

Photosynthesis ecology of tropical forests in Rwanda



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**Uppsats för avläggande av naturvetenskaplig masterexamen i
Miljövetenskap**

30 hp

**Institutionen för biologi och miljövetenskap
Göteborgs universitet**

Naturvetenskapliga
fakulteten



GÖTEBORGS UNIVERSITET

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MSc thesis 30 credits, 2014

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Abstract

The observed climate change is a result of increasing greenhouse gases in the atmosphere from anthropogenic activities which cause changes in the planet's energy balance. Photosynthesis is one of the most important plant physiological processes that link the plant to its environment; using CO₂ as a reactant, assigns a prominent role in carbon sequestration to world forests storing more than half of the global carbon. However, photosynthesis is sensitive to temperature. Therefore the responses and feedbacks of forests to a changing climate will significantly influence atmospheric CO₂ cycle and ultimately future climate.

The basis of variation in photosynthetic capacities among tropical trees is poorly explored and understood, in particular for African forests. In order to reduce this knowledge gap, measurements of leaf traits and gas exchange were conducted on sun and shade leaves of ten tree species growing in two tropical forests in Rwanda in central Africa. Seven species were studied in Ruhande Arboretum, a forest plantation at mid altitude (~1700 m), and six species in Nyungwe National Park, a cooler and higher altitude (at ~2500 m) montane rainforest. Three species were common to both sites and in Nyungwe three species each belonged to the successional groups pioneer and climax species. Climax species had considerably lower maximum rates of photosynthetic carboxylation (V_{cmax}) and electron transport (J_{max}) than pioneer species. This difference was not related to leaf nutrient content, but rather seemed to be caused by differences in within-leaf nutrient allocation between the two successional groups. With respect to N, leaves of climax species invested less N into photosynthetic enzymes and more N into chlorophyll. Across the entire dataset, variation in photosynthetic capacities among species was not related to leaf nutrient content. Photosynthetic capacities, (i.e., J_{max} and V_{cmax}), $J_{\text{max}}:V_{\text{cmax}}$ ratio and P content were significantly higher at the cooler site (Nyungwe). This study contributes important tropical data for global terrestrial biosphere models and suggests that, for montane rainforest trees of different functional types, within-leaf N allocation is a stronger determinant of photosynthetic capacities than leaf nutrient content. Within-leaf N allocation in turn seems to be a leaf trait linked to successional group identity.

Key words: *montane rainforest, nitrogen phosphorous, SPAD, temperature*

Acknowledgement

I am thankful to the Almighty God and His son Jesus Christ for everything. Its only by His Grace and Love I can still type these letters from my computer while writing this thesis.

I am very grateful to my supervisors Johan Uddling Fredin and Göran Wallin. Without their heart full of compassion for success of my studies as well as my welfare both in Sweden in Rwanda, I couldn't have done what you are about to read in following pages. Without having met them I couldn't have been able to realize my dream of continuing my master studies in Europe especially in Sweden.

Special thanks go to Thomas Berg Hasper and Brigitte Nyirambangutse for their help and support in my everyday life in Sweden.

Very special thanks to my friends, colleagues and who have also contributed much during the data collection and analysis of the present study especially Johanna Gårdesten, Felix Niyonzima, Lisa Adolfsson and Donat Nsabimana (also being a local supervisor in Rwanda).

I am so grateful to all members of my research group AiroPlant in Department of Biological and Environmental Sciences at University of Gothenburg, especially Håkan Pleijel, Maria Grundström, Shubhangi Lamba, Tage Vowles, Angelica af Ekenstam, Martina Lutz, Linda Hansson, Åslög Dahl and Mats Röntfors, for their fruitful comments, corrections, encouragements and support during my project also my Master studies at the University of Gothenburg.

Thanks go to Etienne Zibera for his help during field work in Nyungwe National Park and also to Emmanuel for climbing trees to bring down branches for measurement.

I also appreciate support from my family especially of prayers and encouragements during my studies.

I am thankful to the Swedish Government who supported me by a scholarship within the Global university initiative at University of Gothenburg, Sweden. We are grateful to Rwanda Agricultural Board (RAB) Ruhande and Rwanda Development Board (RDB) that authorized data collection in Ruhande Arboretum and Nyungwe National Park, respectively.

Table of contents

Abstract	1
Acknowledgement	2
Table of contents	3
1. Introduction.....	4
1.1 Climate change.....	4
1.2 Photosynthesis.....	4
1.3 Tropical forests	5
1.4 Aims and hypotheses	7
2. Material and Methods	10
2.1 Study sites and plant species.....	10
Nyungwe National Park	10
Ruhande Arboretum.....	10
2.2 Gas exchange measurements	11
2.3 Photosynthesis model parameterisation	12
2.4 Other leaf traits	12
2.5 Statistical analyses	13
3. Results.....	14
4. Discussion.....	20
5. Conclusions.....	22
References.....	23
Appendices.....	31

1. Introduction

1.1 Climate change

The Earth's climate system is strongly regulated by the solar energy and water through exchange of heat and moisture between atmosphere and Earth surface (Chahine 1992; Kandel 2012). Of the incoming solar radiation (Shortwave radiation, SWR), 50% is absorbed by the Earth surface, 30% is reflected back to space by gases and aerosols, clouds and Earth's surface and about 20% is absorbed by the atmosphere (Trenberth et al. 2009; Kandel 2012; Cubasch et al. 2013). A high amount of the radiation emitted by the Earth surfaces (longwave radiations, LWR) is absorbed by the atmosphere constituents including water vapour and other greenhouse gases (GHGs) such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O); and clouds (Montzka et al. 2011; Kandel 2012). Anthropogenic emissions enhance greenhouse effect by increasing the concentrations of GHGs mainly CO₂, CH₄ and N₂O (Montzka et al. 2011; Cubasch et al. 2013). The atmospheric composition and Earth's land cover changes from human activities alter the solar energy fluxes and water balance consequently having a feedback on the Earth's climate system (Ramanathan et al. 2001; Feddema et al. 2005; Hansen et al. 2005).

The evidence of significant human influence on the Earth's climate system is unequivocal (Levitus et al. 2001; IPCC 2013). The atmospheric CO₂ concentration, considered the most important anthropogenic GHG (Meinshausen et al. 2009; Salomon et al. 2010), has been increasing from 280 ppm, at the start of the industrial revolution (~1750), up to the atmospheric concentration of 401.3 ppm in April 2014 (Canadell et al. 2007; www.co2now.org). The main sources of CO₂ emissions are fossil fuel burning, cement production and land use change (Canadell et al. 2007; Raupach et al. 2007; Le Quéré et al. 2009; Heimann 2010). The Earth's global mean surface temperature has also been increasing since the late 1800s, with a warming of 0.85 (0.65-1.06) °C during 1880-2012 period (Hartmann et al. 2013). The Earth's surface temperature decadal records indicate that the past three decades has been successively warmer and the warmest record being the first decade of 21st century. According to the future climate projections, based on a broader range of model simulations and with low to high emissions scenarios, we will reach by 2100 atmospheric CO₂ concentration of 421 ppm and 936 ppm for low and high emissions scenarios respectively (IPCC 2013). The global mean surface temperature is expected to increase by 0.3 °C to 1.7°C and 2.6 °C to 4.8 °C by 2081-2100 for low and high emissions scenarios respectively (Collins et al. 2013). However, changes in temperature, precipitation and wind cycles are also expected to vary differently in different climatic regions across the planet (IPCC 2013).

1.2 Photosynthesis

Photosynthesis is one of the most important physiological processes that link the plant to its environment (Ainsworth and Rogers, 2005; Bonan 2008). Using CO₂ as a reactant, photosynthesis plays a central role in the sequestration of atmospheric CO₂ in terrestrial

ecosystems. Plants have generally shown to respond to elevated CO₂ concentrations by enhancing photosynthesis (Ainsworth and Long 2005; Bonan 2008; Taub 2010). Forests contribute significantly to global carbon sequestration and it was shown that they store ~ 45% of terrestrial carbon and represent ~ 50% of the terrestrial net primary production (NPP). It is reported that in 1990s, forests sequestered ~ 33% of the total anthropogenic carbon emissions from fossil fuel and land use change (Bonan 2008). Many functional processes in plants depend on temperature to be efficient at their optimal levels (Taiz and Zeiger 2010) and so does photosynthesis (Medlyn et al. 2002; Kattge and Knorr 2007; Lloyd and Farquhar 2008). This implies that the responses and feedbacks of vegetation to a changing climate will significantly influence atmospheric CO₂ cycle and ultimately future climate (Cox et al. 2000).

In this regard, different gas fluxes methods are being used to study the interactions of vegetation and atmosphere (Bonan 2008; Newman et al. 2011). These methods include for example the Free Air CO₂ Enrichment (FACE) experiments (Ainsworth and Long 2005), Eddy covariance towers (Baldocchi 2003) and CO₂ springs (Cook et al. 1998; Onoda et al. 2009). Photosynthesis measurements are mostly conducted using portable gas exchange systems providing after measurement on the leaf some important parameters including the CO₂ uptake (A), leaf conductance (g_s) and intercellular CO₂ concentrations (C_i) (Long and Bernacchi 2003). The data gained from these measurements are used in different global terrestrial biosphere models to simulate the apparent and potential responses and feedbacks of ecosystems on climate (Foley et al. 1996; Cox et al. 2000; Cramer et al. 2001).

In global carbon and climate models, terrestrial carbon uptake is modelled using the well-established biochemical model of photosynthesis developed by Farquhar, von Caemmerer & Berry (1980; Sellers et al. 1997). This model is based on a mechanistic approach where the leaf photosynthesis is estimated from the major biochemical processes such as Rubisco activity and ribulose-1,5-bisphosphate (RuBP) regeneration (Bernacchi *et al.*, 2003; Kattge and Knorr, 2007). It assumes that photosynthesis can be limited by Rubisco when the concentration of intercellular CO₂ (C_i) is low or by RuBP regeneration when C_i is high. The latter situation is mainly limited by light driven electron transport rate but can also be limited by enzymatic reactions of the Calvin cycle (other than Rubisco) (Sharkey et al., 2007). The outputs of the model are two important parameters, the maximum rates of photosynthetic carboxylation (V_{cmax}) and electron transport (J_{max}). Values of V_{cmax} and J_{max} vary widely among species and plant functional types (Kattge and Knorr 2007) and depend on environmental factors such as nutrient (Kattge et al. 2009; Domingues et al. 2010; Mercado et al. 2011) and light (Wullschlegel 1993; Carswell et al. 2000) availability.

