

EFFECTS OF WARMING ON PHOTOSYNTHESIS IN TROPICAL MONTANE TREE SPECIES IN RWANDA



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Figure on the front page:

Drone picture of the Rubona research site (intermediate elevation site), Rwanda.

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Abstract

Global warming continues to rise, however, knowledge on effects of warming on photosynthetic physiology is lacking in tropical trees. I investigated the effects of warming on net photosynthesis ($A_{\text{net}25}$) and its underlying biochemical processes - the maximum Rubisco carboxylation ($V_{\text{cmax}25}$) and maximum electron transport ($J_{\text{max}25}$) as well as stomatal conductance (g_{s25}) at common temperature of 25°C in 16 tropical montane tree species with eight early successional (ES) species, and eight late-successional (LS) species. They were grown at three different sites along an elevation gradient, differing by 6.8 °C in mean annual temperature. In addition, I explored the stomatal conductance (g_s), and net photosynthesis (A_{net}) to see the possible effects of warming on these parameters. The $V_{\text{cmax}25}$ remained constant in warm-grown species but the $V_{\text{cmax}25}$ rates of ES species were higher than in LS species by 57, 62, and 46%, at Sigira site High elevation, hereafter denoted “HE”), Rubona site, Mid elevation, hereafter denoted “ME”), Kirehe site, Low Elevation hereafter denoted “LE”) LE site, respectively. The $J_{\text{max}25}$ reduced by 14 and 15% in warm grown species at low-elevation hereafter denoted “LE”, Makera, and mid-Elevation hereafter denoted “ME”, Rubona sites, respectively compared to species grown at high elevation hereafter denoted “HE”, Sigira site. $J_{\text{max}25}$ was higher in ES species than in LS species by 41%, 51%, and 25% at HE site, ME site, and LE site, respectively. The $J_{\text{max}25}/V_{\text{cmax}25}$ ratio declined in warm grown species by 4% at ME site and 11% at LE site, respectively compared to species planted at HE site. The g_s at common temperature 25° C remained unaffected by warming but it was higher in ES species by, 77, 146, and 86% at HE site, ME site, and LE site, respectively. The A_{net} at common temperature 25° C remained constant, LS species had lower A_{net} compared to ES species by 66, 81, and 63%, at HE site, ME site, and LE site, respectively. In summary, these findings showed that tropical montane tree species growing in natural field conditions do not thermally acclimate their Rubisco carboxylation capacity but show some thermal acclimation in maximum electron transport rates. My results also indicate that net photosynthesis is biochemically driven by V_{cmax} . Overall, my results suggest tree species belonging to different successional groups similarly acclimate photosynthesis.

Keywords: Climate change, acclimation, maximum Rubisco carboxylation, maximum electron transport, early successional, late-successional.

Popular scientific summary

The earth climate is getting warmer mainly due to human activities. warming affects biodiversity including forests. Tropical forests are rich in biodiversity and play a significant role in regulating regional climate, and they store up to 55% of carbon in terrestrial biomass. However, tropical forests are more at risk than others especially through the decrease in growth and increased mortality. The net CO₂ assimilation through photosynthesis process is linked to a reduction of tree growth through the decline in photosynthesis. However, our knowledge of photosynthetic responses to warming in tropical species is limited, especially when they are grown in realistic field conditions.

For my master's thesis, I studied the effect of warming on biochemical processes underlying net photosynthesis in 16 tropical tree species with early successional (ES; shade-intolerant and fast-growing) species and late-successional (LS; shade tolerant and slow-growing) grown in three different field plantations along an elevation gradient in Rwanda, central Africa. The air temperature at these three sites differs by up to 6.8 °C.

I found that tropical trees decline one of the two biochemical processes of photosynthesis while the other remained constant. However, the net photosynthesis remained constant in response to warming. Generally, my results show that ES species have higher rates of photosynthetic capacity and photosynthesis than LS species, however, tree species belonging to two successional groups acclimate photosynthesis in a similar manner.

Table of content

Abstract	1
Popular scientific summary.....	2
Table of content	3
Introduction.....	4
1.1 Background	4
1.2 Photosynthesis.....	5
1.3 Effects of warming on Photosynthetic capacity and stomatal conductance.....	6
1.4 Aim.....	7
1.5 Hypotheses	7
Materials and Methods.....	8
2.1 Site description.....	8
2.2 Plant material.....	9
2.3 Gas exchange measurements.....	10
2.4 A-C _i curve fitting.....	10
2.5 Statistical analysis	11
Results.....	12
3.1 Effects of warming on photosynthetic capacity ($V_{\text{cmax}25}$, $J_{\text{max}25}$, and $J_{\text{max}25}/V_{\text{cmax}25}$ ratio) ..	12
3.2 Effects of warming on stomatal conductance (g_s) and Net Photosynthesis (A_{net})	14
Discussion	17
4.1 Effect of growth temperature on photosynthetic capacity	17
4.2 Effect of growth temperature on stomatal conductance (g_s).....	18
4.3 Effect of growth temperature on net photosynthesis (A_{net}).....	19
4.4 Warming sensitivity of successional groups	19
Conclusion	19
Acknowledgment	21
References	22
Supplementary Materials	28

Introduction

1.1 Background

The atmospheric CO₂ concentration is currently estimated at 411 parts per million (ppm), corresponding to a 47% rise from the pre-industrial concentration of 280 ppm, and is expected to continue to rise in the coming years (IPCC, 2013). In addition to carbon dioxide (CO₂), other anthropogenic greenhouse gases (GHGs) such as methane (CH₄), nitrous oxide (N₂O), and NO_x have increased simultaneously, which have led to global warming, changes in intensity and frequency of extreme weather events, such as drought and flooding in different regions of the planet (Ciais et al., 2013). Biodiversity and ecosystem functions are highly affected by climate change through climate-induced changes in species distributions, interaction, and diversity, as well as primary production (Díaz et al., 2019; Weiskopf et al., 2020). At the individual species level, species are exhibiting changes in their morphology, behavior, and phenology (Merilä & Hendry, 2014). However, it is still uncertain how biodiversity and ecosystem functioning are going to respond to these changes in the future when the global average temperature is expected to increase an extra 1–4 °C by the year 2100 (Stocker et al., 2013). It is predicted that tropical forests will be severely affected by extreme weather events such as drought and fires during El Niño years (Cochrane, 2003; Karmalkar et al., 2011; Lewis et al., 2011). Particularly, the temperature rise will lead to critical impacts on tropical forests (Ciais et al., 2013), however, the extent of these impacts on tropical tree species and their community composition is highly uncertain.

Tropical forests are rich in biodiversity and play a significant role in regulating regional climate, and precipitation patterns, as well as the global carbon cycle (Lewis et al., 2011). Moreover, tropical forests store up to 55% of carbon (C) in terrestrial biomass with annual net carbon uptake of about 2.3 Gt C (Pan et al., 2011). They represent also one-third of the earth's terrestrial gross primary productivity (Malhi et al., 2014b; Lewis et al., 2015). However, different studies show that global warming is enhancing the loss of tropical biomass carbon due to slower tree growth, increased tree mortality as well as diminished tree longevity (Bugmann & Bigler, 2011; Brienen et al., 2015; Hubau et al., 2020; Sullivan et al., 2020). Tropical forests are expected to be critically affected by global warming compared to other biomes due to their relatively stable climate throughout the year and their narrow thermal niche (Janzen, 1967; Wright et al., 2009). Moreover, high-montane species are at more risk than lower elevation counterparts, as some studies reported changes in species composition driven by increased mortality of species at higher, cooler elevations (Feeley et al., 2013). In addition, species from lower, hotter elevations were shown to migrate to higher altitudes where they can keep their thermal niches (Duque et al., 2015; Fadrique et al., 2018). However, physiological mechanisms underlying the decrease in tree performance in high elevation trees with warming are not well known.

Photosynthesis and respiration are the two important physiological processes that may underly the reduced performance of trees in response to warming especially in scenarios where

there is a decline in net photosynthesis and increased respiration. However, a recent study on tropical species showed that respiration thermally acclimates and may thus not be the process that limits growth (Mujawamariya et al., 2020). However, there is still a knowledge gap on photosynthetic responses to warming in tropical species, especially when they are grown in realistic field conditions (Mercado et al., 2018; Vårhammar et al., 2015). Therefore, considering the importance of tropical forests and the rate of climate change in the future, it is very important to understand plant photosynthetic responses to warming (Booth et al., 2012; Vårhammar et al., 2015).

1.2 Photosynthesis

Photosynthesis is a process by which plants convert light energy into chemical energy, and during this process, the CO_2 is fixed from the atmosphere and diffuses through the stomata and the intercellular air spaces, and ends up in the chloroplast (Sharkey et al., 2007; Von Caemmerer, 2000). Inside the chloroplast, the photosynthetic enzyme Rubisco catalyzes the carboxylation of ribulose-1,5-bisphosphate (RuBP) by CO_2 and produces the first product of the Calvin cycle, 3-phosphoglyceric acid (PGA). The generated ATP and NADPH by photosynthetic electron transport are utilized to make sugars and starch, and the regeneration of RuBP (Caemmerer, 2000; Lambers et al., 2008; Yamori et al., 2014). Therefore, the net CO_2 assimilation may be limited by either i) the carboxylation activity of the enzyme Rubisco to catalyze the reaction between CO_2 and ribulose bisphosphate (RuBP), ii) the regeneration of RuBP, or iii) by the sink activity through the export of the triose phosphate to produce starch and sugars (glucose and fructose), and regeneration of inorganic phosphate (Kumarathunge et al., 2019; Sharkey et al., 2007; Von Caemmerer, 2000).

