Morphological and chemical leaf traits of tropical montane tree species and their responses to warming

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Declaration

I, Aloysie MANISHIMWE, declare that this thesis titled “Morphological and chemical leaf traits of tropical tree species and their responses to warming” is the outcome of my own PhD by research, except where specifically acknowledged.

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Dedication

I, Aloysie MANISHIMWE, dedicate this thesis
titled “Morphological and chemical leaf traits of
tropical tree species and their responses to
warming”.

To my husband
To my daughter
To my mother
To my late father
To my sister
To my brothers
To my uncles
To my Aunties

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Abstract

Leaf morphological and chemical traits of tropical trees vary along climate gradients, but it is currently unclear how they will respond to a rapidly warming climate and how this will vary among species. Considering this, tropical trees native to East and Central Africa were investigated in three complementary studies in Rwanda. Differently aged trees of both early (ES) and late (LS) successional species from two types of upland forest, Lake Victoria transitional rainforest (LVTF;1600-2000 m a.s.l.) and Tropical montane rainforest (TMF; 2000–3000 m a.s.l.) were included. The three studies were: (i) an elevation gradient study on young trees of 18 species planted at three sites, from 2400 m to 1300 m a.s.l (15.2-20.6 ºC mean annual temperature, MAT); (ii) an elevation gradient study on mature trees of four species growing at five sites from 2700 to 1700 m a.s.l (13.3-19.5 ºC MAT); and (iii) a study of 20 mature tree species growing in 15 permanent monitoring plots in Nyungwe TMF located between 2500 to 1950 m a.s.l. (14.1-16.1 ºC MAT).

The variation in leaf morphology (LMA: leaf mass per area, LA: leaf size or area, leaf W/L: leaf width to length ratio or leaf shape) and leaf macro- and micronutrients were investigated. LA and LMA decreased while leaf W/L increased with decreasing elevation in most species. The decline in LMA with declining elevation was mostly due to decreasing leaf density rather than leaf volume per unit area. The leaf morphological elevation responses of young and mature trees were essentially the same for the four species included in all three studies. The elevation gradient site effects on leaf morphology are likely related to both increasing temperature and vapour pressure deficit (VPD) from high to the low elevation sites.

The most limiting nutrient was P independently of ontogeny, successional strategy, and elevation, although for some species and age classes also K, S, Zn, Cu could potentially be co-limiting. The ratios of most nutrient elements (except K, Ca, Mn) to N were higher in mature compared to young trees. Leaf nutrient resorption efficiency varied among species but not between successional groups.

The juvenile tree leaf concentration of most nutrients across species and sites correlated significantly with its soil concentration (N, P, K, Ca, Mg, Fe, Zn, and Cu) and soil pH (K, Ca, Mg, Fe, Mn, Zn, B, Cu, and Mo). Down the elevation gradient, mass-based leaf contents of Ca, Mg, Fe, B and Mo increased while N, P, Zn, and Cu decreased with most species responding similarly., while the elevation effect of other elements were more variable among species. The nutrients declining down the elevation were all potentially limiting and warming will therefore likely enhance nutrient limitation and possibly lead to reduced productivity of these species. However, no such elevation trend was observed for mature trees. The elevation effect may therefore be transient with age.

In general, and independently of tree age, the variation in both leaf morphology and leaf nutrient content and ratios was considerably larger between species than between sites, despite large site differences in both soil and climate conditions. Several morphological and nutrient variables differed significantly between successional groups, but the species identity explained more the main variation between species than the successional strategy. This study therefore clearly shows the risk of predicting forest responses to climate change using only few species, highlighting the need for more data from tropical trees.

Key words: tropical montane forests, tropical trees, leaf morphology, leaf nutrients, warming climate, climate change.

List of papers

Papers and Manuscripts included in this thesis:
Paper I: **Manishimwe, A**., Ntirugulirwa, B., Zibera, E., Nyirambangutse, B., Mujawamariya, M., Dusenge, M. E., Bizuru, E., Nsabimana, D., Uddling, J., & Wallin, G. (2022). **Warming responses of leaf morphology are highly variable among tropical tree Species**. *Forests*, *13*(2), 1–24. https://doi.org/10.3390/f13020219

Paper II: **Manishimwe, A.;** Ntirugulirwa, B.; Zibera, E.; Bizuru, E.; Nsabimana, D.; Uddling, J.; Wallin, G.; **Leaf nutrient content in young Afromontane trees along an elevation gradient is more controlled by species identity than by soil and climate (***Manuscript***)**

Paper III: Mujawamariya, M.; **Manishimwe, A.**; Ntirugulirwa, B.; Zibera, E.; Ganszky, D.; Ntawuhiganayo B. E.; Nyirambangutse, B.; Nsabimana, D.; Wallin, G.; Uddling, J. (2018). **Climate sensitivity of tropical trees along an elevation gradient in Rwanda**. *Forests* 9, no. 10: 647. https://doi.org/10.3390/f9100647

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The papers and manuscripts and their respective supplementary material are appended at the end of the thesis.