1.3 Tropical forests

Tropical forests cover ~ 8.5 % of the global land area (Lewis et al. 2009). Storing more than half of the carbon in the world's forests (Pan et al. 2011) and contributing roughly one-third of global

terrestrial primary production (Beer et al. 2010), tropical forests play an important role in controlling the rate of ongoing climate change (Lewis 2006; IPCC 2013).

Tropical rainforests constitute a large portion of natural tropical forests and are commonly mentioned when referring to natural forests in this region (Lewis 2006). Tropical rainforests have high biodiversity and are typically composed by comparatively dense stands of tall and evergreen broadleaf trees (Lewis 2006). Canopies are multilayered and complex with strong vertical gradients in light availability (Kenzo et al. 2006). It is thus important to consider variation in photosynthetic capacities (i.e., V_{cmax} and J_{max}) of species belonging to different functional groups and of leaves at different canopy positions in order to better understand carbon dynamics in tropical rainforests (Coste et al. 2005; Kenzo et al. 2006). Models of tropical rainforest dynamics classify tree species with respect to successional order following canopy gap formation (Swaine and Whitmore 1988; Yamamoto 2000). Pioneer species are early successional and shade-intolerant and only grow when there is a gap in the canopy, whereas climax species are late successional and shade-tolerant and have the ability to grow under a closed canopy. While pioneer tree species have been shown to have higher V_{cmax} and J_{max} values than climax species in temperate ecosystems (e.g. Niinemets, Kull & Tenhunen 1998), no clear pattern has yet emerged for tropical rainforest species as one study found no such difference among successional groups (Coste et al. 2005) but another study did (Houter & Pons 2012). Tropical rainforests are similar to temperate forests with respect to variation in photosynthetic capacities with vertical canopy position within closed canopies, with sun leaves in the upper canopy having higher nutrient content and V_{cmax} and J_{max} values than shade leaves in the lower canopy (Carswell et al. 2000; Domingues et al. 2005; Kenzo et al. 2006).

While tropical plant species are adapted to high and, over the year, temporally stable growth temperatures (Clark 2004), the spatial variation in prevailing growth temperature within the range of a given species can be substantial, e.g. along large elevational gradients (Table 1). Presently little is known how tropical trees acclimate to variation in prevailing growth temperature by adjusting their photosynthetic capacities. In controlled experiments, high growth temperature decreased J_{max} and $J_{\text{max}}:V_{\text{cmax}}$ ratio at any given measurement temperature (Yamori, Noguchi & Terashima 2005 and references therein). However, neither J_{max} nor V_{cmax} at 25°C varied with growth temperature in a meta-analysis combining data from 36 different plant species growing in different locations and climatic conditions; a study thus not separating evolutionary adaptation from physiological acclimation (Kattge and Knorr 2007). Better understanding of how individual species acclimate their photosynthetic capacities to differences in prevailing temperature under field conditions is critical in order to more reliably predict terrestrial responses to, and feedbacks on, global warming (Friedlingstein et al. 2006; Booth et al. 2012).

Another potentially important environmental factor controlling natural variation in photosynthetic capacities of tropical trees is leaf nutrient content (Domingues et al. 2010;

Mercado et al. 2011). A meta-analysis used in global terrestrial biosphere models reported on rather strong positive relationships between leaf nitrogen (N) content and photosynthetic capacities in tropical trees (Kattge et al. 2009), but relationships have been weak in studies of tropical montane rainforests (van de Weg et al. 2012) and African rainforests (Meir et al. 2007). With respect to tree growth, tropical rainforests growing on intensely weathered soils are thought to be more strongly limited by phosphorus (P) than by N (Vitousek 1984; Davidson et al. 2007), whereas the opposite has been suggested for montane rainforests (Fisher et al. 2012). There are few studies comparing the limitations of leaf P and N content on photosynthetic capacities of tropical rainforest trees, with one lowland study suggesting that P was somewhat more limiting than N (Meir et al. 2007) and one high-elevation study suggesting the opposite (van de Weg et al. 2012). More data on photosynthetic capacities in relation to leaf N and P content is critically needed to determine the influences of P and N on photosynthesis in African tropical forests.

1.4 Aims and hypotheses

In this study, leaf gas exchange and leaf chemical and morphological traits were measured in mature trees of seven native and three exotic tropical forest tree species in Rwanda (Table 1) in order to increase the knowledge on which factors that control natural variability of photosynthetic capacities in African tropical forests. Data were collected at two sites (high elevation cooler, mid-elevation warmer), and included different successional types (pioneer, climax) and canopy levels (upper, lower).

From the available information regarding variation of photosynthetic capacities in successional groups of temperate ecosystems, variation of photosynthetic capacities of the same plant species growing in different growth temperatures environments and nutrient limitation to photosynthesis in different ecosystems, the following hypotheses were addressed:

- (i) Pioneer species have higher nutrient content, V_{cmax} and J_{max} than climax species.
- (ii) Species growing in cooler habitat have higher V_{cmax} and J_{max} and $J_{\text{max}}:V_{\text{cmax}}$ ratio at a given temperature than same species growing in warmer habitat.
- (iii) Sun leaves have higher nutrient content, V_{cmax} and J_{max} than shade leaves.
- (iv) P is the most limiting nutrient to photosynthesis at mid-elevation site whereas N is the most limiting nutrient to photosynthesis at the montane rainforest site.

Table 1 Description of the tree species included in this study, as well as the stem diameter at breast height (DBH; mean \pm standard deviation) of the trees investigated

Species	Family	Origin	Climax or pioneer species	Site in this study	Natural habitat	Altitude in Rwanda (m)	DBH (cm)
<i>Carapa grandiflora</i> Spargue ^a	Meliaceae	Native	Climax	Nyungwe, Arboretum	Montane rainforests in deep soils on mountain slopes and valleys.	1600-2500	36 \pm 24
<i>Cedrela serrata</i> Royle ^b	Meliaceae	Exotic	-	Arboretum	Native to India, Indonesia, Myanmar and Sri Lanka, and had been introduced to eastern and central Africa. Requires well-drained soils.	1200-2400	19 \pm 7
<i>Corymbia maculata</i> (Hook.) K.D. Hill & L.A.S Johnson ^{b,c}	Myrtaceae	Exotic	-	Arboretum	Fast-growing exotic species introduced from Australia and commonly used in plantations worldwide. Earlier scientific name is <i>Eucalyptus maculate</i> .	1000-2000	19 \pm 2
<i>Entandrophragma excelsum</i> (Dawe & Sprague) ^{a,c}	Meliaceae	Native	Climax	Nyungwe, Arboretum	Montane rainforests in deep soils on mountain slopes and valleys.	1500-2100	36 \pm 10
<i>Hagenia abyssinica</i> (Bruce) J. F. Gmel. ^a	Rosaceae	Native	Pioneer	Nyungwe	Growing in disturbed forests, woody swamps and volcanic soils; on mountain slopes, crests and valleys.	1600-3600	38 \pm 10
<i>Macaranga kilimandscharica</i> (Pax.) ^a	Euphorbiaceae	Native	Pioneer	Nyungwe	Found in fallow fields, disturbed montane forests, in deep soils, dry to humid crests and/or slopes.	1700-2700	25 \pm 8
<i>Pinus patula</i> Schlecht. & Cham. ^{d,e}	Pinaceae	Exotic	-	Arboretum	Native to Mexico. Plantations are mostly found in eastern, central and southern Africa.		40 \pm 9

<i>Podocarpus falcatus</i> (Thunb.) R.Br. ex Mirb ^a	Podocarpaceae	Native	Climax	Arboretum	Montane rainforests in deep drained soils on mountain slopes and crests.	1800-3000	40 ± 9
<i>Polyscias fulva</i> (Hiern.) Harms ^f	Araliaceae	Native	Pioneer	Nyungwe, Arboretum	Upland grassland, riverine, lowland and montane rainforests. Found in both natural and planted forests throughout Rwanda.	1180-2400	43 ± 14
<i>Syzygium guineense</i> (Willd.) DC. ^f	Myrtaceae	Native	Climax	Nyungwe	Sub-montane, montane, upper montane, dry montane and riverine forests.	1000-2130	33 ± 14

^aBloesch, Troupin & Derungs. 2009; ^bOrwa et al., 2009; ^cNsabimana et al. 2009; ^dNduwayezu et al. 2009; ^eFAO 2001, ^fFischer & Killmann 2008

2. Material and Methods

2.1 Study sites and plant species

Data were collected in Nyungwe national park (tropical rainforest forest, also classified as a tropical montane cloud forest; Bruijnzeel, Scatena & Hamilton 2010) and Ruhande Arboretum (plantation) located in Rwanda, central Africa, during January-March 2009 and January-May 2010. An additional measurement campaign to investigate the effect of cutting branches on leaf photosynthetic capacities was conducted in February 2013.

Nyungwe National Park

Nyungwe national park (hereafter called 'Nyungwe'), covering approximately 1000 km², is located between 2°17'-2°49'S and 29°03'-29°29'E in the southwestern part of Rwanda. The altitude ranges from around 1500 to 2925 m. Nyungwe is one of the largest blocks of montane rainforest remaining in Africa (Gharahi Ghehi et al. 2012). It holds more than 260 tree species (24 of which are endemic to the Albertine Rift) that belong to 45 families (Plumptre et al. 2002). At a meteorological station located at Uwinka site (2° 28' 43" S, 29° 12' 00" E, 2465 m altitude; Nsabimana 2009), the average day and night air temperatures were 15.7 °C and 13.5 °C, respectively, the relative humidity was 81%, and annual rainfall was 1879 mm during 2007 - 2013. The difference between the warmest and coldest month was 1.1 °C. The soils derive mainly from schists, micaschists, quartzitic schists and granites (Gharahi Ghehi et al. 2012).

The species investigated in Nyungwe were *Carapa grandiflora*, *Entandrophragma excelsum*, *Hagenia abyssinica*, *Macaranga kilimandscharica*, *Polyscias fulva* and *Syzygium guineense* (Table 1). Species in this site were classified into two succesional groups: climax (*C. grandiflora*, *E. excelsum* and *S. guineense*) and pioneer (*H. abyssinica*, *M. kilimandscharica* and *P. fulva*) species. The most abundant tree species in Nyungwe are *S. guineense* and *M. kilimandscharica*, accounting for 18.2% and 17.5% of trees with ≥ 30 cm diameter at breast-height, respectively (Plumptre et al. 2002). The third most abundant species was *C. grandiflora*, contributing 6.6% of large trees. Contributions of the other three selected species were: *H. abyssinica* 4.2%, *P. fulva* 1.2%, *E. excelsum* 1.0%. Each tree species was measured at three well characterized plots near Uwinka station (Nsabimana 2009), except *E. excelsum* trees which all grew in one single stand nearby.