The most common model to represent net photosynthesis is based on these three biochemical processes and they are represented by the maximum rate of Rubisco carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), and triose phosphate use (TPU) (Von Caemmerer, 2000). However, TPU was recently shown not to be the limiting process to net photosynthesis in plants growing in their current ambient growth conditions (Kumarathunge et al., 2019). The photosynthetic capacity parameters (i.e., V_{cmax} and J_{max}) can be determined from the response of net photosynthesis (A_n) to different levels of intercellular carbon dioxide (C_i) at light saturation, responses commonly known as the A - C_i curve. The V_{cmax} is considered as the initial slope of the A - C_i curve at low CO_2 concentrations (< 400 ppm), which demonstrates the control of rubisco capacity to fix CO_2 , and J_{max} is parameterized from the slope of the curve at higher CO_2 concentrations (> 1000 ppm), (Vårhammar et al., 2015) when the curve begins to plateau (Sage, 1990). Moreover, their rates are impacted by different environmental factors including light, nutrients, carbon dioxide (CO_2) concentration, water availability as well as temperature (Kattge et al., 2007; Kenzo et al., 2006).

1.3 Effects of warming on Photosynthetic capacity and stomatal conductance

Temperature is considered as the main environmental factor regulating many physiological processes in plants (Crous, 2019), including plant primary carbon metabolism (Dusenge et al., 2019). Therefore, plants need to adjust their physiological processes to increasing temperatures if they are to maintain their performance in a warming world (Crous, 2019; Dusenge et al., 2019). Thermal acclimation represents morphological and/or physiological adjustments that maintain or improve the performance of plants in response to change in growth temperature (Lambers, 2008; Smith & Dukes, 2013). Prior studies show that plants can acclimate their photosynthetic capacity rates (i.e., V_{cmax} and J_{max}) in response to long-term changes in temperature (Smith & Dukes, 2017; Way & Yamori, 2014). Previous studies mainly conducted on boreal and temperate species found contrasting results on thermal acclimation of V_{cmax} and J_{max} at common temperature (e.g., at 25°C). Some studies reported an increase of V_{cmax} and J_{max} (Crous et al., 2013; Long & Bernacchi, 2003; Perdomo et al., 2016), while, others did not find any change in response to an increase in growth temperature (Bermudez et al., 2020; Stefanski et al., 2019). Similar findings of constant V_{cmax} and J_{max} with warming are commonly reported in meta-analyses (Kattge et al., 2007; Kumarathunge et al., 2019; Way & Oren, 2010). However, other studies reported a decrease in both V_{cmax} and J_{max} (Dusenge et al., 2020; Wang et al., 2020; Way & Sage, 2008). Studies conducted on a small number of tropical tree species under controlled growth conditions (Crous et al., 2018; Scafaro et al., 2017) and in the field (Carter et al., 2020; Fauset et al., 2019) showed that the V_{cmax} and J_{max} do not change with warming. However, only one study on rubber trees reported that the V_{cmax} and J_{max} increased with warming (Kositsup et al., 2009). Therefore, further studies on a larger number of tree species and under field conditions are needed to improve our understanding of warming effects on photosynthetic capacity in tropical forests.

In many studies, downregulation of the $J_{\text{max}}/V_{\text{cmax}}$ ratio in warm-grown plants compared to cool-grown counterparts is reported (Dusenge et al., 2020, 2015; Kumarathunge et al., 2019; Smith & Dukes, 2017; Stefanski et al., 2019; Way & Yamori, 2014). This is explained by a strong proportional decrease found in J_{max} compared to V_{cmax} in response to growth temperature (Kumarathunge et al., 2019). Studies conducted on tropical tree species found that $J_{\text{max}}/V_{\text{cmax}}$ ratio decline with growth temperature (Fauset et al., 2019; Kositsup et al., 2009) while others reported a constant $J_{\text{max}}/V_{\text{cmax}}$ ratio (Scafaro et al., 2017). These contrasting findings highlight the uncertainty and knowledge gap of responses of photosynthetic capacities to warming, especially for tropical tree species. Therefore, since V_{cmax} and J_{max} are key parameters used for representing photosynthesis in Earth System Models, which are used to predict feedback between vegetation and climate in the future, it is strongly important to understand how photosynthetic capacity respond to warming in more realistic field conditions in tropical trees (Mercado et al., 2018; Way & Yamori, 2014).

Alongside biochemical processes of photosynthesis, the stomatal conductance (g_s) is another important parameter that influences net photosynthesis (A_{net}) as it affects the diffusion of CO₂ inside the leaf (Farquhar & Sharkey, 1982). The decrease of g_s has been associated to vapor

pressure deficit (VPD) which is continuously increasing due to global warming (Grossiord et al., 2020). Previous studies indicated that in warmer condition, the increased VPD initiates the reduction in g_s (Oren et al., 1999), therefore leads to reduction of intercellular CO_2 concentration (C_i) through partial closure of stomata and finally reduction in the A_{net} (Hikosaka et al., 2006; Medlyn et al., 2002; Sage & Kubien, 2007). Some studies assessed responses of g_s to warming, found that g_s declined (Dusenge et al., 2020; Fauset et al., 2019), or increased (Marchin et al., 2016) with growth temperatures, but other studies did not find any effect of warming on g_s (Drake et al., 2015). A meta-analysis conducted by (Way & Oren, 2010) showed that g_s does not have consistent responses to warming. However further studies are still required to fully understand this relationship effect of warming on stomatal conductance.

Tropical tree species have trait differences corresponding to their respective successional strategies (Nyirambangutse et al., 2017; Wright et al., 2004). Past studies have indicated that late-successional (LS; shade tolerant and slow-growing) species may be more sensitive to warming than early-successional (ES; shade-intolerant and fast-growing) species mostly associated with the inherently low g_s and large leaf area which altogether increase their leaf temperatures (Vårhammar et al., 2015). A recent study in tropical tree species also showed that ES species have a higher photosynthetic capacity (V_{cmax} and J_{max}) than LS species (Ziegler et al., 2020). However, there is still not enough knowledge as to whether thermal acclimation of photosynthetic capacity between ES species and LS species differs. The knowledge on the responses of different successional strategies to warming is a key factor in determining how warming may affect community tree composition of tropical forests, and how this may feedback to carbon cycling.

1.4 Aim

By attempting to reduce these knowledge gaps, this study explored the effect of warming on photosynthetic capacity (V_{cmax} and J_{max}) in 16 tropical tree species grown in three different field plantations along an elevation gradient. The air temperature at these three sites differs by up to 6.8 °C.

1.5 Hypotheses

The following hypotheses were tested:

H 1: The maximum Rubisco carboxylation (V_{cmax}) and maximum electron transport (J_{max}) at a common leaf temperature of 25 °C remain unchanged with warming.

H 2: Warming leads to downregulation of the J_{max}/V_{cmax} ratio at a common leaf temperature of 25 °C.

H 3: Net photosynthesis (A_{net}) and stomatal conductance (g_s) at a common leaf temperature of 25 °C decline due to increased vapor pressure deficit (VPD) with growth temperature.

H 4: Early-successional species thermally acclimate better than late-successional species.