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List of Symbols and Abbreviations

ATMF	African Tropical Montane Forest
ANOVA	Analysis of variance
$A_{Shrinkage}$	Fraction of shrinkage in area of senesced
B	Boron
°C	Degree Celsius
°N	Degree North
°S	Degree South
C	Carbon
Ca	Calcium
Cu	Copper
ES	Early succession
Fe	Iron
GLM	General linear model
HE	High elevation site
HSD	Honestly significant difference
IPCC	Intergovernmental panel on climate change
K	Potassium
K	Leaf area factor or Montgomery parameter
KRE	Potassium resorption efficiency (%)
LM	Leaf mass
LS	Late succession
LMA	Leaf mass per area (g m^{-2})
LA	Leaf size or leaf area (cm^{-2})
$L_{A,fresh}$	Area of fresh green leaves
$L_{A,dry}$	Area of dried, senesced leaves
LD	Leaf density (g cm^{-3})
LE	Low elevation site
LiMA	Litter leaf mass per unit area
LL	Leaf length
LT	Leaf thickness
LW	Leaf width
LVA	Leaf volume per unit area (cm^{-3} m^{-2})
LVTF	Lake Victoria Transitional Forest
MAT	Mean annual temperature (ºC)
MAP	Mean annual precipitation (mm)
M a.s.l	Meter above sea level
ME	Mid-elevation site
M_{Loss}	Fraction of mass loss
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
NPP	Nyungwe permanent plots
NRE	Nitrogen resorption efficiency (%)
NuRE	Nutrients resorption efficiency (%)
N	Nitrogen

[N]	Leaf nitrogen concentration
N_A	Area-based Nitrogen (g m^{-2})
N_M	Mass-based Nitrogen (mg g^{-1})
[Nu]	Leaf nutrient concentration
$[Nu]_L$	Nutrient concentration in green leaves
$[Nu]_{LT}$	Nutrient concentration in litter
P	Phosphorus
P_A	Area-based Phosphorus (g m^{-2})
P_M	Mass-based Phosphorus (mg g^{-1})
PRE	Phosphorus resorption efficiency (%)
PPFD	Photosynthetic photon flux density (μmol m^{-2}s^{-1})
S	Sulfur
SPSS	Statistical Package for the Social Sciences
RTREE	Rwanda TRopical Elevation Experiment
TH	Tree height
TMF	Tropical Montane Forest
TMCF	Tropical Montane Cloud Forest
W/L	Width to length ratio or leaf shape
VPD	Vapour pressure deficit (kPa)
Zn	Zinc

1. General introduction

1.1. Tropical montane forests extent, location, and uniqueness

Tropical montane rainforests (TMFs) are unique ecosystems due to their altitudinal zonation and diverse stand structure, dominated by evergreen and semi-deciduous broadleaved tree species (Moser et al., 2008; Richter, 2008). Latitudinal and altitudinal gradients in temperature, precipitation, solar radiation, and geomorphology drive the spatial distribution and diversity of the structure and function of these ecosystems (Salinas et al., 2021; Xu et al., 2017). TMFs are found between latitudes of 23.5°N (tropic of Cancer) and 23.5°S (tropic of Capricorn) and commonly defined as forest within an elevation range between a lower limit of 800 to 1000 m a.s.l. up to 3600-4000 m a.s.l. (Kappelle, 2004; Richter, 2008). Based on satellite data, (Spracklen & Righelato, 2014) estimated the total area of these forests above 1000 m a.s.l. to 880 000 km^2, corresponding to 8% of the tropical forest area. The elevation limits may, however, be lower in the outer tropics, tropical islands and coastal areas while increasing towards the equator and at continental highlands (determined as the Massenerhebung effect or noted as telescoping), as schematically illustrated in Figure 1 (Grubb, 1971; Irl et al., 2016; Kappelle, 2004). As an example, the TMF in central African upland often starts at elevations above 1600-2000 m a.s.l. The TMFs exist at elevations from the tropical temperate climate zone up to the cold altitudinal belts and varies considerably within their distributional area from (i) open mountain woodland in arid to semi-arid zones, to (ii) mountain forests in semi-humid to humid zones, and to (iii) mountain rainforest in per-humid zones (Richter, 2008).

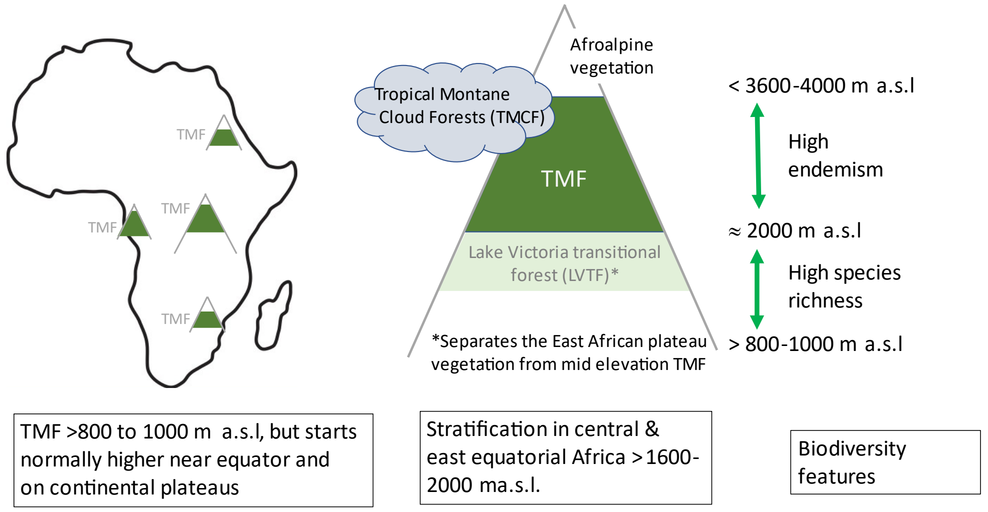


Figure 1. Schematic illustration of tropical montane rainforests (TMFs) distribution in Africa and their general features. The dark green color represents the TMFs while the light green color represents the Lake Victoria transitional forest (LVTF).

