21.0 $\pm$ 0.3
$J_{\text{max}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	OP	53.1 $\pm$ 5.7	55.2 $\pm$ 3.3	88.5 $\pm$ 7.9	84.8 $\pm$ 13.5	107.6 $\pm$ 5.1	75.2 $\pm$ 9.1	77.4 $\pm$ 2.5
	SP	65.1 $\pm$ 7.5	70.2 $\pm$ 10.7	63.6 $\pm$ 4.4	62.3 $\pm$ 8.6	69.3 $\pm$ 5.3	62.9 $\pm$ 10.9	65.6 $\pm$ 1.9
	DP	52.9 $\pm$ 15.3	47.0 $\pm$ 7.0	44.3 $\pm$ 2.8	48.6 $\pm$ 1.7	45.7 $\pm$ 6.5	46.3 $\pm$ 6.8	48.1 $\pm$ 4.7
$J_{\text{max}}/V_{\text{cmax}}$	OP	1.62 $\pm$ 0.05	1.96 $\pm$ 0.27	1.99 $\pm$ 0.09	1.65 $\pm$ 0.07	1.84 $\pm$ 0.17	1.54 $\pm$ 0.12	1.77 $\pm$ 0.07
	SP	1.87 $\pm$ 0.43	2.60 $\pm$ 0.19	3.13 $\pm$ 0.31	2.22 $\pm$ 0.53	1.89 $\pm$ 0.09	2.02 $\pm$ 0.18	2.29 $\pm$ 0.22
	DP	1.49 $\pm$ 0.37	2.52 $\pm$ 0.26	3.29 $\pm$ 0.61	2.71 $\pm$ 0.07	2.15 $\pm$ 0.15	2.64 $\pm$ 0.25	2.48 $\pm$ 0.23
$R_{\text{d}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	OP	0.70 $\pm$ 0.04	0.87 $\pm$ 0.07	0.78 $\pm$ 0.11	0.67 $\pm$ 0.08	0.76 $\pm$ 0.16	0.61 $\pm$ 0.10	0.73 $\pm$ 0.07
	SP	0.72 $\pm$ 0.15	0.50 $\pm$ 0.16	0.63 $\pm$ 0.11	0.54 $\pm$ 0.14	0.65 $\pm$ 0.12	0.58 $\pm$ 0.15	0.60 $\pm$ 0.10
	DP	0.52 $\pm$ 0.08	0.35 $\pm$ 0.06	0.59 $\pm$ 0.16	0.37 $\pm$ 0.04	0.37 $\pm$ 0.02	0.38 $\pm$ 0.02	0.43 $\pm$ 0.04
LCP ( $\mu\text{mol mol}^{-1}$ )	OP	12.9 $\pm$ 0.3	13.4 $\pm$ 0.8	11.1 $\pm$ 1.9	11.4 $\pm$ 0.9	13.0 $\pm$ 2.0	9.6 $\pm$ 1.5	12.9 $\pm$ 0.3
	SP	10.6 $\pm$ 0.8	6.9 $\pm$ 1.9	9.7 $\pm$ 0.9	7.0 $\pm$ 1.6	8.5 $\pm$ 2.4	8.2 $\pm$ 2.9	10.6 $\pm$ 0.8
	DP	6.3 $\pm$ 0.6	5.1 $\pm$ 0.5	6.7 $\pm$ 0.4	6.2 $\pm$ 0.5	5.6 $\pm$ 2.7	5.8 $\pm$ 0.5	6.3 $\pm$ 0.6
Gs ( $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	OP	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.14 $\pm$ 0.01	0.16 $\pm$ 0.05	0.26 $\pm$ 0.08	0.15 $\pm$ 0.05	0.13 $\pm$ 0.03
	SP	0.04 $\pm$ 0.00	0.03 $\pm$ 0.01	0.07 $\pm$ 0.02	0.08 $\pm$ 0.01	0.15 $\pm$ 0.06	0.11 $\pm$ 0.03	0.08 $\pm$ 0.02