Materials and Methods

2.1 Site description

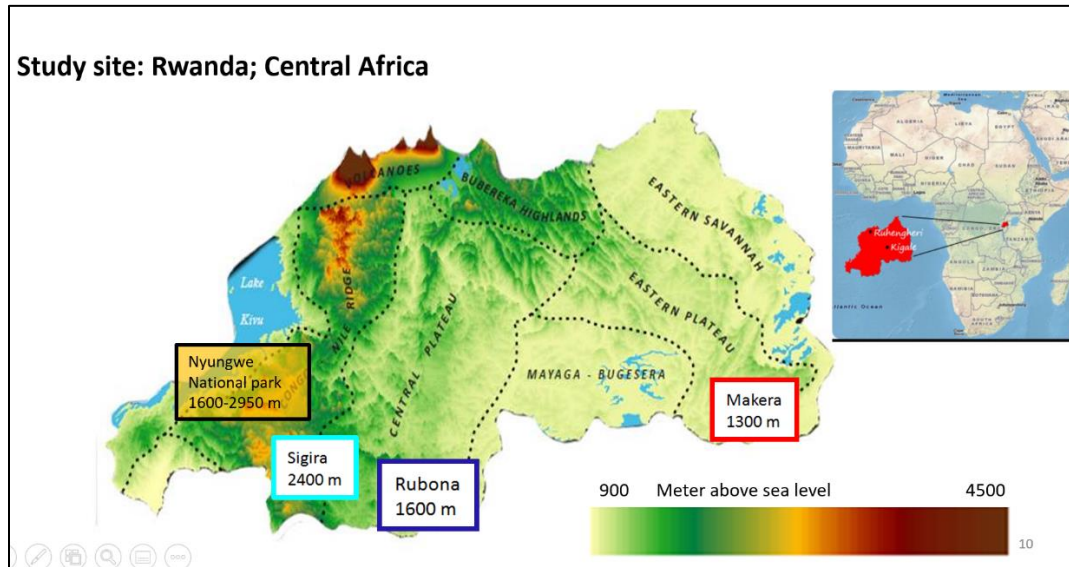


Figure 1: Map of the study area

This study was conducted in experimental tree plantations within the Rwandan-TREE project established in January 2018, at three different sites along an elevation gradient in Rwanda, Central Africa. The three sites demonstrate differences in elevation (1300 – 2400 m.a.s.l.), and climatic conditions (temperature and precipitation). The first site, Sigira is located at the highest elevation (2400 m a. s. l., S2°30'54": E 29°23'44" hereafter denoted "HE") close to Nyungwe National park, in Nyamagabe District, the southwest part of Rwanda. Nyungwe natural forest is one of the largest afro montane rainforest blocks remaining in Africa (Ghehi et al., 2012). The Sigira site is also considered as the control site because the species used in this experiment are native to Nyungwe national Park (Nyirambangutse *et al.*, 2017). The second site, Rubona is installed at mid-elevation (1600 m.a.s.l, S2°28'30"; E29°46'49" hereafter denoted "ME") in Huye District also in the southwestern part of Rwanda. The vegetation in this region is characterized mainly by transitional rain forests. The third and low-elevation site, Makera is located at (1300 m.a.s.l., S2°6'31"; E30°51'16" hereafter denoted "LE") in Kirehe District in the eastern part of Rwanda close to the border with Tanzania. Makera is one of the natural forest complexes, Ibanda-Makera, and characterized by evergreen and semi-evergreen bushland and thicker. These differences in elevation affect also the annual mean air temperature at three sites, Sigira, the highest and coolest site has 15.2 °C, while mid-elevation Rubona and low-elevation Makera have 20.0 °C, and 20.6 °C, respectively. The three sites also show differences in annual precipitation, the highest is found at Sigira (~2100 mm), followed by Rubona (~1700 mm), and lowest at Makera (~1100 mm).

2.2 Plant material

Data were collected on 16 tropical montane tree species with eight early successional (ES) and eight late-successional (LS) from all three sites, and six tree replicates for each species (3x6x16=288) (Table 1). However, two species *Markhamia lutea* and *Ficus thonningii* do not have a clear categorization to either group (Table 1). The plantation was established in January 2018, seedlings were transplanted from the nursery that was located at the intermediate elevation site (Rubona), before being transplanted to experimental plantations at the three sites. Within each plot, plants are spaced by 1.5mx 1.5m. These tree plantations at each site have different water and nutrients treatments, but the data in my thesis were collected in plots of high water (irrigated in Rubona & Makera sites) to match precipitation regimes of the control plots in the Sigira site. All the used plots are not fertilized.

Table 1: Tree species and their successional groups

	Scientific names	Author	Family
Early-successional species			
1	<i>Bridelia brideliifolia</i>	(Pax.) Fedde	Phyllanthaceae
2	<i>Croton megalocarpus</i>	Hutch.	Euphorbiaceae
3	<i>Dombeya rotundifolia</i>	(Hochst.) Planchon	Malvaceae
4	<i>Harungana montana</i>	Spirlet	Hypericaceae
5	<i>Macaranga kilimandscharica</i>	Pax	Euphorbiaceae
6	<i>Maesa lanceolata</i>	Forsk.	Primulaceae
7	<i>Markhamia lutea</i> *	(Benth.) K.Schum.	Bignoniaceae
8	<i>Polyscias fulva</i>	(Hiern) Harms	Araliaceae
Late-successional species			
1	<i>Carapa grandiflora</i>	Sprague	Meliaceae
2	<i>Chrysophyllum gorungosanum</i>	Engl.	Sapotaceae
3	<i>Entandrophragma excelsum</i>	(Dawe & Sprague) Sprague	Meliaceae
4	<i>Faurea saligna</i>		
5	<i>Ficus thonningii</i> *	Blume	Moraceae
6	<i>Prunus africana</i>	(Hook.f.) Kalkman	Rosaceae
7	<i>Syzygium guineense</i>	(Willd.) DC.	Myrtaceae
8	<i>Afrocarpus falcatus</i>	(Thumb) C.N.Page	Podocarpaceae

* Species with unclear categorization in these respective successional groups.

2.3 Gas exchange measurements

Leaf gas exchange measurements were carried out using the LI-6400 portable photosynthesis system, with a 2 x 3 cm leaf cuvette (Li-Cor Inc., Lincoln, NE, USA). These measurements were collected from February to April 2021, between 9 am to 4 pm on sunny and healthy (i.e., without visible damage from herbivores) leaves. However, some trees at the LE site were very tall, and it was difficult to access sunny leaves. Thus, we had to cut a small branch place it in a water-bucket, and re-cut it underwater to reduce the disruption of water transport in the xylem and subsequent stomatal closure as also done by (Dusenge et al., 2015; Ziegler et al., 2020). However, this method was only done on four trees in a total, belonging to *Croton megalocarpus* and *Markhamia lutea* species. Light-saturated net CO₂ assimilation rates (A_n) were measured at varying intercellular CO₂ concentrations (C_i) and generating so-called $A-C_i$ curves. All the $A-C_i$ curves were conducted at a PPFD (photosynthetic photon flux density) of 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, airflow rate of 400 $\mu\text{mol s}^{-1}$, and at a common leaf temperature of 25 °C. In the leaf cuvette, the relative humidity was kept between 60 and 70%. The $A-C_i$ curves were started once the gas exchange was stable at a reference CO₂ concentration of 410 $\mu\text{mol m}^{-2}$, and the CO₂ concentrations were then changed sequentially to 250, 125, 50, 410, 600, 800, 1200, 1600 and 2000 and 410 $\mu\text{mol m}^{-2}$. The leaves of *Afrocarpus falcatus* are small (Figure 2) and do not fit in the entire leaf cuvette, therefore after measurements, I took photos and corrected for leaf area using Image J software.

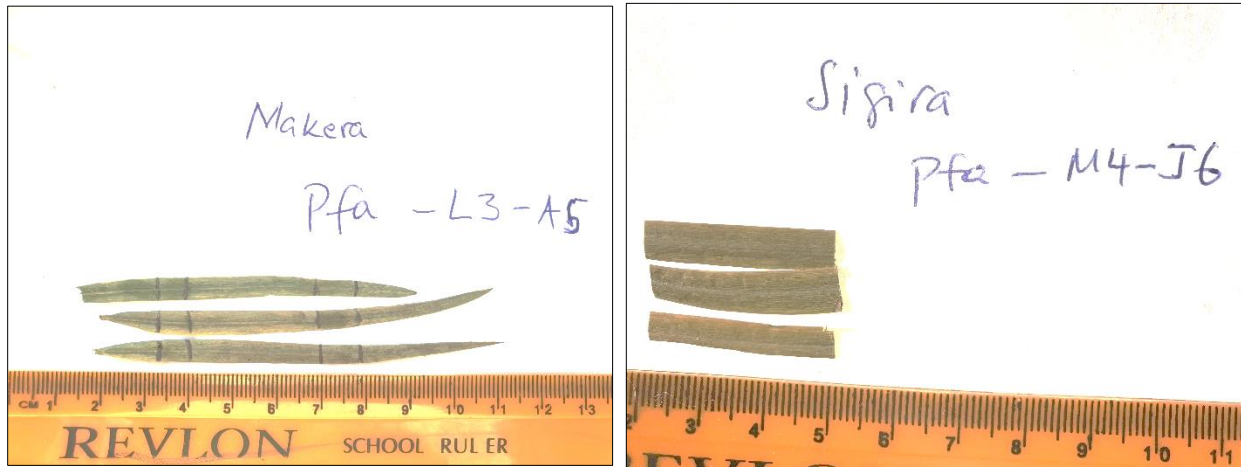


Figure 2: Measurements of *Afrocarpus falcatus* leaves for area correction.

2.4 $A-C_i$ curve fitting

The C₃ biochemical model of photosynthesis by (Farquhar et al., 1980) was used to parameterize both the maximum Rubisco carboxylation (V_{cmax}) and maximum electron transport (J_{max}) from the $A-C_i$ curves. The $A-C_i$ curves were fitted using the *plantecophys* R package for modeling and analysis of Leaf Gas Exchange, and the bilinear fitting method was used (Duursma, 2015). None

of the curves was visibly limited by TPU. The J_{\max} was parameterized only if there were at least two data points that were fitted by the J_{\max} -limited photosynthesis (A_j) curve at any C_i concentration or one single data point at $C_i > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. Based on this criterion, eight curves out of 288 A - C_i curves were not parameterized for J_{\max} . All the curves were parameterized at a leaf temperature of 25 °C. The site-specific atmospheric pressure was considered during the fitting. To explore any potential effects of warming on stomatal conductance (g_s), net photosynthesis (A_{net}), and vapor pressure deficit (VPD_L), I extracted these data in my A - C_i curve measurements. However, there was some site-specific variation in vapor pressure deficit in the leaf cuvette (VPD_L) (Figure S3, Table S4) due to variability in measurements leaf temperature being not always at exactly 25 °C as well as varying relative humidity across sites (Table S4).