TMFs are often separated from the tropical lowland forests by transitional forests determined by e.g., the premontane belt in the northern Andes and by the Lake Victoria transitional forest (LVTF, at approximately 1600-2000 m a.s.l) on the East African plateau (Kappelle, 2004; Kindt et al., 2014). Above the upper montane tree line, the Alpine ecosystem takes over and extend up to ~4500 m a.s.l., above which exists the nival belt with mountain glaciers (Kappelle, 2004). Within the TMF elevation range, the climate and vegetation gradually change upwards and to account for these altitudinal changes these forests often are separated into lower, mid, and upper TMFs. However, sometimes the lower TMF is completely overlapping with the transitional zone, and it should be noted that on this topic, the terminology for different vegetations zones is not completely consistent across the literature (Mata-Guel et al., 2023). At 2200-2600 m a.s.l., there is an upward transition from irregular to more persistent cloud cover known as Tropical Montane Cloud Forests (TMCF, Figure 1), which covers 6.6% of all TMFs (Bruijnzeel & Scatena, 2011; Doumenge et al., 1995; Kappelle, 2004; Scatena; et al., 2010). TMFs are biodiversity hotspots, with highest species richness at elevations below 2000 m a.s.l. while the abundancy of endemic species usually peaks above 2000 m a.s.l. (Kessler & Kluge, 2010; Richter, 2008). African TMFs (ATMFs) have high levels of endemism (Cronin et al., 2014; Platts et al., 2013) but lower species turnover (i.e., lower increase in number of species with increasing sample area) compared to Neotropical and South-East Asian forest. This is due to repeated extinction events during Pleistocene climatic oscillations and isolation due to tectonic uplift on the African continent (Fayolle et al., 2014; Slik et al., 2015). Still, the ATMFs have high species richness and endemism compared to other ecosystems and has attracted attention of conservationists (Plumptre et al., 2007) due to threats from land-use changes (e.g., deforestation, habitat loss and fragmentation) and climate change (e.g. increased temperature and drought) their mutually reinforcing effects (Mata-Guel et al., 2023; Plumptre, 2010;Soh et al., 2019)

1.2. Climate change threats to tropical montane forests

One of the main features of climate change is increased global average temperature during the coming decades in parallel with a changing regional precipitation pattern (IPCC, 2021). The present climate at equatorial tropical Africa is controlled by two main climatic gradients which affect the species composition: i) the rainfall gradient controlling the dry, moist, and wet forests of West and Central Africa (i.e., the entire Guineo-Congolian region) and ii) the elevational temperature gradient controlling the upland and coastal forest of East Africa (Fayolle et al., 2014).

The East African region is characterized by a complex terrain including high mountains and great lakes, located between the monsoon domain of West Africa and the Indian ocean (Ring et al., 2018) and faces some of the largest inter-annual rainfall variations in the world (Camberlin, 2018)**.** When approaching the equator from south or north latitudes, the seasonal variation in temperature decreases (narrow thermal niche), which potentially makes the equatorial species more sensitive to projected future increases in temperature (Camberlin, 2018; Crous, 2019) as these species are adapted to a thermally stable environment (Cuesta et al., 2020; Malhi et al., 2010).

Between 2030 and 2052, it is predicted that Sub-Saharan temperatures (and associated vapour pressure deficit, VPD) and precipitation extremes will increase and exceed global projection at 2 °C scenario above pre-industrial levels (IPCC, 2018; Niang, I. et al., 2014). Furthermore, Central West Africa will exhibit an increase in the number of hot days and variability in precipitation (i.e., slight increase in heavy rainfall, decrease in length of wet days, and increasing drought) while the East Africa will experience increase in precipitation in some regions and a decrease in others (IPCC, 2018; Niang, I. et al., 2014)**.** Therefore, the global warming effects on the precipitation regimes in the Afro-tropics is uncertain (Camberlin et al., 2010; Camberlin, 2018; IPCC, 2021; Ring et al., 2018).

The climate of tropical mountains is normally assigned as cold tropical (mean annual temperature, MAT <18°C) and lacks clear thermal seasons with broader temperature variations than annual ones (Richter, 2016). Under humid conditions, MAT decrease with increasing elevation, typically by 0.60 °C per 100 m (Richter, 2016). Assuming that vegetation is tracking their thermal niche, a possible future temperature increases of 2 ºC during the coming decades would move the altitudinal temperature climate gradient upwards by approximately 300 to 350 m.

For montane species to keep their current functional climatic niche in the future, it is predicted that most species will shift upslope as a response to warming (Platts et al., 2013; Salinas et al., 2021; Zu et al., 2021). The upslope shift of species has been defined as an upward migration of warm-adapted or thermophilic species, a phenomenon known as thermophilization (Johnston et al., 2018) and considered as a pathway of tropical montane species towards extinction if they cannot acclimate to future warming and compete with upslope migrating warm adapted species (Freeman et al., 2021; Salinas et al., 2021). This shift in species composition in relation to elevation has already been observed in TMF located in the Andes (Duque et al., 2015; Fadrique et al., 2018), Taiwan, Mexico, Hawaii, and East Africa (Mata-Guel et al., 2023) and these changes are predicted to continue in the future (Bax et al., 2021). However, less is known about the warming responses of the ATMFs though some studies noted that African lowland tropical forests are more resistant to climatic extremes than Amazonian and Asian forests (Bennett et al., 2021). This is also supported by paleo-ecological records suggesting possible tolerance of ATMFs to warm temperatures because many tree species in these forests have experienced warmer climates in the past (Ivory et al., 2016; Morel & Nogué, 2019).

Generally, plant growth is determined by the interaction between temperature and precipitation but in high mountains, temperature is usually the main factor controlling growth (Mainali et al., 2015). However, a study in Hawaiian tropical montane wet forests found that the effect of temperature on wet TMFs can be modulated by the inter-annual rainfall variations (Lyu et al., 2021; Selmants et al., 2014). This is likely explained by the effect of pronounced seasonality in rainfall (Hiltner et al., 2016; Richter, 2008).

In this regard, it is important to investigate whether ATMFs will resist or acclimate to alarming future warming climate by noting that the future of tropical species will require an investigation of individual-level acclimation capacity (Feeley et al., 2023).