	DP	0.04 $\pm$ 0.01	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00	0.07 $\pm$ 0.02	0.05 $\pm$ 0.01	0.12 $\pm$ 0.05	0.05 $\pm$ 0.01
$A_{\text{n}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	OP	1.80 $\pm$ 0.64	1.57 $\pm$ 0.65	6.68 $\pm$ 0.30	6.83 $\pm$ 1.33	8.22 $\pm$ 1.80	5.62 $\pm$ 1.57	5.12 $\pm$ 0.89
	SP	2.78 $\pm$ 0.41	2.56 $\pm$ 0.35	3.30 $\pm$ 0.81	4.51 $\pm$ 0.55	4.98 $\pm$ 1.11	3.67 $\pm$ 0.35	3.63 $\pm$ 0.42
	DP	2.88 $\pm$ 0.98	2.11 $\pm$ 0.40	1.46 $\pm$ 0.09	2.65 $\pm$ 0.48	2.88 $\pm$ 0.51	2.26 $\pm$ 0.45	2.43 $\pm$ 0.43
	OP	0.11 $\pm$ 0.01	0.15 $\pm$ 0.04	0.08 $\pm$ 0.01	0.15 $\pm$ 0.04	-	0.06 $\pm$ 0.01	0.10 $\pm$ 0.01

<b>P<sub>a</sub></b> (g m <sup>-2</sup> )	<b>SP</b>	0.08 ± 0.00	0.27 ± 0.01	0.06 ± 0.01	0.11 ± 0.02	-	0.02 ± 0.00	0.08 ± 0.02
	<b>DP</b>	0.06 ± 0.03	0.09 ± 0.01	0.02 ± 0.01	0.04 ± 0.00	-	0.02 ± 0.01	0.04 ± 0.00
	<b>OP</b>	0.10 ± 0.02	0.17 ± 0.05	0.09 ± 0.01	0.23 ± 0.06	-	0.06 ± 0.01	0.12 ± 0.02
<b>P<sub>m</sub></b> (%)	<b>SP</b>	0.11 ± 0.00	0.35 ± 0.01	0.13 ± 0.01	0.30 ± 0.03	-	0.03 ± 0.00	0.13 ± 0.03
	<b>DP</b>	0.15 ± 0.01	0.20 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	-	0.07 ± 0.00	0.10 ± 0.02
	<b>OP</b>	2.32 ± 0.11	1.55 ± 0.13	1.16 ± 0.10	1.66 ± 0.07	1.91 ± 0.07	1.75 ± 0.18	1.72 ± 0.02
<b>N<sub>a</sub></b> (g m <sup>-2</sup> )	<b>SP</b>	2.01 ± 0.12	1.47 ± 0.06	0.63 ± 0.07	1.18 ± 0.05	1.00 ± 0.10	1.52 ± 0.11	1.30 ± 0.01
	<b>DP</b>	1.88 ± 0.20	1.35 ± 0.18	0.53 ± 0.01	1.35 ± 0.12	1.25 ± 0.04	1.63 ± 0.03	1.32 ± 0.04
	<b>OP</b>	1.74 ± 0.05	1.65 ± 0.06	1.34 ± 0.05	2.59 ± 0.14	3.68 ± 0.46	1.65 ± 0.15	2.11 ± 0.07
<b>N<sub>m</sub></b> (%)	<b>SP</b>	2.02 ± 0.13	1.96 ± 0.12	1.37 ± 0.07	2.73 ± 0.23	3.39 ± 0.21	2.16 ± 0.15	2.27 ± 0.13
	<b>DP</b>	2.40 ± 0.04	2.82 ± 0.10	1.60 ± 0.02	3.87 ± 0.39	4.48 ± 0.23	3.06 ± 0.17	3.04 ± 0.14

		Plant species						
Leaf traits		<i>C. grandiflora</i>	<i>E. excelsum</i>	<i>S. guineense</i>	<i>C. megalocarpus</i>	<i>D. goetzenii</i>	<i>P. fulva</i>	<i>All</i>