Equations used by the C_3 photosynthesis model are following:

$$A_c = \frac{V_{\text{cmax}}(C_i - \Gamma^*)}{\left[C_i + K_c \left(1 + \frac{O}{K_o} \right) \right]} - R_{\text{day}} \quad [\text{Equation 1}]$$

Where V_{cmax} -limited photosynthesis (A_c), the carboxylation-limited photosynthesis, V_{cmax} is the maximum rate of carboxylation, Γ^* is the CO_2 compensation point in absence of mitochondrial respiration, K_c and K_o are Michaelis-Menten constants of the activity of Rubisco concerning CO_2 and O_2 , and O is the intercellular concentration of O_2 .

$$A_j = \left(\frac{J}{4} \right) \frac{(C_i - \Gamma^*)}{(C_i + 2\Gamma^*)} - R_{\text{day}} \quad [\text{Equation 2}]$$

Where J_{\max} is the rate of electron transport

2.5 Statistical analysis

The effects of growth temperature on photosynthetic capacity (V_{cmax} and J_{\max}), g_s , and A_{net} were tested using a mixed-effects two-way ANOVA with the site and the successional group as the main factors and species as a random factor. All analyses were done using R (R Core Team, 2021). The analyses were done using *asreml* package, and F- and p-values were generated using the conditional Wald- F statistic (Butler et al., 2017). The effects were judged to be statistically significant when the p-value < 0.05. The *Markhamia lutea* (*Mlu*), an *ES* species, and *Ficus thonningii* (*Fth*), *LS* species have shown behaviors of the successional group to which they are not belonging. To assess these behaviors, I conducted statistical analyses in two ways, one analysis including two species and another one excluding them. Statistical analysis and figures including all species were put in the supplementary material, hereafter denoted “S”.

Results

The $J_{\max 25}$, and $J_{\max 25}/V_{\max 25}$ ratio were different among sites (Table 2, S4), while $V_{\max 25}$, g_s , and A_{net} remained constant across sites (Table 2, S4). In addition, the $V_{\max 25}$, $J_{\max 25}$, g_{s25} , and $A_{\text{net}25}$ were different between successional groups (Table 2, S4). However, the combination of site and successional groups was not significant for all parameters investigated ($V_{\max 25}$, $J_{\max 25}$, $J_{\max 25}/V_{\max 25}$ ratio, g_{s25} , $A_{\text{net}25}$) for both analyses including and without *Markhamia lutea* (*Mlu*) and *Ficus thonningii* (*Fth*) species (Table 2, S4).

Table 2: P-values for effects of site, successional group, and their combination, without *Mlu* and *Fth*

Mixed-effects ANOVA			
	Site	Successional group	Site x successional group
$J_{\max 25}$	0.005	0.005	0.244
$V_{\max 25}$	0.412	0.002	0.696
$J_{\max 25}/V_{\max 25}$	0.003	0.208	0.961
g_s	0.326	0.006	0.248
A_{net}	0.429	0.006	0.583

Values in **bold** indicate significant p-values ($p < 0.05$). Maximum Rubisco carboxylation; $V_{\max 25}$, Maximum Electron transport $J_{\max 25}$, $J_{\max 25}/V_{\max 25}$ ratio; $A_{\text{net}25}$, Net Photosynthesis; g_{s25} , stomatal conductance.

3.1 Effects of warming on photosynthetic capacity ($V_{\max 25}$, $J_{\max 25}$, and $J_{\max 25}/V_{\max 25}$ ratio)

The $V_{\max 25}$ did not change across sites in either analysis including all species or without *Mlu* and *Fth* (Table 2, S4, Figure 4b). Successional groups differ in $V_{\max 25}$ rates (Table 2, S4). The $V_{\max 25}$ of ES species was higher than LS species by 57%, 62%, and 46% at HE, ME, and LE, respectively (Figure 4b). The $J_{\max 25}$ decreased by 15 and 14% at ME and LE sites, respectively compared to species planted at the HE site (Figure 3, 4a, S1, Table 2, S4). The $J_{\max 25}$ between successional groups were different without the two species (Table 2). However, the $J_{\max 25}$ of ES and LS species did not differ including two species (Figure S1, Table S4). The $J_{\max 25}$ of ES species was higher than in LS species by 41%, 51%, and 25%, at HE, ME, and LE, respectively. The J_{\max}/V_{\max} ratio declined by 4% and 11% lower at ME and LE sites, respectively compared to species grown at the HE (Figure 4c, Table 2, S4). Successional groups did not differ either with or without *Mlu* and *Fth* (Table 2, S4).

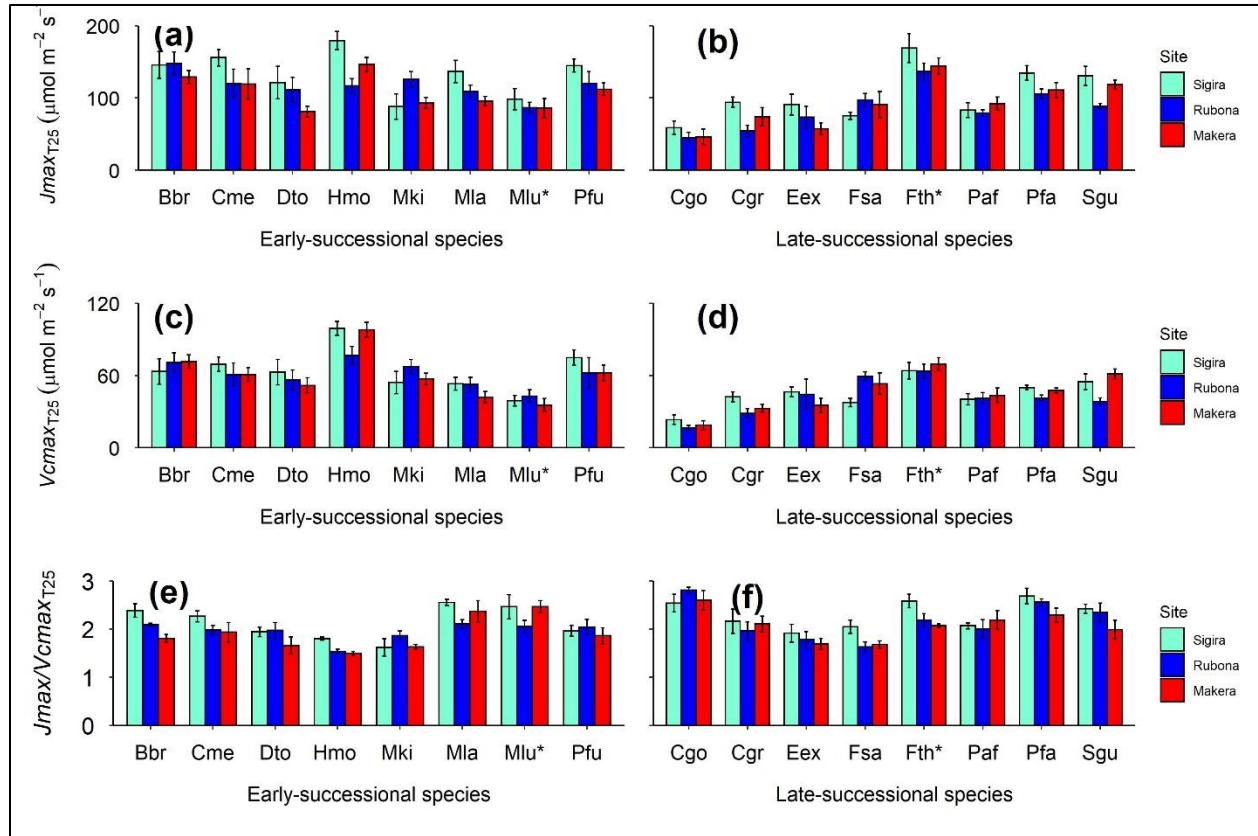


Figure. 3 Photosynthetic capacity in eight early-successional (a,c,e) and eight late-successional species (b,d,f) grown at three different sites in Rwanda-TREE. Maximum electron transport rate (J_{max25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (a, b), Maximum carboxylation rate of Rubisco (V_{cmax25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (c,d) and (e,f) ratio of J_{max25} to V_{cmax25} (J_{max25}/V_{cmax25}) measured at a common leaf temperature of 25°C. Colors represent different sites (Aquamarine, high-elevation, Sigira site, HE; Blue, mid-elevation Rubona site, ME; Red, low-elevation Makera site, LE). Means \pm SE. $n = 4-6$. Abbreviations on the x-axis represent the 16 species (see full names in Table 1)