Ruhande Arboretum

The Ruhande Arboretum (hereafter called 'Arboretum') is also located in the southwestern part of Rwanda at 2°36'S, 29°44'E and an altitude of around 1700 m. It was established in 1933 after displacement of the farming population from that area. The plantation includes 227 tree species with approximately 177 exotic and 50 native tree species and covers an area of 200 ha that is

subdivided into around 500 plots of 50 x 50 m; most of them are monospecific. It is considered the best arboretum in Africa (REMA 2009). At a meteorological station ca 2 km from the Arboretum (2° 35' 28" S, 24° 44' 39" E, 2465 m altitude; Nsabimana 2009), the average day and night air temperatures were 20.8 °C and 17.1 °C, respectively, the relative humidity was 74% and annual rainfall was 1231 mm during 2006 – 2013. The difference between the warmest and coldest month was 1.5 °C. The soil type is Ferralsols originating from the parent material of schists and granites mixed with micaschist and quartzite (Nsabimana et al. 2009).

The species investigated in Arboretum were *Carapa grandiflora*, *Cedrela serrata*, *Entandrophragma excelsum*, *Corymbia maculata*, *Pinus patula*, *Podocarpus falcatus* and *Polyscias fulva* (Table 1). *C. serrata*, *C. maculata* and *P. patula* are exotic species while the other four species are native. Each tree species was measured at three or (for *P. fulva*) six replicated plots. The four native species originate from Nyungwe, providing an opportunity for comparisons of the same species growing at the two different sites. In this study, three species were investigated at both sites: *C. grandiflora*, *E. excelsum* and *P. fulva*.

2.2 Gas exchange measurements

Field measurements of leaf gas exchange were conducted using a portable leaf gas exchange instrument, LI-6400 (LI-COR Inc., Lincoln, NE, USA). Mature leaves without visible damage were selected and measured for responses of net photosynthetic rate (A_n) to ten different intercellular CO₂ concentrations (C_i ; so called $A-C_i$ curves) and to five different levels of photosynthetic photon flux density (PPFD). The $A-C_i$ curves were conducted at PPFD of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while PPFD responses were measured at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$ concentration of air entering the leaf chamber. For leaves of *C. grandiflora*, stomatal conductance fell rapidly at high CO₂ and therefore a shorter $A-C_i$ protocol, still with at least two of the three highest CO₂ concentrations included, was used. The range of CO₂ concentration in air entering the leaf chamber was 60-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the PPFD response range was 0-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements in darkness were conducted after five minutes, to allow at least partial acclimation of respiration to darkness (R_d). Measurements were conducted at 20° C in Nyungwe and at 25° C in the warmer Arboretum. The leaf-to-air vapor pressure deficit was kept below a certain level at all times (1.5 kPa in Nyungwe and 2.0 kPa in Arboretum). Measurements were conducted only if the starting value of stomatal conductance was over 0.05 $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$.

Three trees (one at each of three replicate plots) of each of the 13 species by site combinations were selected, except for *P. fulva* in Arboretum where six trees and plots were used. On each tree, two sun leaves from the upper canopy and two shade leaves from the lower canopy were measured. The total number of leaves measured was thus 168.

Upper canopy leaves were made accessible by cutting rather large branches (1-2 m) which were put in a bucket of water to avoid disruption of water transport within the xylem (Domingues et

al. 2010). Shade leaves were measured on attached branches at accessible height in some species, but usually branches had to be cut. We explicitly assessed if there was a short-term impact of cutting a branch on photosynthetic capacities by conducting $A-C_i$ measurements on leaves of reachable branches before and ten minutes after the branches were cut, with the leaf inside the leaf chamber all the time. Across 11 leaves in three species measured in Nyungwe (3 leaves of *C. grandiflora*, 4 leaves of *M. kilimandscharica*, 4 leaves of *P. fulva*), we found no significant effect of the cut on V_{cmax} and a quite small negative effect (-8%) on J_{max} (Table 2).

2.3 Photosynthesis model parameterisation

The photosynthesis model by Farquhar et al. (1980), with modifications of photosynthetic temperature dependencies (Bernacchi et al. 2001), was used to parameterize the photosynthetic capacities V_{cmax} and J_{max} from $A-C_i$ curve data by the least-square method. Internal leaf conductance for CO_2 was not measured and therefore apparent V_{cmax} and J_{max} values were determined based on C_i rather than on the CO_2 concentration at the chloroplast. The uncertainty of the values of curvature of the light-response (0.9) and quantum yield (0.3 mol electrons mol photons⁻¹) of electron transport used when calculating J_{max} from actual electron transport (J) have only a slight effect on the estimated value of J_{max} (Medlyn et al. 2002). The only *a priori* restriction made to $A-C_i$ fitting (i.e. V_{cmax} and J_{max} determination) was that data points with C_i below 100 $\mu\text{mol mol}^{-1}$ were forced to be V_{cmax} -limited.

Values of V_{cmax} and J_{max} reported here were all determined for a reference temperature of 25 °C, although measurements in Nyungwe were conducted at 20 °C. According to the temperature response functions of the model used, the increases in V_{cmax} and J_{max} at 25 °C compared to 20 °C were +57% and +35%, respectively. These temperature adjustments of Nyungwe data were justified by very similar temperature sensitivities of V_{cmax} (+60%) and J_{max} (+31%) in the 20-25 °C range measured in six Rwandan tree species in another study (Vårhammar 2011).

The apparent quantum yield of photosynthesis was determined as the slope of the response of photosynthesis to PPFD in the range 25-75 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.4 Other leaf traits

All leaves measured for gas exchange were also analyzed for N and P content. Dry mass of leaf discs with known area was determined after drying to constant weight at 70 °C. The N content of dried leaves was determined using an elemental analyzer (EA 1108; Fison Instruments, Rodano, Italy). Leaf P content was determined by first extracting and oxidizing leaf P into phosphate (Valderrama 1981) and then measuring phosphate content by spectrophotometry (Ames 1966). A subset of 18 leaf samples were also analyzed for P content using inductively coupled plasma mass spectrometry (Basic Suite 1VE1; ACME Analytical Laboratories, Vancouver, BC, Canada). As the latter P analysis method indicated incomplete conversion of

leaf P into phosphate by the former method, the relationship between the two methods ($R^2 = 0.83$; range: 0.06-0.45% P of dry mass) was used to adjust data obtained by the former method for all leaves.

All leaves were also measured for length, width, thickness and SPAD value, except *P. patula* leaves for which SPAD was not possible to measure. The SPAD value is a proxy of leaf chlorophyll content (Uddling et al. 2007), optically measured using a SPAD meter (SPAD model 502; Minolta corporation, Ltd., Osaka, Japan).

2.5 Statistical analyses

Data were statistically analyzed by analysis of variance (ANOVA) appropriate for the different comparisons, using SAS software version 9.3.1 (SAS Institute, Cary, NC, USA). All 13 populations (species by site combinations) were analyzed with ‘population’ as main plot factor and ‘canopy level’ as sub-plot factor. Sites were compared using data of three species common to both sites in a test with ‘site’ and ‘species’ as main plot factors and ‘canopy level’ as sub-plot factor. Successional groups in Nyungwe were compared in a test with ‘species’ nested within ‘successional group’ and ‘canopy level’ as a sub-plot factor.

Differences between species in Nyungwe and Arboretum with respect to slopes and intercepts of the relationship between J_{\max} and V_{\max} were tested using ANCOVA.

Table 2. Effect of cutting branches on the maximum rates of photosynthetic carboxylation (V_{\max}) and electron transport (J_{\max}). Values shown are mean effects \pm 95% confidence intervals

Species	<i>n</i>	Effect of cut (%)	
		V_{\max}	J_{\max}
<i>C. grandiflora</i>	3	-9 \pm 31	-7 \pm 25
<i>M. capensis</i>	4	3 \pm 10	-7 \pm 4
<i>P. fulva</i>	4	-4 \pm 10	-10 \pm 1
<i>All species</i>	11	-3 \pm 9	-8 \pm 6

3. Results

All physiological, chemical and morphological variables studied except area-based N content ($P = 0.15$) differed significantly ($P \leq 0.02$) among site by species combinations (Appendix 1 and 2). Across all data, relationships between area- or mass-based nutrient content (N, P) and photosynthetic capacities (V_{cmax} , J_{max}) were weak ($r^2 \leq 0.13$) and statistically non-significant (Table 3). Analyzing data by site, one positive (V_{cmax} vs. mass-based P) and one negative (V_{cmax} vs. area-based N) relationship were significant at $P \leq 0.05$, but this is about what could be expected by pure chance, given the total number of 16 relationships (Appendix 3). Analyzing data for each site by species combination separately, many significant positive relationships between nutrient content (both P and N) and photosynthetic capacities were found in the Arboretum, but not in Nyungwe (Appendix 4).

Pioneer tree species had significantly higher V_{cmax} (+64%; $P = 0.005$) and J_{max} (+75; $P = 0.003$) values than climax tree species (Fig. 1a-b). Neither area- or mass-based leaf nutrient content nor N:P ratio or leaf mass per area (LMA) differed between the two successional groups ($P \geq 0.16$; Fig. 1c-d; Appendix 1 and 5). Quantum yield was 71% higher in pioneer compared to climax species ($P = 0.003$), although pioneer species had lower chlorophyll content than climax species, as judged by their 31% lower SPAD values ($P < 0.060$; Fig. 1e). Respiration in darkness did not differ among successional groups ($P = 0.34$).

Exotic species in the Arboretum were not easily classified into pioneer and climax trees. However, species with high photosynthetic capacities tended to have low SPAD values also at this site. For example, among those species, *C. serrata* had intermediate values of V_{cmax} and J_{max} but low SPAD values, and *C. maculata* had the highest V_{cmax} and J_{max} values but intermediate SPAD values. Across the entire dataset (i.e., both Arboretum and Nyungwe data), photosynthetic capacities and SPAD were strongly negatively related ($P \leq 0.002$; Fig. 2).