<b>LMA</b> (g m <sup>-2</sup> )	<b>OP</b>	88.3 ± 4.2	98.9 ± 5.5	185.5 ± 5.7	70.1 ± 5.6	47.2 ± 3.2	99.2 ± 3.4	98.2 ± 3.7
	<b>SP</b>	67.6 ± 5.3	65.2 ± 6.4	122.8 ± 22.9	43.8 ± 6.6	30.4 ± 6.1	51.1 ± 11.7	63.5 ± 9.8
	<b>DP</b>	56.3 ± 1.8	48.8 ± 1.1	99.3 ± 16.8	28.9 ± 4.6	17.8 ± 0.7	28.6 ± 2.9	47.1 ± 4.5
<b>Leaf length</b> (cm)	<b>OP</b>	16.3 ± 0.3	16.4 ± 1.7	11.4 ± 0.4	11.4 ± 0.6	9.3 ± 0.3	9.6 ± 0.6	12.4 ± 0.4
	<b>SP</b>	17.9 ± 0.4	21.4 ± 1.1	12.7 ± 0.3	13.6 ± 0.7	13.6 ± 0.8	11.0 ± 1.1	15.0 ± 0.6
	<b>DP</b>	17.7 ± 0.5	21.7 ± 0.9	13.8 ± 0.5	12.7 ± 1.8	11.7 ± 0.7	13.1 ± 0.6	15.3 ± 0.6
<b>Leaf width</b> (cm)	<b>OP</b>	6.23 ± 0.33	7.14 ± 0.47	3.12 ± 0.23	5.90 ± 0.31	8.39 ± 0.47	4.31 ± 0.24	5.85 ± 1.10
	<b>SP</b>	5.91 ± 0.13	8.69 ± 0.57	3.84 ± 0.11	6.69 ± 0.04	12.92 ± 0.65	5.04 ± 0.38	7.18 ± 0.23
	<b>DP</b>	6.54 ± 0.06	8.51 ± 0.44	4.13 ± 0.48	8.09 ± 0.80	10.12 ± 0.20	5.45 ± 0.26	7.28 ± 0.28
<b>Leaf thickness</b> (mm)	<b>OP</b>	0.25 ± 0.01	0.27 ± 0.00	0.29 ± 0.02	0.26 ± 0.01	0.25 ± 0.01	0.29 ± 0.01	0.27 ± 0.01
	<b>SP</b>	0.21 ± 0.01	0.21 ± 0.01	0.27 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.24 ± 0.02	0.22 ± 0.01
	<b>DP</b>	0.17 ± 0.01	0.18 ± 0.00	0.21 ± 0.01	0.16 ± 0.00	0.18 ± 0.01	0.19 ± 0.02	0.18 ± 0.01
<b>Leaf size</b> (cm <sup>2</sup> )	<b>OP</b>	64.3 ± 3.5	60.1 ± 1.0	10.5 ± 1.3	22.9 ± 4.2	42.3 ± 21.6	18.0 ± 0.7	36.4 ± 4.6
	<b>SP</b>	71.2 ± 7.2	135.7 ± 16.3	16.5 ± 2.6	48.4 ± 9.4	104.3 ± 22.9	31.2 ± 6.4	67.9 ± 10.6
	<b>DP</b>	67.5 ± 3.2	110.1 ± 1.6	16.6 ± 3.8	55.8 ± 4.9	40.3 ± 4.9	26.8 ± 4.4	52.0 ± 2.0
<b>Leaf angle</b> (°)	<b>OP</b>	66 ± 2	27 ± 2	44 ± 3	52 ± 2	34 ± 5	56 ± 3	47 ± 1
	<b>SP</b>	33 ± 2	25 ± 2	29 ± 2	30 ± 5	20 ± 2	20 ± 7	26 ± 3
	<b>DP</b>	24 ± 1	16 ± 1	16 ± 1	25 ± 3	17 ± 3	14 ± 2	19 ± 1
<b>SPAD</b>	<b>OP</b>	51.2 ± 0.8	44.3 ± 4.4	52.0 ± 1.5	34.3 ± 1.8	31.0 ± 1.0	32.9 ± 1.1	41.0 ± 0.9
	<b>SP</b>	60.3 ± 2.5	54.9 ± 4.9	50.5 ± 2.9	43.6 ± 3.6	33.6 ± 2.0	31.9 ± 1.3	45.8 ± 2.6