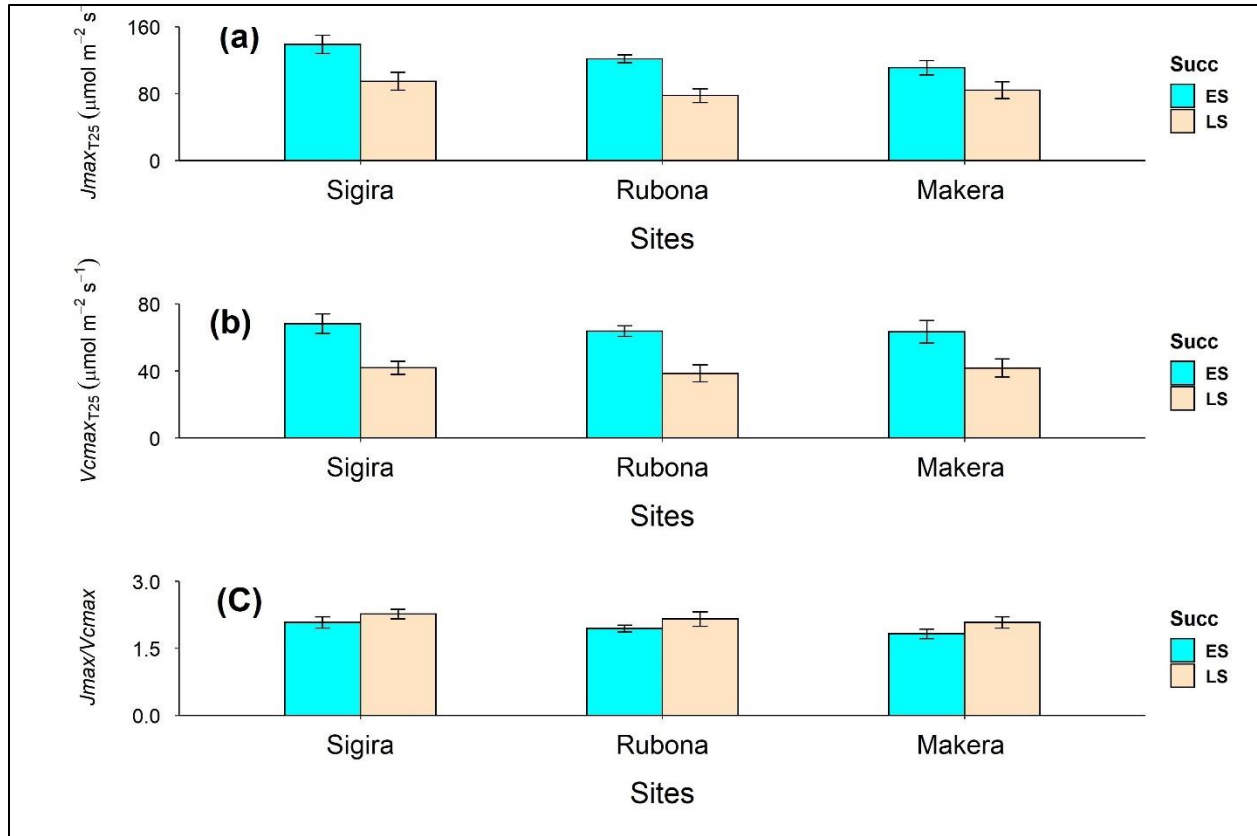


Figure. 4 Photosynthetic capacity at a common temperature of 25°C in seven early-successional and seven late-successional species grown at three different sites in Rwanda-TREE. Averages of maximum electron transport rate (J_{max25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (a), Maximum carboxylation rate of Rubisco (V_{cmax25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (b) and J_{max25}/V_{cmax25} ratio (c). Colors represent successional groups (Beige, early-successional, Bisque, late-successional). the x-axis represents different sites (Sigira site, high-elevation, HE; Rubona site, mid-elevation, ME; Makera site, low-elevation LE). Means \pm SE. $n=7$ for ES and $n=7$ for LS. (Tukey's post hoc test, $P < 0.05$).

3.2 Effects of warming on stomatal conductance (g_s) and Net Photosynthesis (A_{net})

The g_s at 25 °C remained constant across sites (Figure 6, Table 2, S4). Successional groups did not differ when all species are included (Table 2), however, after excluding the *Mlu* and *Fth* species, the g_{s25} was higher in ES species by 77%, 146%, and 86% at HE, ME, and LE sites, respectively (Figure 6). There was no effect of warming on A_{net} at 25 °C since it remained constant across all sites (Figure 6b, Table 2, S4), but LS had lower A_{net25} compared to ES species by 63%, 81%, and 66% at LE, ME, and HE sites, respectively.

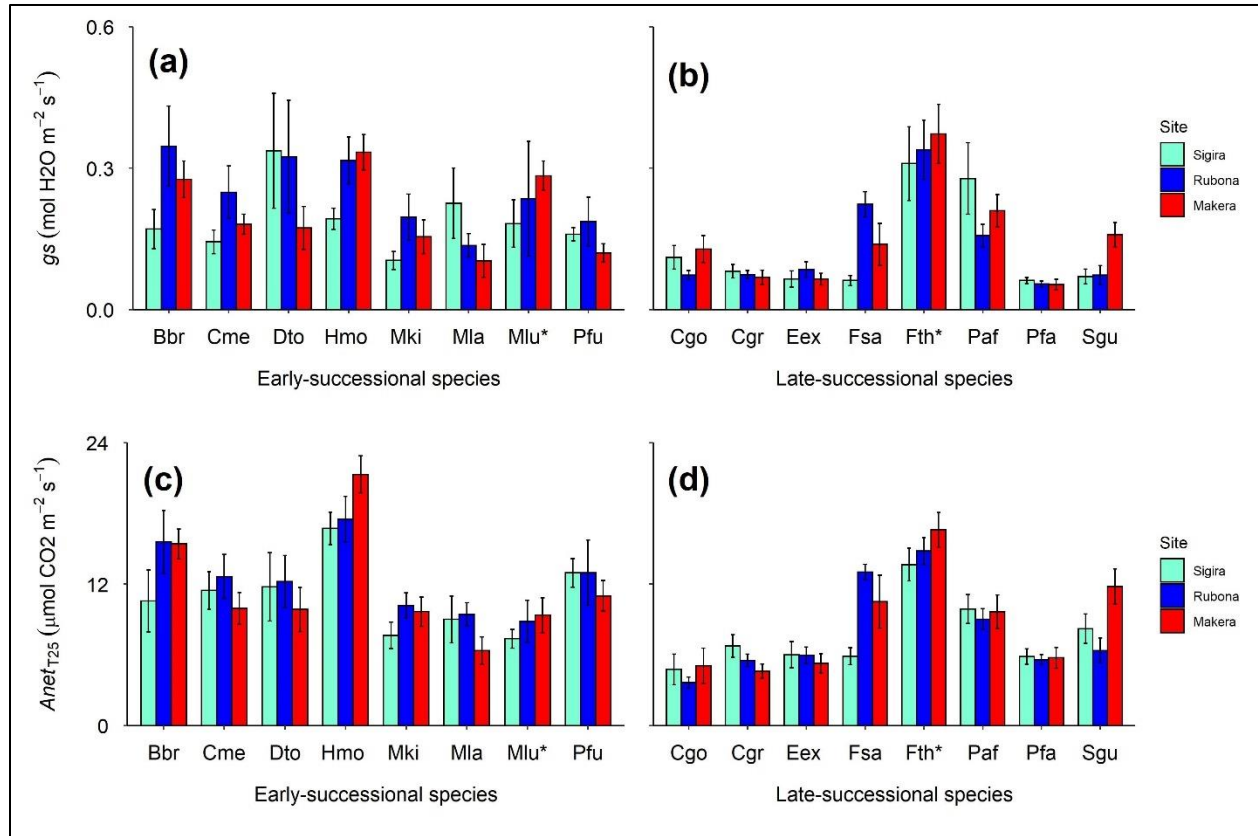


Figure. 5 Stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) (a,b), and Net Photosynthesis (A_{net} , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) (c,d) of eight early-successional (a,c) and eight late-successional species (b,d) measured at the reference CO_2 concentration of $410 \mu\text{mol m}^{-2}$ when the gas exchange was stable and at a common leaf temperature of 25°C . Colors represent different sites (Aquamarine, high-elevation, Sigira site, HE; Blue, mid-elevation Rubona site, ME; Red, low-elevation Makera site, LE). Means \pm SE. $n = 4-6$. Abbreviations on the x-axis represent the 16 species (see full names in Table 1)