1.3. Tropical montane forest productivity

TMFs provide diverse ecosystem services including regulation of climate, water, carbon and nutrient cycling and storage (Salinas et al., 2021). The carbon storage of both montane and lowland Afrotropical forests was significantly higher than in Neotropical forests (Cuni-Sanchez A. et al., 2021). It has been shown that the ATMFs store as much above ground carbon

(149.4 t ha^{-1}) as their lowland counterparts (Cuni-Sanchez A. et al., 2021; Nyirambangutse et al., 2017), which is much higher than previously thought (Spracklen & Righelato, 2014). Some studies also indicate that the productivity of the ATMFs is high compared to TMFs on other continents (Nyirambangutse et al., 2017; Sierra Cornejo et al., 2021). On the other hand, under warming climate, it is predicted that these carbon storing forests will become sources of carbon, since soil microbial activity and respiration will increase and therefore enhance CO_2 releases in the atmosphere (Chen et al., 2017; Nottingham et al., 2019).

In general, phosphorus (P) has been thought as the limiting element for forest production in the tropics (Vitousek, 1984) due to old, strongly weathered soils (Vitousek & Farrington, 1997). However, nitrogen (N) has been suggested to increasingly limit forest production with increasing elevation, due to reduced mineralisation at lower temperature (Fisher et al., 2013; Wolf et al., 2011). For instance, in humid TMFs, soil N content and soil organic carbon (SOC) increased (with SOC increasing more than N) while soil N isotopic natural abundance (δ15N) declined with elevation worldwide due to increased immobilisation and reduced mineralisation at higher altitudes (Gay et al., 2022). By contrast, some studies found that trees also in TMFs are mostly limited by P (Allen et al., 2020; Cárate-Tandalla et al., 2018), whereas other studies suggested a co-limitation by N and P (Fisher et al., 2013; Tanner et al., 1990; Vitousek & Farrington, 1997).

These inconsistent results emphasize the need to investigate the limiting nutrients in TMFs and how elevated temperature may affect the limitation balance between N and P. Furthermore, little is known on how other potentially limiting nutrients may affect tropical forest trees (Townsend et al., 2011), especially during warming. Yet, different macro- and micronutrients play different metabolic roles, for example through their constituents and activators of enzymes and facilitator of transport over membranes essential for all physiological processes including photosynthesis, development of new leaves and allocation within trees (Uchida, 2000).

2. Literature review

2.1. Leaf traits, their importance for productivity and response to climate change

Leaves are the core organ driving the functioning of terrestrial ecosystems through their interception of solar radiation (Falster & Westoby, 2003) used to synthetise carbohydrates, the process known as photosynthesis (Bolhàr-Nordenkampf & Draxler, 1993). This process is ultimately controlled by the morphological, chemical and physiological states and processes in the leaves (Wright et al., 2004). Leaf morphological and chemical traits correlate with each other, and they differ across species and environmental gradients (Wang et al., 2013). The relationships between these traits constitute the universal leaf economic spectrum consisting of key structural, chemical, and physiological properties. These traits include but are not limited to LMA, leaf thickness (LT), equivalent to leaf volume per unit area (LVA) in flattened leaves, leaf density (LD), , leaf size (here expressed as area of individual leaves, LA), and leaf shape or leaf width to length (W/L) ratio and leaf contents of N, P , and other macro-and micronutrients (Meziane & Shipley, 2001; Shipley et al., 2006; Wright et al., 2004). Intra- and interspecific variations of all these traits (Figure 2) and their responses to warming were investigated in this study.

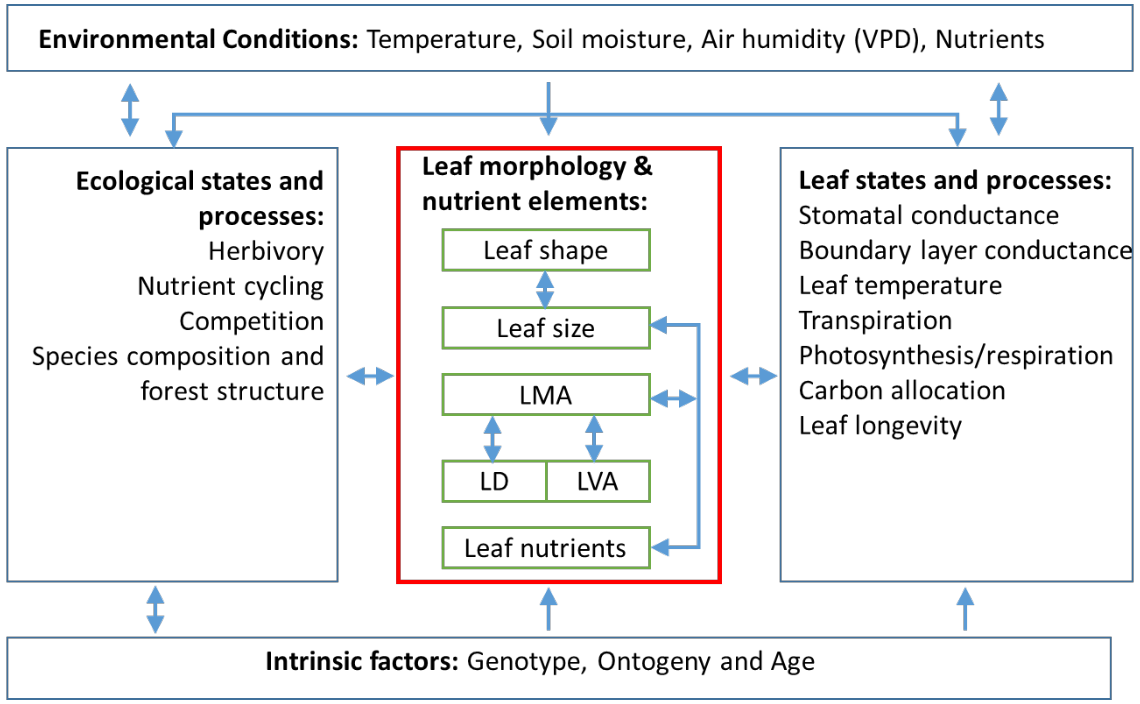


Figure 2. Conceptual relationship between leaf morphology and nutrient elements traits and how they relate to some environmental factors, other leaf traits, species genetics, and ecological states and processes. The red frame encloses the focus area of this thesis, in which the respective acronyms represent: LMA, leaf mass per unit area; LD, leaf density; LVA, leaf volume per unit area.