	<b>DP</b>	69.6 ± 0.6	64.8 ± 2.4	57.9 ± 1.5	44.3 ± 1.6	34.3 ± 3.9	31.9 ± 0.4	50.6 ± 0.9

		Plant species						
		<i>C. grandiflora</i>	<i>E. excelsum</i>	<i>S. guineense</i>	<i>C. megalocarpus</i>	<i>D. goetzenii</i>	<i>P. fulva</i>	<i>All</i>
<b>Tree traits</b>								
<b>RGR</b>	<b>OP</b>	2.31 ± 0.07	2.32 ± 0.04	3.09 ± 0.15	1.72 ± 0.02	2.39 ± 0.17	2.79 ± 0.07	2.35 ± 0.07
	<b>SP</b>	2.23 ± 0.11	2.45 ± 0.12	2.54 ± 0.11	1.43 ± 0.11	2.29 ± 0.11	2.17 ± 0.02	2.17 ± 0.04
	<b>DP</b>	1.25 ± 0.05	1.53 ± 0.12	1.59 ± 0.06	0.08 ± 0.28	1.15 ± 0.12	1.13 ± 0.14	1.18 ± 0.04
<b>Roots Weight</b> (g)	<b>OP</b>	35.6 ± 3.2	26.8 ± 1.2	41.9 ± 5.6	34.5 ± 1.2	41.1 ± 7.4	30.2 ± 2.7	33.7 ± 1.5
	<b>SP</b>	26.7 ± 4.7	29.9 ± 3.7	18.7 ± 1.5	18.0 ± 2.2	26.6 ± 5.1	17.3 ± 0.8	23.0 ± 2.4
	<b>DP</b>	10.6 ± 0.8	12.5 ± 1.2	7.1 ± 0.3	5.1 ± 1.9	8.1 ± 0.8	5.6 ± 0.7	8.5 ± 0.5
<b>Stem Weight</b> (g)	<b>OP</b>	30.0 ± 2.1	31.9 ± 0.8	33.0 ± 6.4	30.7 ± 0.6	32.0 ± 2.7	51.9 ± 2.9	34.7 ± 1.7
	<b>SP</b>	34.3 ± 2.1	36.9 ± 3.9	27.9 ± 1.7	32.2 ± 3.4	40.2 ± 2.7	27.0 ± 1.0	33.2 ± 0.6
	<b>DP</b>	12.4 ± 0.9	14.6 ± 1.7	9.3 ± 0.3	9.6 ± 2.0	14.0 ± 2.1	11.3 ± 1.6	12.0 ± 0.8
<b>Branches+Petioles</b> (g)	<b>OP</b>	4.52 ± 0.32	3.82 ± 0.63	9.52 ± 0.40	5.59 ± 0.25	3.22 ± 0.11	4.58 ± 1.30	4.95 ± 0.36
	<b>SP</b>	6.48 ± 0.33	6.45 ± 0.66	5.94 ± 0.80	3.38 ± 0.55	2.52 ± 1.09	4.25 ± 0.78	4.82 ± 0.38
	<b>DP</b>	2.79 ± 0.01	2.85 ± 0.44	1.51 ± 0.34	0.97 ± 0.49	1.14 ± 0.14	1.14 ± 0.43	1.85 ± 0.21
<b>Leaves Weight</b> (g)	<b>OP</b>	17.0 ± 1.7	14.6 ± 2.3	21.2 ± 3.0	6.5 ± 0.6	2.6 ± 0.9	12.3 ± 1.5	12.3 ± 1.3
	<b>SP</b>	23.3 ± 1.4	21.8 ± 2.7	16.9 ± 1.1	7.9 ± 2.1	3.8 ± 0.8	8.7 ± 1.4	13.8 ± 1.5
	<b>DP</b>	10.7 ± 0.3	11.1 ± 1.2	4.9 ± 1.0	2.6 ± 1.4	1.1 ± 0.4	1.7 ± 0.6	5.9 ± 0.3
<b>Total Weight</b> (g)	<b>OP</b>	87.5 ± 5.0	77.5 ± 4.5	106.0 ± 15.5	77.6 ± 1.3	78.9 ± 10.0	99.2 ± 5.4	86.0 ± 3.0
	<b>SP</b>	91.1 ± 5.0	95.3 ± 8.3	69.8 ± 2.1	61.7 ± 4.5	73.2 ± 8.7	57.4 ± 1.3	75.0 ± 2.3
	<b>DP</b>	36.8 ± 1.5	41.3 ± 4.5	23.0 ± 1.7	18.4 ± 5.8	24.4 ± 3.4	19.8 ± 3.3	28.4 ± 1.8