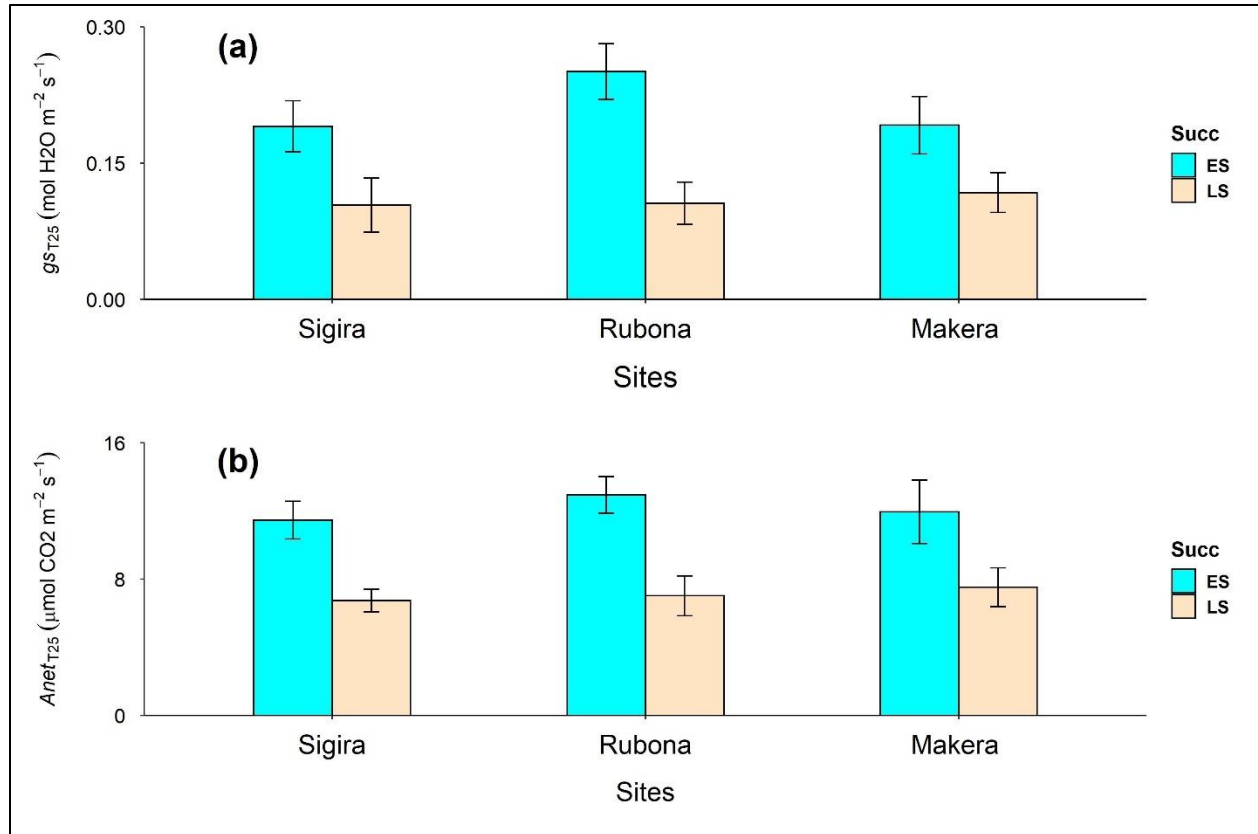


Figure. 6 Averages of stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) (a), and Net Photosynthesis (A_{net} , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) (b) measured at a common leaf temperature of 25°C for seven early- successional and seven late-successional. Colors represent successional groups (Beige, early-successional, Bisque, late-successional). the x-axis represents different sites (Sigira site, high-elevation, HE; Rubona site, mid-elevation, ME; Makera site, low-elevation LE). Means \pm SE. $n=7$ for Early successional and $n=7$ for Late successional. (Tukey's post hoc test, $P < 0.05$).

Discussion

This field study explored the effects of warming on photosynthetic capacity (i.e., $V_{\text{cmax}25}$ and $J_{\text{max}25}$), stomatal conductance (g_s), as well as net photosynthesis (A_{net}) in 16 tropical montane tree species grown at three different sites along an elevation gradient in the Rwanda-TREE project. $V_{\text{cmax}25}$ and $J_{\text{max}25}$ showed contrasting warming responses. The $J_{\text{max}25}$ and $J_{\text{max}25}/V_{\text{cmax}25}$ ratio significantly declined in warm-grown species compared to species grown at higher, and cooler HE site (Figure 4a, c, Table 2). However, $V_{\text{cmax}25}$, g_{s25} , and $A_{\text{net}25}$ remained constant across three sites whether *Mlu* and *Fth* are included or not (Table 2, S4). The successional groups did not show differences in response to warming, however, early successional (ES) species demonstrated higher rates in $J_{\text{max}25}$, $V_{\text{cmax}25}$, g_{s25} , and $A_{\text{net}25}$ than late-successional (LS) species across all three sites (Figure 4a, b, 6a, b, Table 2, S4). But with all species included, only $V_{\text{cmax}25}$ and A_{net} rates were different between ES and LS species (Table S4).

4.1 Effect of growth temperature on photosynthetic capacity

In this study, the $V_{\text{cmax}25}$ remained constant across the sites (Figure 4b, Table 2), and this was in line with our first hypothesis. These results confirm what has been found by different field studies conducted in boreal and temperate species (Bermudez et al., 2020; Lamba et al., 2018; Stefanski et al., 2019) as well as in meta-analyses (Kumarathunge et al., 2019; Way & Yamori, 2014). Similar results were also found on tropical species in controlled environments (Crous et al., 2018) and field studies (Carter et al., 2020; Fauset et al., 2019). However, these results are in contradiction with other studies that reported a decrease of V_{cmax} mainly based on the optimality theory (Wang et al., 2020). The optimality theory suggests that in warmer conditions, the photosynthetic enzymes are very active and fast so that the net CO_2 assimilation will be done with lower photosynthetic enzymes compared to cool grown plants (Smith & Keenan, 2020). Therefore, as photosynthetic capacity (i.e., V_{cmax} and J_{max}) involves also enzymatic reactions, then the photosynthetic capacity of warmer grown plants should decrease (Wang et al., 2020). Previous studies have shown that V_{cmax} rates are strongly linked to nutrients content especially leaf nitrogen, and changes in V_{cmax} with warming were shown to correlate with changes in the leaf N and Rubisco (Kattge et al., 2009). Results in my thesis would have been more informative on the potential mechanisms driving these non-significant changes in V_{cmax} rates with warming if, at least, the data on nitrogen concentration were included. Unfortunately, the leaf nitrogen concentration was not analyzed in this study due to time constraints.

The J_{max} decreased across sites with increasing growth temperature (Figure 4a, Table 2). Similar findings were reported in boreal and temperate species in controlled growth conditions (Dusenge et al., 2020; Way & Sage, 2008), but contradict results from field studies (Bermudez et al. 2020; Stefanski et al. 2019). Surprisingly, other studies on tropical species found J_{max} to remain constant in controlled growth conditions (Crous et al., 2018) and the field conditions (Fauset et al., 2019; Scafaro et al., 2017) or to increase in response to warming (Kositsup et al., 2009). The current findings are not in the agreement with the first hypothesis because J_{max} did not remain

constant as hypothesized. The decrease in J_{\max} with warming is the response supported by the optimality theory (Smith & Keenan, 2020; Wang et al., 2020). In addition, the acclimation of J_{\max} found in this study can also be driven by the high sensitivity of J_{\max} to the temperature that was reported in tropical montane tree species (Vårhammar et al., 2015). This is due to its dependence on electron transport in the thylakoid membrane, and at high temperatures, it becomes destabilized, and membranes become more fluid. Therefore, the ATP/NADPH required to regenerate RuBP can be reduced and hence reduction of carbon assimilation (Vårhammar et al., 2015; Way & Oren, 2010). The high daily maximum temperature combined with high photosynthetic photon flux density (PPFD) at lower elevation sites (Mujawamariya et al., 2020) may have led to extreme leaf temperature which may have led to a decline in J_{\max} . The decline in J_{\max} in warm grown species has also been linked to a decline in leaf nitrogen and pigments (chlorophyll and carotenoid) concentrations that have led to a decrease in RuBP regeneration in warm grown species (Crous et al., 2018; Dusenage et al., 2020; Scafaro et al., 2017; Way & Sage, 2008). Unfortunately, the leaf nitrogen was not measured in this study to be able to confirm these correlations. Further field studies should consider the measurements of chlorophyll to have a clear understanding of warming effects on J_{\max} .

This study found that the J_{\max}/V_{\max} ratio declined significantly with a warming which confirms our second hypothesis (Figure 4c, Table 2). The same results were also reported in previous studies (Bermudez et al., 2020; Crous et al., 2018; Dusenage et al., 2020, 2015; Kattge et al., 2007; Kumarathunge et al., 2019; Stefanski et al., 2019). However, there are also other studies contrasting with our results and indicated no change in J_{\max}/V_{\max} ratio due to warming (Scafaro et al., 2017). Nevertheless, some studies on tropical species, similarly, found to decline the J_{\max}/V_{\max} ratio due to growth temperature (Crous et al., 2018; Kositsup et al., 2009). The decline of the J_{\max}/V_{\max} ratio in my study is driven by the decrease of J_{\max} while V_{\max} remained constant, in other studies the decline was justified by the fact that J_{\max} proportionally decreased more than V_{\max} (Sage & Kubien, 2007; Way & Yamori, 2014). The decline in J_{\max}/V_{\max} ratio has been also associated with the adjustments in the allocation of leaf nitrogen (N) between V_{\max} and J_{\max} processes (Hikosaka et al., 2006; Scafaro et al., 2017). Unfortunately, due to time constraints, the leaf nitrogen was not measured to confirm this partitioning of N between J_{\max} and V_{\max} .