Trees growing in Nyungwe (cooler habitat) had significantly higher V_{cmax} and J_{max} than trees growing in Arboretum (warmer habitat), when comparing species measured at both sites (Fig. 3a-b). The difference was stronger for J_{max} (+46%; $P < 0.001$) than for V_{cmax} (+18%; $P = 0.052$), and consequently the $J_{\text{max}}:V_{\text{cmax}}$ ratio was 17% higher in Nyungwe (2.2) than in Arboretum (1.9; $P = 0.002$). Neither the intercept ($P = 0.86$) nor the slope ($P = 0.16$) of the relationship between J_{max} and V_{cmax} differed between the two sites when including only data from the three species common to both sites (Fig. 4a). However, when including data for all species, the J_{max} vs. V_{cmax} relationship was significantly steeper in Nyungwe than in Arboretum ($P = 0.006$; Fig. 4b). Area-based leaf P content was higher (+79%; $P < 0.001$) in Nyungwe compared to Arboretum, while N content did not significantly differ ($P \geq 0.10$; Fig. 3c-d). Consequently, the N:P ratio was lower in Nyungwe (9.2 ± 0.4 ; \pm SE) compared to Arboretum (15 ± 1.1 ; \pm SE; $P < 0.001$; Appendix 6). Leaves were 16% longer in Nyungwe compared to Arboretum ($P = 0.007$) while thickness, width and LMA did not differ ($P \geq 0.15$; Appendix 1 and 6).

Although not statistically significant in the comparisons of sites and successional groups based on subsets of the total data set, both V_{cmax} (+21%) and J_{max} (+27%) were significantly higher for sun leaves compared to shade leaves across all data ($P \leq 0.001$, Appendix 1-2). Area-based P (+14%) and N (+18%) content were higher in sun leaves compared to shade leaves ($P \leq 0.009$). Sun leaves were thicker (+8%), shorter (-8%) and had higher LMA (+23%) compared to shade leaves ($P \leq 0.001$). Sun leaves had higher apparent quantum yield than shade leaves (+15%; $P = 0.012$), although SPAD values did not differ between canopy levels ($P = 1.00$). Respiration was higher in sun leaves compared to shade leaves (+46%; $P < 0.001$).

Table 3 Parameters of the relationships between photosynthetic capacities (y-axis; V_{cmax} or J_{max}) and area- or mass-based foliar nitrogen and phosphorous content (x-axis; P_a , N_a , P_m , N_m) across all species. Each data point represents one site by species by canopy level combination (i.e., $n = 13 \times 2 = 26$). See Table 3 for explanations of x and y variables

Nutrient content (P_a & N_a : g m^{-2} ; P_m & N_m : % of dry mass)	Photosynthetic parameter ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Slope	y-intercept	r^2	P value
P_a	V_{cmax}	-6.1	51	0.00	0.83
N_a	V_{cmax}	-1.9	54	0.00	0.73
P_m	V_{cmax}	52.5	40	0.07	0.18
N_m	V_{cmax}	6.4	36	0.07	0.19
P_a	J_{max}	38.8	97	0.01	0.58
N_a	J_{max}	0.3	105	0.00	0.98
P_m	J_{max}	178.5	72	0.13	0.065
N_m	J_{max}	15	73	0.06	0.23

Symbols and abbreviations of leaf traits: V_{cmax} , maximum rate of carboxylation of Rubisco at 25°C ($\mu\text{mol m}^{-2} \text{s}^{-1}$); J_{max} , maximum rate of electron transport at 25°C ($\mu\text{mol m}^{-2} \text{s}^{-1}$); N_m , leaf mass-based nitrogen content (% of dry mass); P_m , leaf mass-based phosphorus content (% of dry mass); N_a , leaf area-based nitrogen content (g m^{-2}); P_a , leaf area-based phosphorus content (g m^{-2})

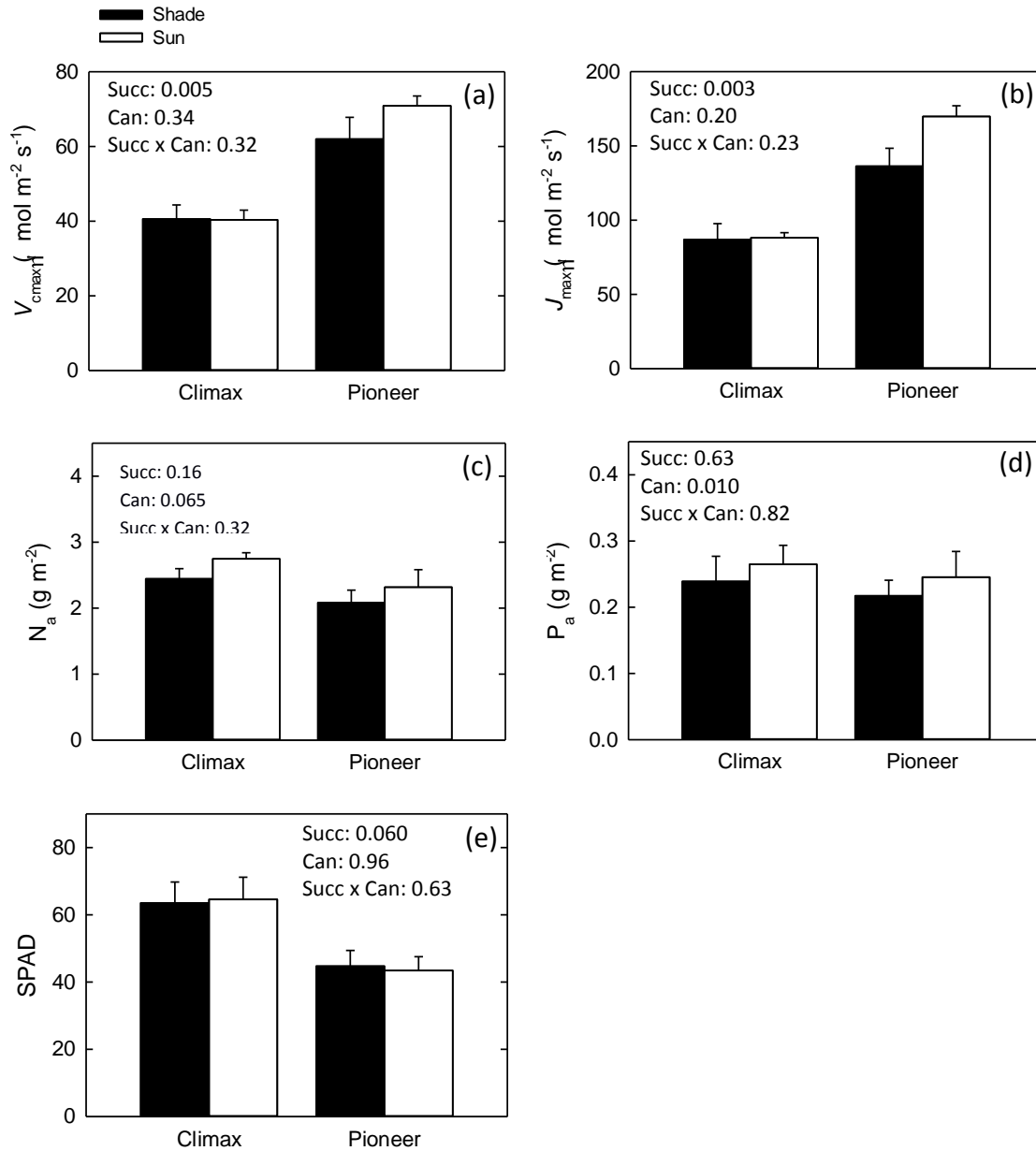


Fig. 1 Photosynthetic capacities, leaf nutrient content and SPAD values of different successional types. Comparison of the maximum rates of photosynthetic (a) carboxylation (V_{cmax}) and (b) electron transport (J_{max}) as well as area-based foliar (c) nitrogen (N_a) and (d) phosphorous (P_a) content (g m^{-2}) and (e) SPAD values between three pioneer and three climax species in Nyungwe montane rainforest. P values of an analysis of variance for the effects of successional group (Succ), canopy position (Can) and their interaction are shown in the figure. Error bars represent the standard error ($n = 3$)

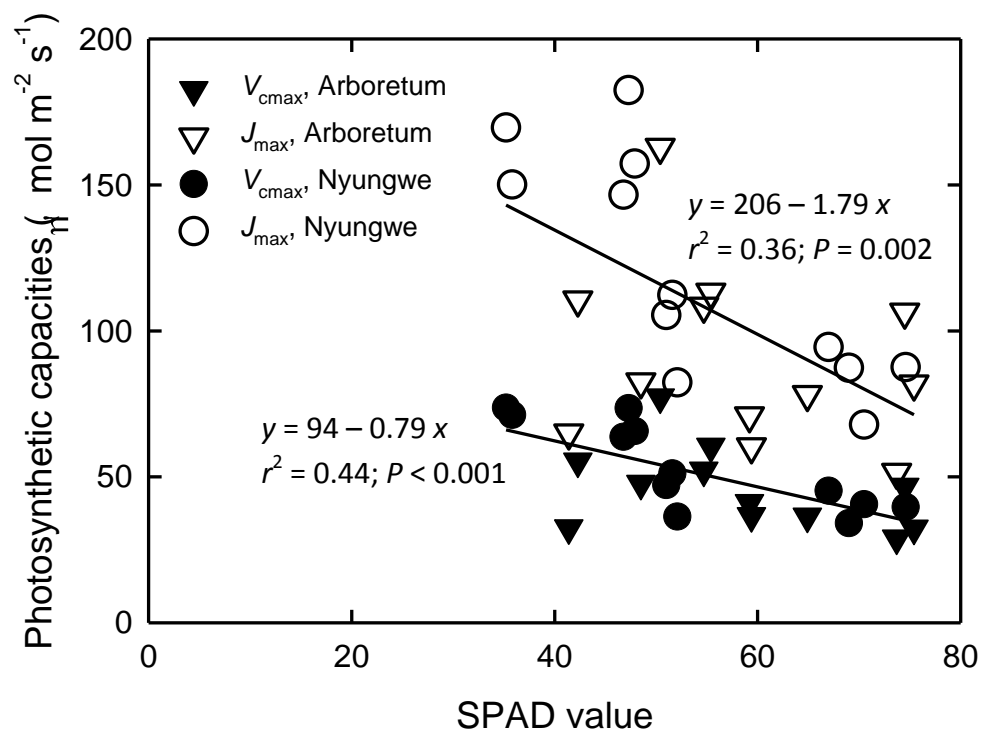


Fig. 2 Photosynthetic capacities in relation to SPAD values. Relationships between SPAD value (a proxy of chlorophyll content) and the maximum rates of photosynthetic carboxylation (V_{cmax}) and electron transport (J_{max}). Each data point represents one site by species by canopy level combination ($n = 24$)