Leaf traits vary within and between individuals, species and ecosystem, e.g. between crown positions, ontogenetic stages, successional stages and forest types (Derroire et al., 2018; Reich et al., 2003). Dynamic successional stages including early (ES), intermediate and late successional (LS) groups characterise tropical forests, including TMFs (Carson et al., 2008; Coelho et al., 2016; Fan et al., 2019). ES and LS species have acquisitive and conservative strategies, respectively (Lohbeck et al., 2015). Conservative LS species are characterized by high LMA, low nutrient concentrations, low herbivory rate, low light demanding or shade tolerant and low photosynthetic rates as opposed to characteristics of acquisitive ES species (Poorter et al., 2004; Schönbeck et al., 2015).

Previous experiments with tropical trees have indicated that ES species respond more positively (or less negatively) to warming than LS species (Cheesman & Winter, 2013; Slot & Winter, 2018). This has been shown for plant growth (Cheesman & Winter, 2013) as well as for photosynthesis (Slot & Winter, 2018; Tarvainen et al., 2021; Vårhammar et al., 2015). Moreover, the plasticity of leaf traits including LMA to changes in light environment was found to be greater in ES compared to LS species in Panamian rainforest shrubs (Valladares et al., 2000) while the plasticity was higher in LS than in ES tropical lowland tree species (Rozendaal et al., 2006). However, whether also leaf morphological and chemical traits show larger acclimation capacity to warming in tropical montane ES compared to LS tree species remains unknown.

Leaf morphological and chemical traits were found to be affected by ontogenetic developmental changes of trees. Mature trees typically exhibit higher LMA than young trees (Houter & Pons, 2012; Ji et al., 2021; Mediavilla et al., 2014; Sean C. Thomas & Winner,

2002) while leaf size of young trees was small compared to mature trees in a sunlit site of tropical lowland rain forest (Ishida et al., 2005). By contrast, young or small understory trees had larger leaf size than mature or large canopy trees for most studied species in this tropical lowland rain forest (Thomas & Ickes, 1995) with larger, and thinner leaves capturing more light under large canopy trees (He & Yan, 2018). However, the reverse pattern or no trend in leaf size was observed in the same forest when comparing young or small understory trees versus mature or large canopy trees (Thomas & Ickes, 1995).

The difference in leaf size of young versus mature species in sunlit sites and leaf size of young or small understory versus mature or canopy tree species are likely driven by the species-specific shade tolerance and carbon allocation capacity (Ntawuhiganayo et al., 2020). Moreover, the ontogenetic changes in crown exposure affected the plasticity of ES versus LS tropical low land species (Rozendaal et al., 2006). Leaf N concentrations were higher in mature than in young trees in some studies (He & Yan, 2018; Houter & Pons, 2012; Mediavilla et al., 2014), but the opposite has also been observed (Ji et al., 2021).

2.1.1. Response of LMA and its components to warming climate

Leaf mass per area (LMA) is recognised as a good indicator of plant performance with respect to, e.g., photosynthetic and respiratory rates, chemical composition, resistance to herbivory (De La Riva et al., 2016) and forest productivity (Marron et al., 2005). LMA can respond to environmental factors through changes in both LD and LVA (Figure 3) (Coble & Cavaleri, 2017; Poorter et al., 2009; Villar et al., 2013; Zhang et al., 2020). LMA mostly decreased with increasing temperature, water availability and nutrient stress through changes in LD rather than in LVA (Poorter et al., 2009). Drought, however, increased LMA sand this occurred mostly through changes in LVA (Lambers et al., 2008; Poorter et al., 2009). Furthermore, Choong et al. (1992) reported that LVA contribute more than LD to variation in LMA of tropical tree species, regardless of varying environmental factor. A given change in temperature has a larger effect on LMA in tropical species compared to boreal species (Poorter et al., 2009). However, the LMA responses of tropical tree species to climate change are poorly understood due to general scarcity of data and large-scale manipulative warming experiments in the tropics (Cavaleri et al., 2015; Chapman et al., 2021; Poorter et al., 2009).

Earlier studies conducted on leaf states and processes along elevation gradients in the Andes found an increase in LMA with elevation and thus a decrease with warming (Neyret et al., 2016; van de Weg et al., 2009). However, LMA also increased with tree height (Cavaleri et al., 2010), and tree height declined with increasing elevation (Girardin et al., 2014; Milla & Reich, 2011). In addition, LMA declined with increasing leaf W/L ratio (Lin et al., 2020). While LMA and area-based leaf N and P content positively correlated in global datasets (Osnas et al., 2013, 2018; Wright et al., 2004), elevational trends in leaf N and P content are mixed showing increase or no change in spite of a consistent LMA increase (Kitayama & Aiba, 2002; Körner, 1989; Tanner et al., 1998; van de Weg et al., 2009; Vitousek et al., 1988). These inconsistent global patterns and elevational trends of LMA with other factors complicate causal interpretations.