4.2 Effect of growth temperature on stomatal conductance (g_s)

The g_{s25} remained constant across the sites (Figure 6a, Table 2). Similar findings have been also found in previous studies (Drake et al., 2015). These findings are in contrast with the third hypothesis, however, other studies indicated the decrease of g_{s25} in response to growth temperature as we hypothesized (Fauset et al., 2019; Lamba et al., 2018). The decrease of g_{s25} is directed by short time increased vapor pressure deficit (VPD) through stomata partial closure as a mechanism of water-saving through transpiration (Grossiord et al., 2020; Lange et al., 1971; Medlyn et al., 2002; Oren et al., 1999). In this study, VPD in the leaf cuvette (VPD_L) unintentionally increased at warm sites (Figure S3, Table S4) due to variability in measurements leaf temperature being not always at exactly 25 °C as well as varying relative humidity across sites (Table S4). Therefore,

this variability in VPD_L may have masked any potential acclimation response of g_s in my study. More accurate measurements of g_s are needed to assess the thermal acclimation of g_s in this experiment.

4.3 Effect of growth temperature on net photosynthesis (A_{net})

The A_{net25} at the common temperature (25° C) remained unchanged across sites (Figure 6b, S2b, Table 2, S4). While these findings contradict our fourth hypothesis, they show that the net photosynthesis is probably driven by V_{cmax} and g_s that remained constant also in this study. These findings are in agreement with other studies indicating that at ambient CO_2 concentration, photosynthesis is limited by Rubisco carboxylation rather than RuBP regeneration (Hikosaka et al., 1999, 2006).

4.4 Warming sensitivity of successional groups

The ES species were different from the LS species in V_{cmax} , A_{net} (Figure 4b,6b S1b, 2b, Table 2, S4), J_{max} , and g_s rates (Figure 4a,6a Table 2). The exclusion of *Mlu* and *Fth* showed a strong difference between ES and LS than analysis with all species. This indicates the possible exchange or swap of these two species in the successional groups they are not belonging to and suggesting likely errors that can be generated by the inclusion of two species. The ES species exhibited higher rates than the LS species for all these parameters tested. These results are in contradiction with the fourth hypothesis because I did not find any interaction between the successional group and site, suggesting no differences in thermal acclimation of photosynthetic capacity between the two groups. However, ES species showed higher photosynthetic capacity (J_{max} and V_{cmax}), stomatal conductance (g_s), and net photosynthesis (A_{net}) than LS species but similar J_{max}/V_{cmax} ratio, and these findings were recently reported in a study from Nyungwe forest where the species used in this study come from (Ziegler et al., 2020).

Conclusion

This study showed downregulation of J_{max} , while V_{cmax} , g_s , and A_{net} remained constant in 14 species grown at mid and lowest sites across along elevation gradient. In all parameters explored, late-successional species showed lower rates than early successional species however, there were no interactions between site and successional groups. Considering the high number of species used in my study, I can conclude that tropical montane tree species growing in natural field conditions do not thermally acclimate their Rubisco carboxylation capacity but show some thermal acclimation in maximum electron transport rates. My results also indicate that net photosynthesis is biochemically driven by V_{cmax} . Overall, my results show that tropical tree species can adjust investments to Rubisco and electron transport capacity to physiologically acclimate to a warmer environment (i.e. that they are not fixed and unable to thermally acclimate, as sometimes

suggested). And these results suggest also that tree species belonging to different successional groups similarly acclimate photosynthesis. Although there is uncertainty to what extent these trees will acclimate their electron transport rates because global warming is expected to increase in the future which will have negative effects especially tropical growth primary productivity.

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Supplementary Materials

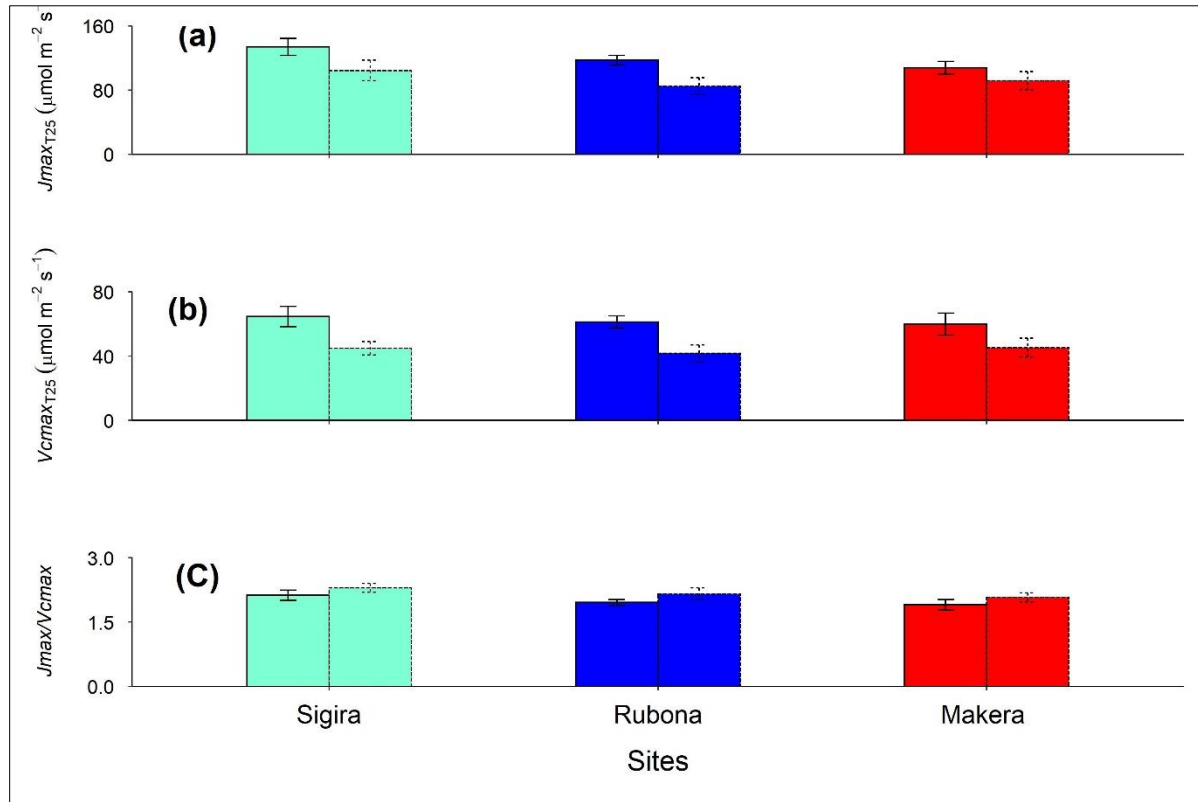


Figure S1. Photosynthetic capacity at a common temperature of 25°C in early-successional and late-successional species grown at different sites in Rwanda-TREE. Averages of maximum electron transport rate (J_{max25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (a), and Maximum carboxylation rate of Rubisco (V_{cmax25} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) (b) and J_{max25}/V_{cmax25} ratio (c). Bar lines represent successional groups (early- successional, solid; late- successional, dashed). Colors represent different sites (Aquamarine, high-elevation, Sigira site, HE; Blue, mid- elevation Rubona site, ME; Red, low-elevation Makera site, LE). Means \pm SE. $n=8$ for ES and $n=8$ for LS.