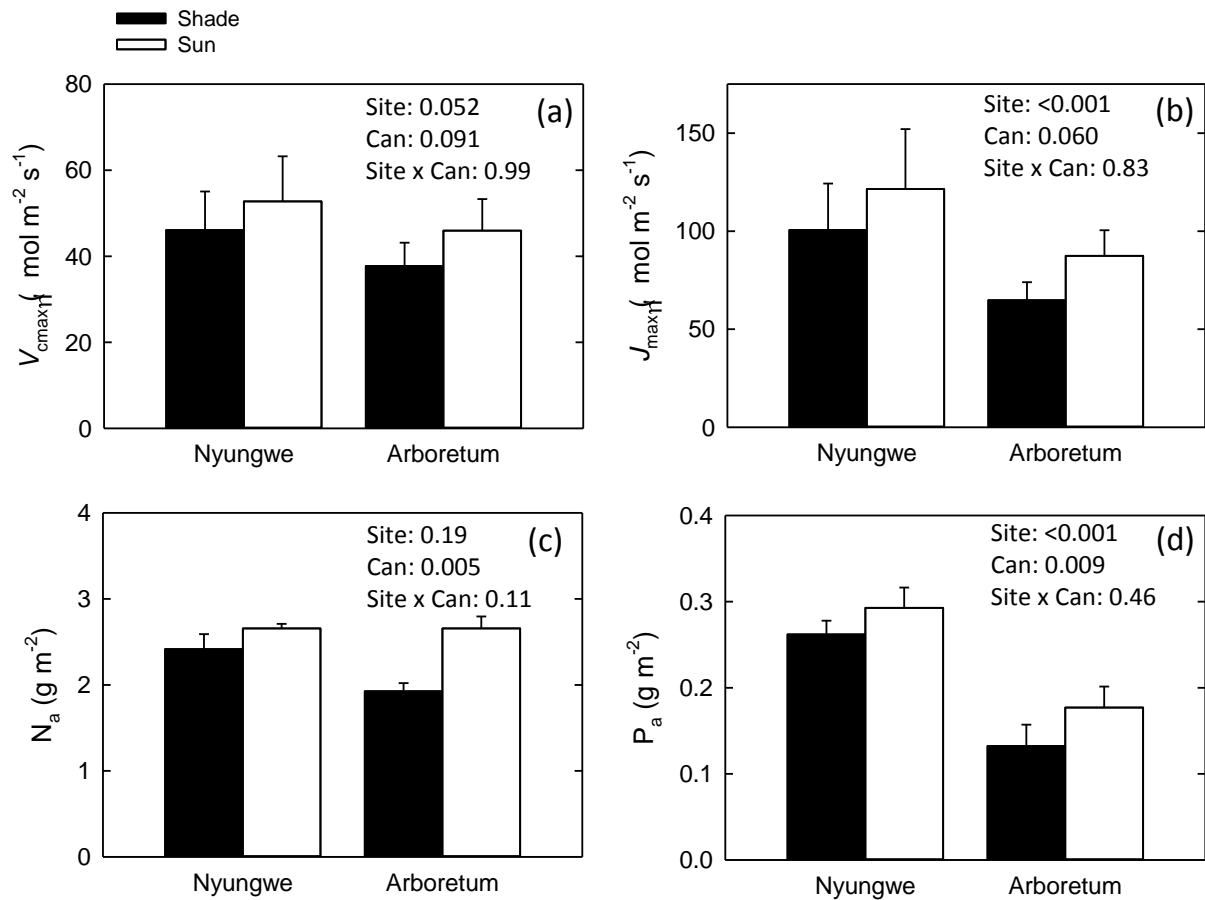


Fig. 3 Photosynthetic capacities and leaf nutrient content of the same species at two different sites. Comparison of the maximum rates of photosynthetic (a) carboxylation (V_{cmax}) and (b) electron transport (J_{max}) as well as area-based foliar (c) nitrogen (N_a) and (d) phosphorous (P_a) content (g m^{-2}) between Nyungwe rainforest and Arboretum Ruhande plantation, based on data from species studied at both sites (*Carapa grandiflora*, *Entandrophragma excelsum* and *Polyscias fulva*). P values of an analysis of variance for the effects of Site, canopy position (Can) and their interactions are shown in the figure. Error bars represent the standard error ($n = 3$)

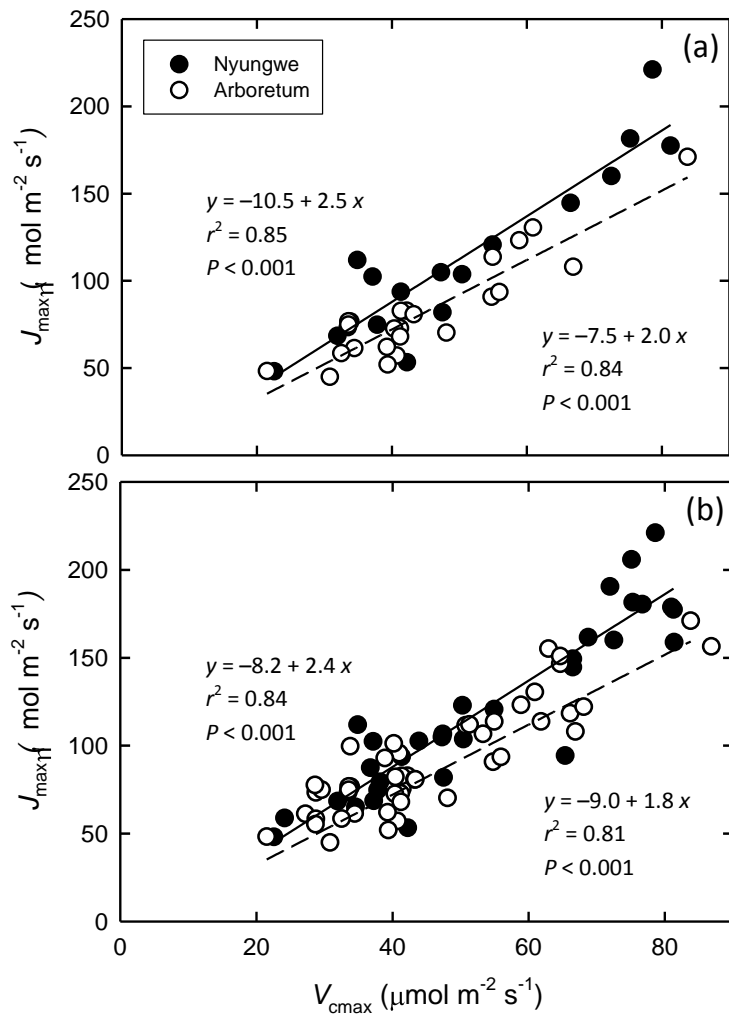


Fig. 4 Relationship between the maximum rates of photosynthetic electron transport (J_{\max}) and carboxylation (V_{cmax}) at 25 °C (a) for the three species studied at both Nyungwe rainforest and Arboretum plantation, or (b) for all species measured at each site (7 species in Arboretum, 6 species in Nyungwe; see Table 1 for information of the species studied at each site). The solid and dashed lines represent the regressions for Nyungwe and Arboretum data, respectively. Each data point represents the average for each tree and canopy position

4. Discussion

There is a shortage of data on photosynthetic capacities (i.e., V_{cmax} and J_{max}) of African tropical rainforest tree species. Meir et al. (2007) is the only study that we are aware of to report such data, and since it derived photosynthetic capacities from PPFD response measurements (i.e., not $A-C_i$ curves) its V_{cmax} values have comparatively large uncertainty. Values of V_{cmax} , J_{max} and $J_{\text{max}}:V_{\text{cmax}}$ ratio determined in the present study lie within the upper range of those reported for tropical rainforest tree species earlier (Carswell et al. 2000, Coste et al. 2005, Domingues et al. 2005, Meir et al. 2007, van de Weg et al. 2012). The rather high $J_{\text{max}}:V_{\text{cmax}}$ ratios found in this study may be explained by the relatively low temperature of the mid and high elevation sites of the present study compared to the temperatures of tropical lowland sites. Species growing in lower temperature usually have higher $J_{\text{max}}:V_{\text{cmax}}$ ratio at a given measurement temperature (Yamori et al. 2005; Kattge and Knorr 2007).

Pioneer and climax species exhibited large differences in photosynthetic capacities, but not in total leaf nutrient content (Fig. 1). Pioneer tree species had 64% higher V_{cmax} values and 75% higher J_{max} values compared to climax species, but the mass- and area-based N and P contents of the two groups were similar (Fig. 1; Appendix 1 and 5). This indicates that pioneer and climax tree species have markedly different allocation of nutrients within their leaves. With respect to N, this hypothesis is supported by the two successional groups exhibiting strongly contrasting investments in photosynthetic enzymes (linked to V_{cmax} and J_{max}) and chlorophyll (linked to SPAD values). Climax species seem to invest less N into photosynthetic enzymes and more N into chlorophyll (SPAD values 45% higher) compared to pioneer species (Fig. 1), a trade-off that held true for the entire dataset (Fig. 2). The use of SPAD values as indicators of interspecific variation in leaf chlorophyll content is supported by a study showing that a general SPAD–chlorophyll relationship could be used for 13 tropical rainforest tree species with large variation in leaf morphology (Coste et al. 2010).

It is well known that species growing in low light conditions and leaves positioned in the lower canopy exhibit within-leaf N allocation that favors efficient light-harvesting at low radiation at the expense of high maximum photosynthetic capacity at saturating radiation (Evans 1989; Niinemets et al. 1998). Our results further indicate that late successional species, adapted to low light conditions when trees are young, keep this within-leaf N allocation also in sun leaves of overstorey canopy trees. If confirmed by direct measurements of leaf chlorophyll content and for additional species, this implies that within-leaf N allocation is to a large extent a genetic adaptation linked to successional group identity, rather than a plastic trait that readily acclimates to prevailing light conditions. Perhaps it is even a leaf trait that may help to mechanistically explain successional progression, with climax species readily growing in the rather ‘bright’ shadow of pioneer species (low chlorophyll content) but pioneer species perishing in the ‘dark’ shadow of climax species (high chlorophyll content) at later successional stages?

Higher photosynthetic capacities in pioneer compared to climax species are commonly observed in temperate tree species (e.g. Niinemets et al. 1998), while results on tropical rainforest tree species are mixed. A greenhouse study on seedlings of 14 tropical rainforest tree species in French Guiana detected no clear-cut differences in photosynthetic capacities among groups of different shade tolerance (Coste et al. 2005), while a study on juvenile and mature trees of 17 tropical rainforest tree species in Central Guyana found that photosynthetic capacities increased with species gap dependence (Houter & Pons 2012). Clearly, data from a broader range of species are thus critically needed to further test the hypothesis based on the present study: that variation in photosynthetic capacities is linked to successional group identity. Similarly to here, however, the study in French Guiana also found that the large interspecific variation in photosynthetic capacities was related to differences in relative within-leaf allocation of N, and not to total leaf N content per se (Fig. 1; Coste et al. 2005). Furthermore, the study in Central Guyana found that photosynthetic capacity per unit chlorophyll, but not leaf area-based N content, increased with species gap dependence (Houter & Pons 2012). These studies thus support the hypothesis that within-leaf nutrient allocation is more important than total leaf nutrient content in explaining variation in photosynthetic capacities among tropical tree species.