2.1.2. Response of leaf size and leaf shape to warming climate

Leaf size and shape have an influence on energy interception, leaf temperature, thickness of boundary layer (Leigh et al., 2017), gas exchange (i.e., CO_2, H_2O) and primary productivity (Li, Reich, et al., 2020; Li, Zou, et al., 2020). Two elevation gradient studies in Mediterranean and temperate climates found LA to decline with increasing elevation due to declining temperature and its covariance with tree height and other traits affected by elevation (Milla & Reich, 2011; Nichlos et al., 2019). In addition, there were significant spatial patterns in leaf shape where leaves became shorter and rounder towards cooler climates, i.e. leaf W/L ratio increases (Li, Zou, et al., 2020) whereas LA increased with increasing MAT and or annual precipitation but correlated more with precipitation than with temperature (Li, Zou, et al., 2020; Nichlos et al., 2019; Peppe et al., 2011; Wright et al., 2017).
Severe drought and high VPD together caused a reduction in LA in a study on drought induced responses in Maize (Devi et al., 2022). Moreover, individual trees of the same species grown under high VPD had smaller LA (1/3 size) and slightly greater vein and stomatal density than species grown under low VPD (Carins Murphy et al., 2014). Therefore, the conflicting influences of temperature and VPD on LA may complicate the prediction of changes in LA in areas such as the tropics where both factors are likely to increase in intensity with a changing climate (Wright et al., 2017). In a global review study, stomatal conductance declined while transpiration increased under high VPD and led to reduced photosynthesis and growth (Grossiord et al., 2020).

2.1.3. Demand, supply, and limitation of leaf nutrients

Nutrients are important for all physiological process in the leaves, with implications for tree growth and development (Karthika et al., 2018; Uchida, 2000). They are allocated to leaves directly from acquisition of new nutrients (i.e., root uptake) from the soil (Mitra, 2017) and from the internal reallocation of nutrients from old senescing leaves (i.e., nutrient resorption) to be used in new developing leaves (Killingbeck, 1996). Previously, studies have focused mainly on N and P to study growth limitation of TMFs trees (see section 1.3). However, plants require other nutrients (K, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo and Ni) than N and P for growth and other vital plant functions (Ågren, 2008; White & Brown, 2010) and these can potentially also be limiting (Sobrado, 2014). Recent studies suggested that K, Ca and Mg are potential future growth-limiting elements and increasingly required nutrients in response to rising N and P availability in TMFs (Velescu et al., 2021; Wilcke et al., 2019). Studies of these other elements in TMFs are rare (Benner et al., 2010) and it is therefore difficult to conclude whether other than N and P elements also can limit or co-limit productivity of these forests. Among plants elements, N, P, K, Ca, Mg, and S are macronutrients needed in high quantities together with structural nutrients (i.e., C, H, and O) while the rest are micronutrients needed in low quantities, but all are known as essential nutrients (Karthika et al., 2018; Mitra, 2017).

Both deficiency and excess, causing limitations or toxicity, respectively, of nutrient contents lead to disorders of various physiological processes, sometime expressed as visual morphological symptoms such as leaf chlorosis, and plant stunting, etc (Karthika et al., 2018;

Millaleo et al., 2010; Uchida, 2000). However, the plant demand for other nutrients than N are assumed to have general stoichiometric relationships with N in the leaves (Ågren, 2008; Marler, 2021; Tian et al., 2019), which are maintained (Sobrado, 2014) through coordinated nutrient uptake (Marastoni et al., 2019). Knecht & Göransson, 2004 suggested that these relationships are similar across all terrestrial plants. Thus, leaf nutrient ratios can be used as a diagnostic tool to determine nutrient limitations in ecosystems (Ericsson, 1994; Linder, 1995). It is suggested that a limiting nutrient has a ratio to N below the optimal range (Sumner, 1978). Therefore, focusing on the full set of mineral nutrients seems imperative (Townsend et al., 2011) to explore if global climate change affect these relationships (Litton et al., 2020; Tian et al., 2019).

In the early stage of tree development, leaf nutrient contents may be increased due to high uptake of nutrients by roots of growing young trees until maturity when leaf nutrients start to decline in relation to increased leaf area index (LAI) and increasing shading (Ishida et al., 2005; Sorensen, 2000). Leaf nutrients may also decline during plant ontogeny due to their dilution in greater production of carbon-rich constituents such as cell walls and starch (Ishida et al., 2005; Sorensen, 2000). In addition, leaf nutrient are resorbed from older senescing leaves to new forming leaves before abscission (Killingbeck, 1996; van Heerwaarden et al., 2003). The percentage of the leaf nutrient pool that is resorbed before the old leaf is lost through abscission is known as nutrient resorption efficiency (NuRE) and it is controlled by nutrient concentration, stoichiometry relations and nutrient limitation (Chen et al., 2021; Ji et al., 2018; Wang et al., 2021; Zeng et al., 2017).

Resorption efficiency of N and P (NRE and PRE) were in the range between 26% and 65% approximatively, however, there are few studies on resorption efficiency for non NP- nutrients (Chen et al., 2021; Liu et al., 2014; Vergutz et al., 2012). NuRE differed among nutrients and species, however, NuRE of other nutrients than N and P has not been much analysed (Liu et al., 2014; Marler, 2021; Vergutz et al., 2012; Yan et al., 2016). In addition, few studies have investigated the effect of successional strategy on NuRE and results were contrasting. NRE did not change with overstory succession and resorption efficiency of phosphorus and potassium (PRE and KRE) decreased with overstory succession (Chen & Chen, 2022). Contrary, ES species showed higher NRE and PRE than LS (Zeng et al., 2017) while another study reported significantly lower NRE and PRE in ES compared to LS species (Fahey et al., 1998).