<b>Root/Shoot ratio</b>	<b>OP</b>	0.75 ± 0.10	0.55 ± 0.02	0.68 ± 0.01	0.82 ± 0.02	1.12 ± 0.19	0.44 ± 0.03	0.70 ± 0.05
	<b>SP</b>	0.42 ± 0.07	0.46 ± 0.04	0.39 ± 0.05	0.43 ± 0.08	0.58 ± 0.10	0.44 ± 0.03	0.45 ± 0.05
	<b>DP</b>	0.43 ± 0.02	0.45 ± 0.01	0.44 ± 0.01	0.38 ± 0.03	0.53 ± 0.03	0.42 ± 0.02	0.44 ± 0.01
<b>Wood density</b> (g/cm <sup>3</sup> )	<b>OP</b>	0.68 ± 0.00	0.55 ± 0.02	0.67 ± 0.03	0.68 ± 0.01	0.48 ± 0.02	0.43 ± 0.02	0.58 ± 0.01
	<b>SP</b>	0.52 ± 0.02	0.57 ± 0.01	0.68 ± 0.03	0.68 ± 0.02	0.45 ± 0.01	0.37 ± 0.01	0.54 ± 0.01
	<b>DP</b>	0.55 ± 0.01	0.54 ± 0.01	0.64 ± 0.01	0.59 ± 0.02	0.35 ± 0.02	0.37 ± 0.03	0.51 ± 0.01

<b>Base diameter</b> (cm)	<b>OP</b>	1.35 ± 0.03	1.56 ± 0.03	1.13 ± 0.06	1.24 ± 0.04	1.29 ± 0.02	1.83 ± 0.06	1.40 ± 0.02
	<b>SP</b>	1.42 ± 0.06	1.70 ± 0.10	1.19 ± 0.03	1.14 ± 0.04	1.38 ± 0.03	1.52 ± 0.04	1.39 ± 0.03
	<b>DP</b>	0.90 ± 0.03	1.14 ± 0.04	0.75 ± 0.03	0.63 ± 0.08	0.95 ± 0.03	1.14 ± 0.06	0.91 ± 0.02
<b>Stem height</b> (cm)	<b>OP</b>	47.4 ± 2.1	47.8 ± 0.7	81.6 ± 4.1	89.8 ± 3.4	111.9 ± 2.0	70.5 ± 3.4	74.8 ± 1.2
	<b>SP</b>	87.8 ± 3.5	51.9 ± 0.9	108.7 ± 14.2	114.2 ± 7.8	164.9 ± 6.1	71.0 ± 4.0	99.8 ± 3.7
	<b>DP</b>	57.1 ± 2.2	48.7 ± 3.3	78.6 ± 3.5	91.9 ± 1.8	124.6 ± 2.5	68.2 ± 3.8	77.8 ± 1.1

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$V_{\text{cmax}}$ : maximum rates of photosynthetic carboxylation,  $J_{\text{max}}$ : maximum rates of electron transport,  $R_{\text{d}}$ : leaf dark respiration, **LCP**: light compensation point,  $g_{\text{s}}$ : stomatal conductance,  $A_{\text{n}}$ : light-saturated net  $\text{CO}_2$  assimilation,  $P_{\text{a}}$ : area-based Phosphorus content,  $P_{\text{m}}$ : mass-based Phosphorus content,  $N_{\text{a}}$ : Nitrogen content per unit leaf area,  $N_{\text{m}}$ : Nitrogen content per unit leaf mass, **LMA**: leaf mass per unit leaf area, **SPAD**: proxy for leaf chlorophyll content.