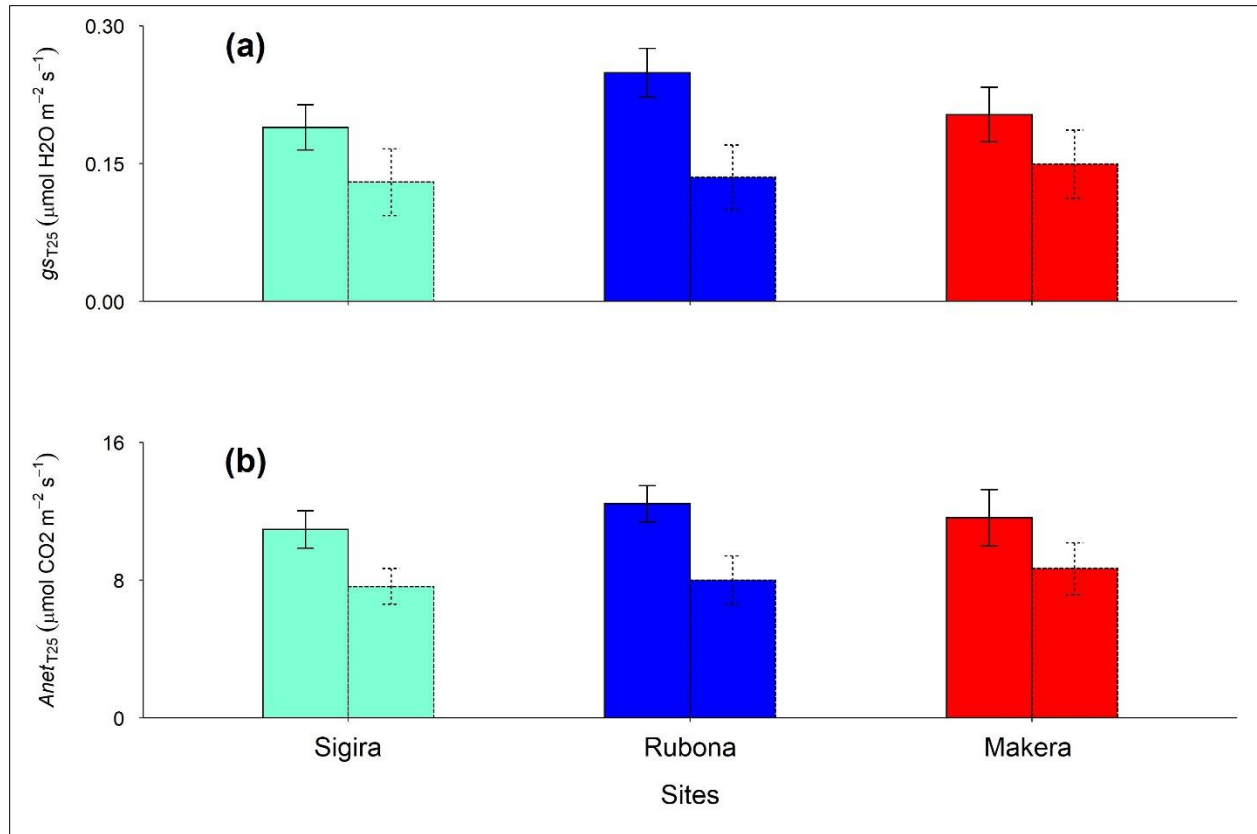


Figure S2. Averages of stomatal conductance (g_s , $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) (a), and Net Photosynthesis (A_{net} , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) (b) measured at a common leaf temperature of 25°C for 16 species. Bar lines represent successional groups (early- successional, solid; late- successional, dashed). Colors represent different sites (Aquamarine, high-elevation, Sigira site, HE; Blue, mid- elevation Rubona site, ME; Red, low-elevation Makera site, LE). Means \pm SE. $n=8$ for Early successional and 8 for Late successional.

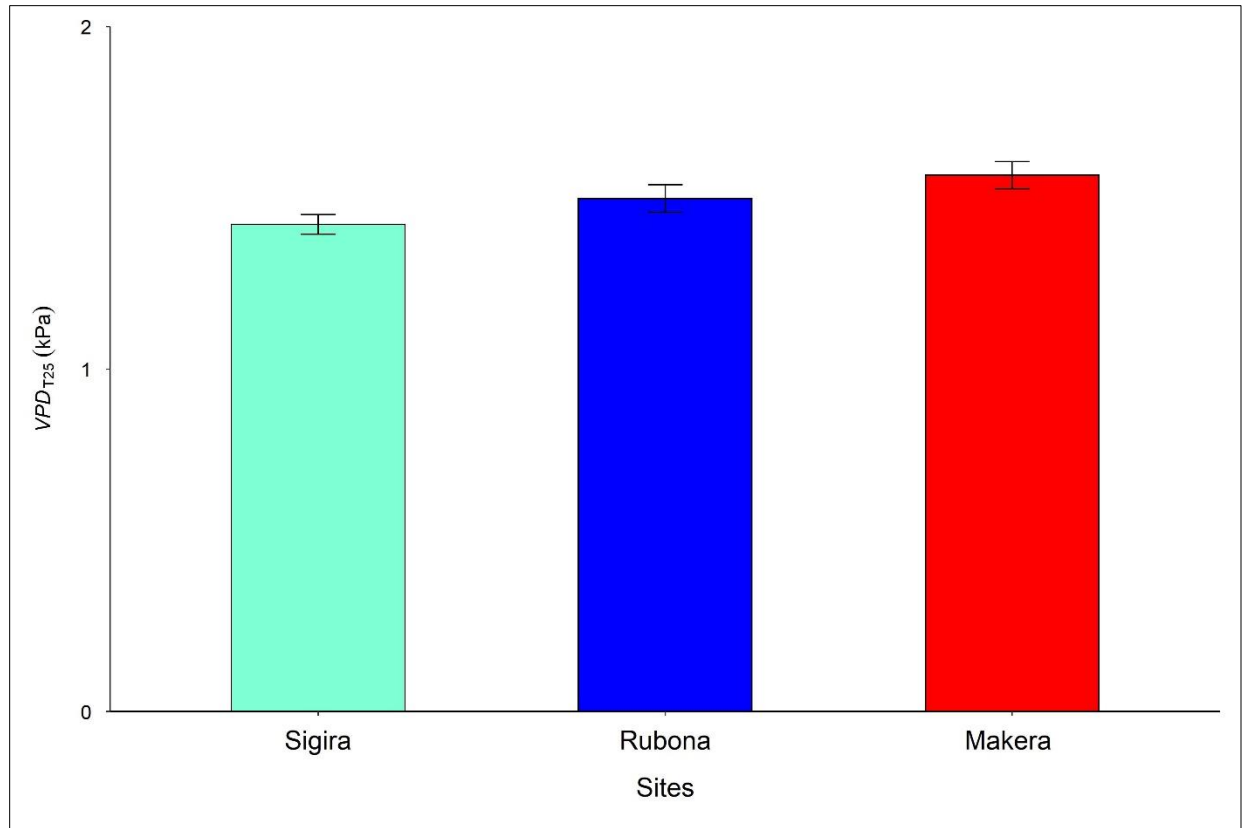


Figure S3. Averages of Vapor Pressure Deficit (VPD, kPa) (c) measured at a common leaf temperature of 25°C. Colors represent different sites (Aquamarine, high-elevation, Sigira site, HE; Blue, mid-elevation Rubona site, ME; Red, low-elevation Makera site, LE). Means \pm SE. n=16.

Table S4. P-values for effects of site, successional group, and their combination including all species.

Mixed-effects ANOVA			
	Site	Successional group	Site x successional group
V_{cmax}	0.470	0.027	0.512
J_{max}	0.001	0.070	0.253
J_{max}/V_{cmax}	0.001	0.250	0.921
gs	0.262	0.079	0.276
A_{net}	0.246	0.058	0.525
VPD	0.004	0.640	0.747

S6. Mixed-effects ANOVA with all species

Wald tests for fixed effects.

Response: Vcmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	216.500	216.500		0.00000
Site	2	27.9	0.769	0.775	A	0.47045
Succ	1	14.0	6.094	6.094	A	0.02705
Site:Succ	2	27.9	0.684	0.684	B	0.51269

Wald tests for fixed effects.

Response: Jmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	264.400	264.400		0.000000
Site	2	28.4	8.481	8.507	A	0.001272
Succ	1	14.0	3.829	3.829	A	0.070685
Site:Succ	2	28.5	1.442	1.442	B	0.253135

Wald tests for fixed effects.

Response: Jmax.Vcmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	847.30	847.30		0.00000
Site	2	28.3	7.96	7.94	A	0.00183
Succ	1	14.0	1.44	1.44	A	0.25063
Site:Succ	2	28.4	0.08	0.08	B	0.92109

Wald tests for fixed effects.

Response: Anet

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	133.600	133.600		0.00000
Site	2	28.1	1.480	1.471	A	0.24676
Succ	1	14.0	4.260	4.260	A	0.05805
Site:Succ	2	28.1	0.658	0.658	B	0.52567

Wald tests for fixed effects.

Response: gs

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	79.100	79.100		0.000000
Site	2	28.5	1.407	1.401	A	0.262850
Succ	1	14.0	3.583	3.583	A	0.079169
Site:Succ	2	28.5	1.344	1.344	B	0.276796

Wald tests for fixed effects.

Response: VPD

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	14.0	3113.00	3113.00		0.000000
Site	2	28.1	6.53	6.54	A	0.00467
Succ	1	14.0	0.23	0.23	A	0.64093
Site:Succ	2	28.1	0.29	0.29	B	0.74765

S6. Mixed-effects ANOVA without Mlu and Fth

Wald tests for fixed effects.

Response: Vcmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	12.0	265.800	265.800		0.00000
Site	2	23.9	0.908	0.919	A	0.41249
Succ	1	12.0	13.750	13.750	A	0.00299
Site:Succ	2	24.0	0.367	0.367	B	0.69669

Wald tests for fixed effects.

Response: Jmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	11.9	361.80	361.80		0.000000
Site	2	24.3	6.49	6.55	A	0.005300
Succ	1	11.9	11.73	11.73	A	0.005064
Site:Succ	2	24.4	1.49	1.49	B	0.244451

Wald tests for fixed effects.

Response: Jmax.Vcmax

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	12.0	706.70	706.70		0.000000
Site	2	24.4	7.15	7.12	A	0.00367
Succ	1	12.0	1.77	1.77	A	0.20832
Site:Succ	2	24.5	0.04	0.04	B	0.96132

Wald tests for fixed effects.

Response: Anet

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	12.0	158.200	158.200		0.000000
Site	2	24.1	0.885	0.874	A	0.42986
Succ	1	12.0	10.570	10.570	A	0.00692
Site:Succ	2	24.1	0.551	0.551	B	0.58365

Wald tests for fixed effects.

Response: gs

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	12.1	106.700	106.700		0.000000
Site	2	24.4	1.174	1.171	A	0.32685
Succ	1	12.1	10.670	10.670	A	0.00669
Site:Succ	2	24.4	1.473	1.473	B	0.24891

Wald tests for fixed effects.

Response: VPD

	Df	denDF	F.inc	F.con	Margin	Pr
(Intercept)	1	12.0	2915.00	2915.00		0.00000
Site	2	24.1	5.84	5.86	A	0.00844
Succ	1	12.0	0.50	0.50	A	0.49346
Site:Succ	2	24.1	0.53	0.53	B	0.59793