2.1.4 Response of leaf nutrients to elevation and warming

The few available studies of covering most of the macro- and micronutrients along elevational gradients in TMFs found variable trends in leaf nutrients depending on the study. For example, leaf N, P, K, and S declined with increasing elevation (Tanner et al., 1998) while no trend was detected for leaf Mg and Ca (Soethe et al., 2008; Wu et al., 2019) and leaf N, P and K decreased upwards (Soethe et al., 2008). Cu and Mn availability increased while N and K availability decreased with elevation and trends of nutrients ratios were different across species and elements (Litton et al., 2020). However, in this latter study, despite the availability of nutrients in relation to elevation gradients warming, the leaf N and K increased, and Mn decreased while no trend for P with warming was found (Litton et al., 2020). These altitudinal

trends may also vary depending on whether foliar nutrients are expressed on area or mass basis (Körner, 1989; Martin et al., 2020; Vitousek et al., 1988) or whether the soil is old or young (Vitousek & Farrington, 1997).

A global meta-analysis of warming experiments showed increases in net N mineralization, net nitrification rate, N pools and N fluxes with increasing temperature (Bai et al., 2013). By contrast, other warming experiments caused a decline in leaf N, P Reich & Oleksyn, 2004), leaf N, P, K (Fyllas et al., 2009), leaf N (Wu et al., 2019) and litter N concentration with warming (Cusack et al., 2016; Tully & Lawrence, 2010) while no consistent warming effect trend on P, K, Ca and Mg was found (Wu et al., 2019). Therefore, leaf nutrients (e.g., N, K, Ca, P, Mg) were differently affected by warming or elevation as responses differed between species, sites, periods and warming experiment types in tropical forests (Asner et al., 2014; Reich & Oleksyn, 2004; Tian et al., 2019; Townsend et al., 2007; Wu et al., 2019). Considering the suggested variation between species, within species and within individual plants differences in the leaf morphological and chemical traits relationships and responses to warming climate, it is important to deepen our understanding of ecosystem functioning under climate change and study a broad range of species and traits because TMFs are characterised by a great structural and functional diversity.

2.2. Elevation gradient as field laboratory to study climate effects in the tropics

Most past studies along elevation gradients investigated different species occurring at different elevations (Martin et al., 2020; Neyret et al., 2016; van de Weg et al., 2009) and thus cannot distinguish between plastic acclimation and inherent differences among species. This could be resolved in studies using an elevation gradient approach with tree species naturally occurring along the whole elevation gradient or, most preferably, trees of the same genotypes planted at different sites along the elevation gradient. The use of elevation gradients in the tropics to investigate climate change effects under ecologically realistic conditions has been supported and recommended by other researchers (Cavaleri et al., 2015; Malhi et al., 2010). However, in tropical mountains, other variables such as precipitation, cloud cover, soil moisture, pH etc., might also covary with elevation (Moser et al., 2008; Richter, 2016). These other abiotic factors may constrain the warming responses since they affect species traits (e.g., LMA, nutrient concentration, root to shoot ratio,) and therefore influence their shifts along the elevation gradient (Singh Ramesh et al., 2023). It is therefore essential to consider the warming responses along elevation gradients in relation to other factors as well.

3. Research Gaps

The predicted future increase in temperature and variations in rainfall will most likely affect species allocation of carbon and other nutrients, in turn influencing species competitiveness and tree community composition in tropical rainforests (Bonal et al., 2016). However, little is currently known regarding the warming responses of traits controlling productivity and carbon stocks of African TMFs (ATMFs) (Boehmer, 2011; Cuni-Sanchez A. et al., 2021; Nyirambangutse et al., 2017; Salinas et al., 2021) since these forests are poorly investigated (Salinas et al., 2021). Leaf morphological and chemical traits play a great role in the leaf economic spectrum (Wright et al., 2004) and have been suggested to be good indicators of productivity (Marron et al., 2005). Most previous studies that explored the warming responses of ATMFs focused on leaf physiological responses (Table 1 ;Dusenge et al., 2021; Mujawamariya, 2021; Sierra Cornejo et al., 2021; Tarvainen et al., 2021; Vårhammar et al., 2015). Although, several studies also included LMA, leaf N and leaf P to explain physiological responses, no detailed studies of morphology or nutrients have been conducted in these studies. Especially, no study have include a full set of the essential nutrient elements in a large number of species. Current knowledge on warming responses of leaf morphology and nutrients is primarily based on global meta-analysis (Peppe et al., 2011; Poorter et al., 2009; Wright et al., 2017) and the available studies on trees from tropical forests were mostly conducted in other continents than Africa (Doughty et al., 2018; Li, Zou, et al., 2020; van de Weg et al., 2009; Vitousek et al., 1988). These previous studies mostly compare different species growing in different climates making it difficult to separate between species-specific and ecosystem responses.

4. Aims and hypotheses

This thesis focused on tree species mainly present in the East and Central African mid TMF and the LVTF vegetation zones along an elevation gradient ranging from 1300 to 2700 m a.s.l where there is currently no persistent cloud cover, but irregular cloud cover may periodically be found.

The overall aim of this thesis project was to investigate warming responses of leaf morphological and chemical traits of common species found in ATMF and LVTF using an elevation gradient approach, and how these traits may differ between species, successional groups, and tree ages. The results shall contribute to better understanding of how these forests may respond to climate warming and provide additional data to physiological, mortality and survival data to inform future tropical forests restoration, and afforestation initiatives. Hypotheses related to these aims are presented in the four papers of this thesis, dealing with leaf morphology (Papers I, III, IV) and nutrients (Papers II, III, IV). The superscripts letters represent different parts of each hypothesis referred to in the results and discussion parts.