In the three species studied at both sites, photosynthetic capacities were significantly higher at the cooler site (Nyungwe) compared to the warmer site (Arboretum; Fig. 3a-b). This is in line with the hypothesis that plants growing in cool environments make relatively larger N investments into photosynthetic enzymes compared to plants growing in warm environment in order to compensate for the low enzyme activity and slower membrane processes at low temperature (von Caemmerer 2000; Yamori et al. 2005). Another possible explanation of higher photosynthetic capacities and $J_{\max}:V_{\max}$ ratio at the cooler site is the higher leaf P content in Nyungwe compared to Arboretum (Fig. 3d). However, P limitations seem less likely to explain the differences in photosynthetic capacities or $J_{\max}:V_{\max}$ ratio between the two sites, as there was no significant correlation between leaf P content and J_{\max} or V_{\max} across all data (Table 3). The enhanced $J_{\max}:V_{\max}$ ratio at low growth temperature is more likely a true temperature response, observed earlier in controlled experiments (Yamori et al. 2005) as well as across observations in a broad range of plant species (Kattge and Knorr 2007). This response acts to maintain a balance between the rates of photosynthetic carboxylation and RuBP regeneration (two rates with markedly different instantaneous temperature responses; Medlyn et al. 2002), thus maximizing photosynthesis at prevailing temperature conditions (Hikosaka et al. 1997; von Caemmerer 2000).

As expected, and in line with earlier studies (Ellsworth and Reich 1993; Carswell et al. 2000; Kenzo et al. 2006), LMA, area-based nutrient content and photosynthetic capacities were higher in sun leaves than in shade leaves (Appendix 1-2). However, the rather moderate enhancement of area-based leaf nutrient content (14-18%) and photosynthetic capacities (21-27%) in upper compared to lower canopy contradicts the commonly used modeling assumption that nutrient allocation and variation in photosynthetic capacities within the canopy are proportional to the

absorption of photosynthetically active radiation (e.g. Field 1983). Our study thus adds to the growing number of reports that PPFD decreases more rapidly than N content with canopy depth in real canopies (e.g. Lloyd et al. 2010; Tarvainen et al. 2013).

Lack of significant relationships between leaf nutrient content and photosynthetic capacities across the entire dataset of the present study (Table 3) contrasts with the rather strong positive N–photosynthetic capacity relationships of a global meta-analysis, also including tropical trees (Kattge et al. 2009). However, earlier studies in tropical montane (van de Weg et al. 2012) and African (Meir et al. 2007) rainforests reported results similar to those presented here (i.e. weak or non-significant leaf nutrient concentration dependencies of V_{cmax} and J_{max}). Furthermore, as mentioned above, photosynthetic capacities were related to within-leaf N allocation rather than to total area-based leaf N content in seedlings of 14 tropical rainforest species in French Guiana (Coste et al. 2005). Clearly, more studies are needed to better understand nutrient limitations of photosynthesis in tropical tree species. Our results indicate that interspecific variation in within-leaf nutrient allocation may play a key role, at least for N (Fig. 1 and 2). Furthermore, the finding that photosynthetic capacities were commonly related to leaf nutrient content within (Appendix 4) but not among tree species (Table 3, Appendix 3) indicates that while total nutrient may be an important determinant of intraspecific variation in photosynthetic capacities, interspecific variation across species belonging to different successional groups is largely controlled by other factors, such as differences in within-leaf nutrient allocation.

5. Conclusions

By measuring leaf gas exchange and leaf chemical and morphological traits in mature trees of ten tropical forest tree species in Rwanda, the present study increases the knowledge of natural variability of photosynthetic capacities in tropical tree species in Africa and, thus, contributes critical tropical data for global carbon and climate models. We found that climax tree species had considerably lower photosynthetic capacities than pioneer tree species. This difference was not related to leaf nutrient content, but rather seemed to be caused by differences in within-leaf N allocation between the two successional groups. With respect to N, leaves of climax species invested less N into photosynthetic enzymes (as judged by lower V_{cmax} and J_{max} values) and more N into chlorophyll (as judged by higher SPAD values). Correlations between photosynthetic capacities and nutrient content across species and sites were very weak ($r^2 \leq 0.13$). These results suggest that, for montane rainforest trees of different functional types, successional group identity is a better predictor of interspecific variation in photosynthetic capacities than total leaf nutrient content. We also found that photosynthetic capacities and $J_{\text{max}}:V_{\text{cmax}}$ ratio were significantly higher in Nyungwe (cooler site) than in Arboretum (warmer site) and that sun leaves had higher photosynthetic capacities than shade leaves.

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Appendices

Appendix 1 Leaf traits of species included in this study (mean \pm standard error; $n = 3$ or 6)

Site	Species	Canopy position	N	V_{cmax}	J_{max}	$J_{\text{max}}/V_{\text{cmax}}$ ratio	QY	R_d	N_m	P_m	N_a	P_a
Arboretum	<i>Cg</i>	Sun	3	36.4 \pm 2.9	78.1 \pm 2.4	2.16 \pm 0.10	0.030 \pm 0.001	1.03 \pm 0.13	1.83 \pm 0.08	0.111 \pm 0.029	2.38 \pm 0.15	0.155 \pm 0.026
		Shade	3	28.9 \pm 3.8	51.5 \pm 5.0	1.82 \pm 0.23	0.025 \pm 0.001	0.42 \pm 0.12	2.02 \pm 0.25	0.115 \pm 0.023	1.74 \pm 0.21	0.099 \pm 0.021
	<i>Cm</i>	Sun	3	77.2 \pm 13.5	162.9 \pm 12.3	2.18 \pm 0.20	0.049 \pm 0.004	2.01 \pm 0.13	1.78 \pm 0.11	0.088 \pm 0.011	2.65 \pm 0.11	0.135 \pm 0.022
		Shade	3	52.2 \pm 17.7	108.5 \pm 24.8	2.23 \pm 0.22	0.037 \pm 0.006	1.59 \pm 0.22	1.64 \pm 0.12	0.056 \pm 0.006	2.06 \pm 0.06	0.070 \pm 0.003
	<i>Cs</i>	Sun	3	55.3 \pm 3.4	110.6 \pm 2.1	2.02 \pm 0.11	0.038 \pm 0.003	1.31 \pm 0.06	2.48 \pm 0.31	0.119 \pm 0.019	2.52 \pm 0.79	0.116 \pm 0.030
		Shade	3	32.4 \pm 4.5	65.0 \pm 5.3	2.03 \pm 0.12	0.037 \pm 0.002	0.69 \pm 0.03	3.61 \pm 0.34	0.256 \pm 0.095	2.13 \pm 1.29	0.113 \pm 0.049
	<i>Ee</i>	Sun	3	41.1 \pm 0.2	70.9 \pm 7.5	1.71 \pm 0.19	0.029 \pm 0.005	1.43 \pm 0.16	1.72 \pm 0.07	0.092 \pm 0.004	2.79 \pm 0.07	0.150 \pm 0.008
		Shade	3	36.6 \pm 2.1	60.3 \pm 1.5	1.94 \pm 0.27	0.019 \pm 0.008	0.61 \pm 0.04	1.76 \pm 0.09	0.103 \pm 0.000	1.98 \pm 0.11	0.116 \pm 0.001
	<i>Pfal</i>	Sun	3	46.7 \pm 9.9	106.4 \pm 6.0	2.45 \pm 0.33	0.032 \pm 0.003	1.96 \pm 0.52	1.45 \pm 0.11	0.196 \pm 0.056	3.65 \pm 0.59	0.478 \pm 0.138
		Shade	3	32.3 \pm 3.3	81.8 \pm 5.6	2.55 \pm 0.09	0.030 \pm 0.002	0.93 \pm 0.32	1.51 \pm 0.03	0.207 \pm 0.063	3.78 \pm 0.21	0.508 \pm 0.149
	<i>Pful</i>	Sun	6	60.4 \pm 5.0	113.2 \pm 14.7	1.83 \pm 0.11	0.041 \pm 0.007	1.16 \pm 0.10	1.86 \pm 0.14	0.154 \pm 0.013	2.80 \pm 0.12	0.226 \pm 0.011
		Shade	6	47.7 \pm 4.5	82.5 \pm 9.8	1.74 \pm 0.08	0.050 \pm 0.000	0.44 \pm 0.11	2.22 \pm 0.15	0.201 \pm 0.030	2.06 \pm 0.35	0.181 \pm 0.032
	<i>Pp</i>	Sun	3	61.4 \pm 5.1	128.4 \pm 11.7	2.08 \pm 0.17	0.038 \pm 0.001	2.23 \pm 0.53	1.68 \pm 0.08	0.135 \pm 0.016	3.26 \pm 0.56	0.266 \pm 0.070
		Shade	3	37.5 \pm 4.5	72.7 \pm 8.7	2.03 \pm 0.09	0.028 \pm 0.001	0.95 \pm 0.05	1.47 \pm 0.06	0.152 \pm 0.013	1.70 \pm 0.24	0.123 \pm 0.068
Nyungwe	<i>Cg</i>	Sun	3	39.6 \pm 9.4	87.5 \pm 21.2	2.11 \pm 0.13	0.035 \pm 0.005	0.85 \pm 0.02	1.90 \pm 0.19	0.227 \pm 0.022	2.76 \pm 0.36	0.321 \pm 0.013

		Shade	3	34.1 ± 0.4	87.3 ± 12.4	2.29 ± 0.07	0.017 ± 0.001	0.69 ± 0.08	2.04 ± 0.11	0.235 ± 0.017	2.33 ± 0.23	0.266 ± 0.013
	<i>Ee</i>	Sun	3	45.2 ± 3.8	94.4 ± 9.8	2.09 ± 0.10	0.035 ± 0.006	1.16 ± 0.26	2.23 ± 0.11	0.217 ± 0.025	2.58 ± 0.30	0.246 ± 0.009
		Shade	3	40.6 ± 4.6	67.8 ± 8.3	1.82 ± 0.19	0.028 ± 0.009	0.99 ± 0.09	2.26 ± 0.06	0.229 ± 0.016	2.75 ± 0.09	0.287 ± 0.018
	<i>Ha</i>	Sun	3	73.6 ± 5.6	169.7 ± 8.8	2.20 ± 0.13	0.052 ± 0.003	1.16 ± 0.11	3.55 ± 0.18	0.354 ± 0.038	1.79 ± 0.10	0.177 ± 0.003
		Shade	3	71.2 ± 3.0	150.1 ± 45.6	2.35 ± 0.37	0.052 ± 0.005	1.05 ± 0.11	3.66 ± 0.08	0.366 ± 0.003	1.72 ± 0.11	0.171 ± 0.010
	<i>Mk</i>	Sun	3	65.6 ± 11.2	157.3 ± 27.6	2.26 ± 0.04	0.048 ± 0.004	1.03 ± 0.09	2.08 ± 0.14	0.203 ± 0.006	2.54 ± 0.22	0.249 ± 0.013
		Shade	3	51.1 ± 9.3	112.3 ± 27.0	2.26 ± 0.04	0.044 ± 0.003	0.90 ± 0.05	2.20 ± 0.21	0.230 ± 0.022	2.36 ± 0.23	0.248 ± 0.031
	<i>Pfulv</i>	Sun	3	73.5 ± 3.6	182.5 ± 22.0	2.47 ± 0.18	0.049 ± 0.005	1.10 ± 0.08	2.11 ± 0.10	0.252 ± 0.007	2.63 ± 0.37	0.311 ± 0.031
		Shade	3	63.6 ± 13.5	146.6 ± 22.7	2.39 ± 0.18	0.041 ± 0.004	0.90 ± 0.07	2.02 ± 0.18	0.217 ± 0.016	2.17 ± 0.11	0.233 ± 0.007
	<i>Sg</i>	Sun	3	36.3 ± 7.6	82.3 ± 20.4	2.34 ± 0.19	0.027 ± 0.006	1.02 ± 0.04	2.16 ± 0.34	0.169 ± 0.028	2.90 ± 0.13	0.227 ± 0.018
		Shade	3	47.1 ± 9.7	105.4 ± 22.1	2.24 ± 0.09	0.026 ± 0.008	0.82 ± 0.09	2.19 ± 0.34	0.158 ± 0.020	2.26 ± 0.36	0.164 ± 0.023

Appendix 1, continued

Site	Species	Canopy position	N	LMA	Thickness (mm)	Length (cm)	Width (cm)	SPAD
Arboretum	<i>Cg</i>	Sun	3	130 ± 4	0.34±0.02	19.5±1.6	6.8±0.6	64.9 ± 2.2
		Shade	3	86 ± 1	0.28±0.01	27±1.6	7.8±0.5	73.7 ± 3.4
	<i>Cs</i>	Sun	3	150 ± 7	0.17±0.01	14.3±0.8	3.3±0.2	50.4 ± 1.0
		Shade	3	127 ± 11	0.16±0.03	14.5±0.3	3.7±0.1	54.7 ± 3.3
	<i>Cm</i>	Sun	3	114 ± 52	0.34±0.00	15±1.5	3.2±0.3	42.3 ± 4.2
		Shade	3	61 ± 38	0.30±0.02	17.1±1.1	3.4±0.1	41.4 ± 3.4

	<i>Ee</i>	Sun	3	162 ± 4	0.34±0.00	21.1±1.3	10.1±0.9	59.2 ± 3.7
		Shade	3	112 ± 1	0.28±0.02	22.2±0.5	9±0.7	59.4 ± 1.7
	<i>Pf</i>	Sun	6	251 ± 29	0.52±0.03	13.1±0.8	6.2±0.6	74.5 ± 5.7
		Shade	6	249 ± 10	0.38±0.03	15.0±1.1	7.1±0.7	75.4 ± 2.8
	<i>Pfalc</i>	Sun	3	150 ± 9	0.38±0.02	4.5±0.7	0.4±0.0	55.4 ± 1.9
		Shade	3	99 ± 20	0.40±0.04	6.8±0.1	0.6±0.1	48.5 ± 1.2
	<i>Pp</i>	Sun	3	193 ± 31	0.56±0.07	17.5±0.8	0.7±0.3	-
		Shade	3	111 ± 14	0.46±0.01	17.4±0.6	0.5±0.2	-
Nyungwe	<i>Cg</i>	Sun	3	143 ± 9	0.34±0.02	23.7±0.3	6.8±0.4	74.6 ± 5.2
		Shade	3	114 ± 6	0.27±0.01	24.0±1.5	12.9±6.1	69.0 ± 6.9
	<i>Ee</i>	Sun	3	115 ± 9	0.27±0.01	27.4±1.7	11.2±1.4	67.0 ± 5.6
		Shade	3	122 ± 4	0.28±0.01	30.6±1.3	12.9±0.8	70.5 ± 3.1
	<i>Ha</i>	Sun	3	51 ± 5	0.22±0.01	17±0.9	5.4±0.2	35.2 ± 2.6
		Shade	3	47 ± 3	0.22±0.01	16.2±1.1	5.1±0.2	35.8 ± 1.9
	<i>Mk</i>	Sun	3	122 ± 3	0.31±0.01	11.8±1.1	5.9±0.8	47.9 ± 3.8
		Shade	3	107 ± 3	0.26±0.02	12.3±1.0	6.5±0.7	51.6 ± 0.5
	<i>Pf</i>	Sun	3	124 ± 14	0.41±0.03	15.8±1.8	5.1±0.7	47.3 ± 1.3
		Shade	3	108 ± 5	0.40±0.02	14.5±1.5	5.1±0.3	46.8 ± 0.4
	<i>Sg</i>	Sun	3	139 ± 14	0.29±0.01	8.4±1.3	3.6±0.2	52.1 ± 5.0
		Shade	3	103 ± 4	0.26±0.04	9.9±0.7	4.1±0.5	51.0 ± 6.1

Abbreviations of species: *Cg*, *Carapa grandiflora*; *Cm*, *Corymbia maculata*; *Cs*, *Cedrela serrata*; *Ee*, *Entandrophragma excelsum*; *Ha*, *Hagenia abyssinica*; *Mk*, *Macaranga kilimandscharica*; *Pp*, *Pinus patula*; *Pfalc*, *Podocarpus falcatus*; *Pfulv*, *Polyscias fulva*; *Sg*, *Syzygium guineense*

Symbols and abbreviations of leaf traits: V_{cmax} , maximum rate of carboxylation of Rubisco at 25°C ($\mu\text{mol m}^{-2} \text{s}^{-1}$); J_{max} , maximum rate of electron transport at 25°C ($\mu\text{mol m}^{-2} \text{s}^{-1}$); QY, apparent quantum yield of photosynthesis at 20°C and 25°C in Nyungwe and Arboretum, respectively ($\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$); R_d , dark respiration at 20°C and 25°C in Nyungwe and Arboretum, respectively ($\mu\text{mol m}^{-2} \text{s}^{-1}$); LMA, leaf mass per unit leaf area (g m^{-2}); N_m , leaf mass-based nitrogen content (% of dry mass); P_m , leaf mass-based phosphorus content (% of dry mass); N_a , leaf area-based nitrogen content (g m^{-2}); P_a , leaf area-based phosphorus content (g m^{-2}); SPAD, a proxy of leaf chlorophyll content

Appendix 2 *P* values of an analysis of variance for the effects of Population (13 site by species combinations), Canopy position (upper vs. lower) and their interaction on different leaf traits of tropical tree species in Rwanda. See Appendix 1 for explanations of leaf traits

Leaf trait	Sources of variation		
	Population	Canopy	Population x Canopy
V_{cmax}	< 0.001	< 0.001	0.24
J_{max}	< 0.001	< 0.001	0.63
$J_{max}:V_{cmax}$ ratio	0.002	0.34	0.81
QY	< 0.001	0.011	0.50
LMA	< 0.001	< 0.001	0.057
P_m	< 0.001	0.27	0.20
N_m	< 0.001	0.046	0.012
P_a	< 0.001	0.009	0.49
N_a	0.15	< 0.001	0.16
N:P ratio	< 0.001	0.80	0.58
SPAD	< 0.001	1.00	0.35
Thickness	< 0.001	< 0.001	0.086
Length	< 0.001	< 0.001	0.14
Width	< 0.001	0.19	0.47

Appendix 3 Parameters of the relationships between photosynthetic capacities (y-axis; V_{cmax} or J_{max}) and leaf area- or mass-based foliar nitrogen and phosphorous content (x-axis; P_a , N_a , P_m , N_m) for each site (Nyungwe and Arboretum). Each data point represents one species by site by canopy level combination. Six and seven species were studied in Nyungwe ($n = 12$) and Arboretum ($n = 14$), respectively. See Appendix 1 for explanations of x and y variables.

Site	Nutrient content (P_a & N_a : $g\ m^{-2}$; P_m & N_m : % of dry mass)	Photosynthetic parameter ($\mu mol\ m^{-2}\ s^{-1}$)	Slope	y-intercept	r^2	<i>P</i> value
Arboretum	P_a	V_{cmax}	-5.3	47.2	0.00	0.859
	N_a	V_{cmax}	5.2	32.8	0.06	0.385
	P_m	V_{cmax}	-87.1	58.6	0.12	0.222
	N_m	V_{cmax}	-4.2	54.3	0.03	0.559
	P_a	J_{max}	32.8	86.0	0.02	0.620
	N_a	J_{max}	19.8	42.1	0.18	0.126
	P_m	J_{max}	-143.1	112.7	0.07	0.372
	N_m	J_{max}	-12.3	116.0	0.05	0.440

Nyungwe	P_a	V_{cmax}	-90.0	75.2	0.09	0.330
	N_a	V_{cmax}	-24.7	112.8	0.37	0.035
	P_m	V_{cmax}	147.9	18.3	0.38	0.033
	N_m	V_{cmax}	14.4	19.3	0.32	0.057
	P_a	J_{max}	-177.9	163.3	0.06	0.458
	N_a	J_{max}	-56.7	256.3	0.30	0.067
	P_m	J_{max}	318.7	44.4	0.27	0.085
	N_m	J_{max}	28.4	53.0	0.19	0.161

Appendix 4. Parameters of the relationships between photosynthetic capacities (y-axis; V_{cmax} or J_{max}) and leaf area- or mass-based foliar nitrogen and phosphorous content (x-axis; P_a , N_a , P_m , N_m) for each species by site combination. Twelve leaves were measured for each species by site combination (3 trees, 2 sun leaves and 2 shade leaves per tree; $n = 12$), except for *Polyscias fulva* in the Arboretum which had the double replication (6 trees; $n = 24$). See Appendix 1 for abbreviations of species and explanations of x and y variables.

Site	Species	Nutrient content	Photosynthetic parameters ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Slope	y-intercept	r^2	P value
Arboretum	<i>Cg</i>	P_a	V_{cmax}	88.3	21.9	0.44	0.025
		N_a	V_{cmax}	2.5	28.5	0.04	0.557
		P_m	V_{cmax}	92.8	22.4	0.27	0.101
		N_m	V_{cmax}	-12.0	56.1	0.23	0.132
		P_a	J_{max}	224.6	36.4	0.42	0.031
		N_a	J_{max}	23.0	19.1	0.49	0.017
		P_m	J_{max}	85.8	55.9	0.03	0.589
		N_m	J_{max}	-11.7	88.2	0.03	0.596
	<i>Cm</i>	P_a	V_{cmax}	110.0	53.6	0.03	0.604
		N_a	V_{cmax}	15.9	27.2	0.06	0.430
		P_m	V_{cmax}	303.6	42.8	0.07	0.401
		N_m	V_{cmax}	90.9	-90.8	0.43	0.021
		P_a	J_{max}	435.8	91.8	0.17	0.173
		N_a	J_{max}	37.8	46.6	0.14	0.226
		P_m	J_{max}	998	63.6	0.32	0.055
		N_m	J_{max}	170.2	-155.5	0.60	0.003
	<i>Cs</i>	P_a	V_{cmax}	-33.8	47.7	0.02	0.634
		N_a	V_{cmax}	-1.2	46.7	0.02	0.659
		P_m	V_{cmax}	-21.0	47.8	0.04	0.547
		N_m	V_{cmax}	-7.1	65.6	0.17	0.184

		P_a	J_{max}	4.7	87.3	0.00	0.972
		N_a	J_{max}	1.1	85.3	0.00	0.833
		P_m	J_{max}	-76.0	102.1	0.14	0.226
		N_m	J_{max}	-18.7	144.8	0.34	0.047
	<i>Ee</i>	P_a	V_{cmax}	107.6	24.4	0.16	0.223
		N_a	V_{cmax}	6.18	24.1	0.25	0.118
		P_m	V_{cmax}	107.7	24.4	0.16	0.223
		N_m	V_{cmax}	11.2	19.6	0.06	0.484
		P_a	J_{max}	-180.0	87.1	0.02	0.760
		N_a	J_{max}	11.8	39.9	0.16	0.380
		P_m	J_{max}	-180	87.1	0.02	0.760
		N_m	J_{max}	-45.8	148.0	0.20	0.315
	<i>Pf</i>	P_a	V_{cmax}	101.9	34.3	0.32	0.006
		N_a	V_{cmax}	7.9	36.4	0.25	0.019
		P_m	V_{cmax}	-30.4	61.7	0.02	0.583
		N_m	V_{cmax}	-9.2	75.0	0.05	0.311
		P_a	J_{max}	208.0	58.9	0.23	0.044
		N_a	J_{max}	14.6	67.8	0.15	0.118
		P_m	J_{max}	-40.4	112.0	0.00	0.797
		N_m	J_{max}	-26.1	159.3	0.06	0.348
	<i>Pfalc</i>	P_a	V_{cmax}	24.4	27.5	0.12	0.269
		N_a	V_{cmax}	4.1	24.2	0.04	0.511
		P_m	V_{cmax}	51.1	29.2	0.09	0.346
		N_m	V_{cmax}	37.9	-16.7	0.17	0.178
		P_a	J_{max}	12.7	87.8	0.02	0.657
		N_a	J_{max}	0.89	90.8	0.00	0.911
		P_m	J_{max}	33.5	87.3	0.02	0.630
		N_m	J_{max}	27.1	53.9	0.06	0.461
	<i>Pp</i>	P_a	V_{cmax}	5.7	49.8	0.00	0.964
		N_a	V_{cmax}	10.3	23.7	0.43	0.028
		P_m	V_{cmax}	5.7	49.8	0.00	0.964
		N_m	V_{cmax}	71	-62.0	0.58	0.007
P_a		J_{max}	218.1	52.8	0.45	0.049	
N_a		J_{max}	30.8	26.2	0.82	0.0003	
P_m		J_{max}	67.1	93.8	0.01	0.819	
N_m		J_{max}	151.8	-138.4	0.63	0.006	
Nyungwe	<i>Cg</i>	P_a	V_{cmax}	99.2	8.3	0.13	0.219
		N_a	V_{cmax}	9.44	12.4	0.25	0.083
		P_m	V_{cmax}	-83.2	55.8	0.09	0.328

		N_m	V_{cmax}	9.3	18.2	0.05	0.473
		P_a	J_{max}	152.5	42.3	0.08	0.425
		N_a	J_{max}	19.8	33.2	0.25	0.144
		P_m	J_{max}	-170.8	122.7	0.07	0.459
		N_m	J_{max}	22.8	38.0	0.08	0.429
	<i>Ee</i>	P_a	V_{cmax}	-18.0	48.2	0.03	0.678
		N_a	V_{cmax}	1.1	39.9	0.00	0.894
		P_m	V_{cmax}	-58.6	56.2	0.17	0.270
		N_m	V_{cmax}	-9.2	63.6	0.02	0.650
		P_a	J_{max}	-210	130.5	0.56	0.052
		N_a	J_{max}	-18.2	128.4	0.09	0.397
		P_m	J_{max}	-190.8	119.3	0.39	0.134
		N_m	J_{max}	-29.1	146.1	0.05	0.552
	<i>Ha</i>	P_a	V_{cmax}	-340.7	131.9	0.11	0.346
		N_a	V_{cmax}	-8.0	86.2	0.01	0.746
		P_m	V_{cmax}	-92.6	105.5	0.10	0.372
		N_m	V_{cmax}	-18.3	137.6	0.17	0.234
		P_a	J_{max}	-	-	-	-
		N_a	J_{max}	-	-	-	-
		P_m	J_{max}	-	-	-	-
		N_m	J_{max}	-	-	-	-
	<i>Mk</i>	P_a	V_{cmax}	267.9	-7.2	0.27	0.081
		N_a	V_{cmax}	31.5	-18.9	0.36	0.038
		P_m	V_{cmax}	202.8	15.1	0.08	0.359
		N_m	V_{cmax}	20.2	15.1	0.10	0.304
		P_a	J_{max}	773.2	-49.6	0.39	0.071
		N_a	J_{max}	93.3	-94.6	0.41	0.062
		P_m	J_{max}	1172.2	-108.1	0.31	0.121
		N_m	J_{max}	37.0	53.7	0.07	0.502
	<i>Pf</i>	P_a	V_{cmax}	55.8	55.4	0.07	0.476
		N_a	V_{cmax}	7.4	52.8	0.09	0.395
		P_m	V_{cmax}	256.2	9.5	0.27	0.125
		N_m	V_{cmax}	46.6	-27.8	0.55	0.014
		P_a	J_{max}	276.4	89.4	0.29	0.109
		N_a	J_{max}	30.5	91.1	0.28	0.114
		P_m	J_{max}	521.4	40.6	0.20	0.195
		N_m	J_{max}	79.5	-3.0	0.29	0.109
	<i>Sg</i>	P_a	V_{cmax}	-58.6	53.0	0.04	0.555
		N_a	V_{cmax}	-13.3	75.7	0.23	0.116

		P_m	V_{cmax}	-54.0	50.1	0.02	0.656
		N_m	V_{cmax}	-10.1	63.5	0.12	0.266
		P_a	J_{max}	-173.1	124.3	0.06	0.487
		N_a	J_{max}	-29.1	165.7	0.21	0.158
		P_m	J_{max}	-168.6	118.9	0.04	0.547
		N_m	J_{max}	-24.6	145.3	0.13	0.269

Appendix 5 *P* values of an analysis of variance for the effects of successional group (Succ), Species identity, Canopy position and their relevant interactions on different leaf traits of tropical tree species in Rwanda. See Appendix 1 for explanations of leaf traits

Leaf trait	Sources of variation				
	Succ	Species	Canopy	Canopy x Species	Canopy x Succ
V_{cmax}	0.005	0.41	0.34	0.73	0.32
J_{max}	0.003	0.67	0.20	0.81	0.23
$J_{max}:V_{cmax}$ ratio	0.21	0.26	0.82	0.62	0.66
QY	0.003	0.54	0.067	0.69	0.51
R_d	0.34	0.12	0.017	0.98	0.88
LMA	0.26	< 0.0001	0.001	0.069	0.39
P_m	0.29	< 0.0001	0.92	0.66	0.89
N_m	0.40	< 0.0001	0.61	0.97	0.95
P_a	0.63	< 0.0001	0.010	0.013	0.82
N_a	0.16	0.058	0.065	0.46	0.81
N:P ratio	0.50	0.0007	0.81	0.87	0.93
SPAD	0.060	< 0.0001	0.96	0.83	0.63
Thickness	0.73	< 0.0001	0.033	0.20	0.48
Length	0.38	< 0.0001	0.39	0.80	0.17
Width	0.29	0.0033	0.19	0.65	0.23

Appendix 6 *P* values of an analysis of variance for the effects of species identity (Species), study site (Site), canopy position (Canopy) and their relevant interactions on different leaf traits of tropical tree species in Rwanda. See Appendix 1 for explanations of leaf traits

Leaf trait	Sources of variation						
	Site	Species	Site x Species	Canopy	Canopy x Site	Canopy x Species	Canopy x Site x Species
V_{cmax}	0.052	<0.001	0.39	0.097	0.10	0.61	0.95
J_{max}	<0.001	<0.001	0.030	0.060	0.83	0.79	0.80

$J_{\max}:V_{\text{cmax}}$ ratio	0.002	0.093	0.11	0.12	0.30	0.70	0.093
LMA	0.79	0.64	0.20	0.002	0.045	0.71	0.62
P_m	<0.001	0.048	0.23	0.93	0.43	0.85	0.17
N_m	0.10	0.70	0.17	0.33	0.50	0.84	0.30
P_a	<0.001	0.095	0.037	0.009	0.46	0.12	0.27
N_a	0.19	0.62	0.48	0.006	0.11	0.66	0.51
SPAD	0.34	<0.001	0.058	0.45	0.81	0.28	0.29
N:P ratio	<0.001	0.009	0.041	0.79	0.96	0.90	0.83
Thickness	0.15	<0.001	0.67	0.004	0.046	0.39	0.34
Length	0.007	<0.001	0.028	0.012	0.20	0.33	0.099
Width	0.27	0.003	0.17	0.28	0.23	0.64	0.43