Table 1. Summary of warming responses of leaf and tree level traits in ATMFs. Documented traits are LMA (leaf mass per unit area), LA (leaf area that stands for leaf size), LW/LL (leaf width to leaf length ratio that stands for leaf shape), N (nitrogen), P (phosphorus), gs (stomatal conductance), A_n (net photosynthesis, and R_d (dark respiration). The warming effects for gs, A_n R_d are expressed for common measurement temperatures (e.g., 25 °C) for plant grown at different temperatures. Studied species include LS (late successional species or strategies), and ES (early successional species/strategies) planted in pots in chambers: Ch-ST (chamber short term), Ch-LT (chamber long term), or planted in pots or in sites along an elevation gradient: Elev (elevation gradient) or studied in their natural habitats following an elevation gradient design.

Leaf level traits											Tree level			
Experi-ment	Plant mate-rial	Age	LMA	LA	LW/ LL	N	P	gs	A_n	R_d	Growth	Morta-lity	Description*	References
Ch - ST	Pot	Young							↘				Two native LS and one ES species	Vårhamar et al. 2015
Ch - LT	Pot	Young	→			↘		↘ ↗	↘ ↗	↘			In all species for LMA and R_d, in 1ES for area based N; ↘ in 1ES and ↗ in 1 ES with mixed strategy for An and gs at T_{opt}.	Wittemann et al. 2022
Elev	Pot	Young				↘			↘	↘	↗ →		1 ES and 1 LS, Biomass increase in ES, but not effect in LS	Dusenge et al. 2021
Elev	Pl	Young								↘			In 9 ES, 5 LS and in 2 with mixed strategies, independent of LMA, area based N, and P changes	Mujawamariya et al. 2021
Elev	Pl	Young							↘				In both ES (10 species) and LS (8 species) but highly in LS	Mujawamariya et al.2023
Elev	Pl	Young									↗	↗	Higher Growth in ES (11 species) than in LS (9 species) and Mortality in LS than in ES	Ntirugulirwa et al. 2023 Preprint
Elev	Pl	Young	↘	↘	↗								In both ES (10 species) and LS (8 species) for LMA and LL/LW but only in 1/3 to 1/2 of species; in ES but mostly not in LS for LA	Manishimwe et al. 2022
Elev	Pl	Young				→ ↘ ↗	→ ↘						→ across all species on both area and mass basis, but also ↘ in 5 species and ↗ in 2 species for N while ↘ in 1 species for P on mass basis.	Manishimwe et al. In prep.
Elev	Nat, Same Sp	Mature				↘	↘		↘				In 3 LS and 3 ES in A and area based N and P	Dusenge et al 2015
Elev	Nat, Same Sp	Mature	→			→	→	↘	↘				In 2 LS than in 2 ES but only in dry seasons	Mujawamariya et al. 2018
Elev	Nat, Dif Sp	Mature				↗					↗		Based on leaf C:N ratio	Sierra cornejo et al. 2021
Elev	Nat, Dif Sp	Mature				↗ ↘	↗ ↘				↗		Both N & P peaked at 1800 m; ↗ at > 1800 m and ↘ at < 1800 m	Okello et al. 2022

* Short description of studied native african tropical montane forests (ATMFs) species, etc

↗ : increase with warming or down the elevation gradient

↘ : decrease with warming or down the elevation gradient

→ No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites).

4.1. Leaf morphology

The aim was to explore how leaf morphological traits are influenced by climate, how they vary among species, how they correlate with leaf N and P, and if their warming responses are affected by the species successional strategy (ES and LS, respectively). By investigating LMA, LA, leaf W/L ratio, the contributions of LVA and LD variation to changes in LMA and the relationship between leaf morphology and leaf N and P, the following hypotheses (H) synthesised from the papers were addressed:

H #1: LMA is higher in LS species and mature trees than in ES species and young trees (Paper I, III, IV). It decreases at warmer sites, with larger contributions from shifts in LD than from shifts in LVA (leaf thickness). The warming-induced shift is similar in young and mature trees (Paper I and III)· but larger in ES compared to LS species (Paper I)

H #2: Leaf size (LA) increases at warmer sites and this change is similar in young and mature trees but more pronounced in ES compared to LS species (Paper I and III). It is higher in young than in mature trees (Papers I, III, IV).

H #3: LMA is negatively and positively related to mass-based and area-based leaf N and P, respectively (Paper I).

4.2. Foliar nutrients

The aim was to explore how leaf nutrient content varies between species, successional groups and sites with different elevation and temperature gradient. Patterns between different nutrients, their ratio to N and their resorption efficiency were also investigated.

The following hypotheses (H) synthesised from the papers were tested:

H #4: Except for N/P ratio, leaf nutrients balance is maintained along the elevation gradients (Paper II);

H #5: Leaf nutrient content generally decrease with warming or decreasing elevation (Paper II, III) and N limitation decline while P limitation increase with declining elevation (Papers II, III, IV);

H #6: Leaf nutrient concentrations and N based nutrient ratios are higher in ES compared to LS species and in mature compared to young trees (Paper II, III, IV) but variation among trees is more strongly related to species than to sites (Paper II);

H #7: Resorption efficiency, is higher for P and K compared to enzyme-bound N and S, higher for macro- compared to micronutrients, and higher for LS compared to ES species (Paper IV).

5. Materials and methods

I have combined data from three complementary studies: (1) A young tree elevation gradient study with plantation at three sites along an elevation gradient (Paper I and II); (2) A mature tree elevation gradient study with mainly naturally regenerated trees of four species grown at five sites (Paper III, and a small part of I ; and (3) A study of mature tree species from 15 permanent plots in Nyungwe montane forests established by Nyirambangutse et al. 2017 (Paper IV and a small part of I). The location, coordinates and elevations are shown in Figure 3, Table 2). In the first two studies using an elevation gradient, a step down in the gradient is meant to represent a possible future climate warming scenario. The leaf morphology measurements and the nutrients analysis followed the same protocol in all three studies for common traits.

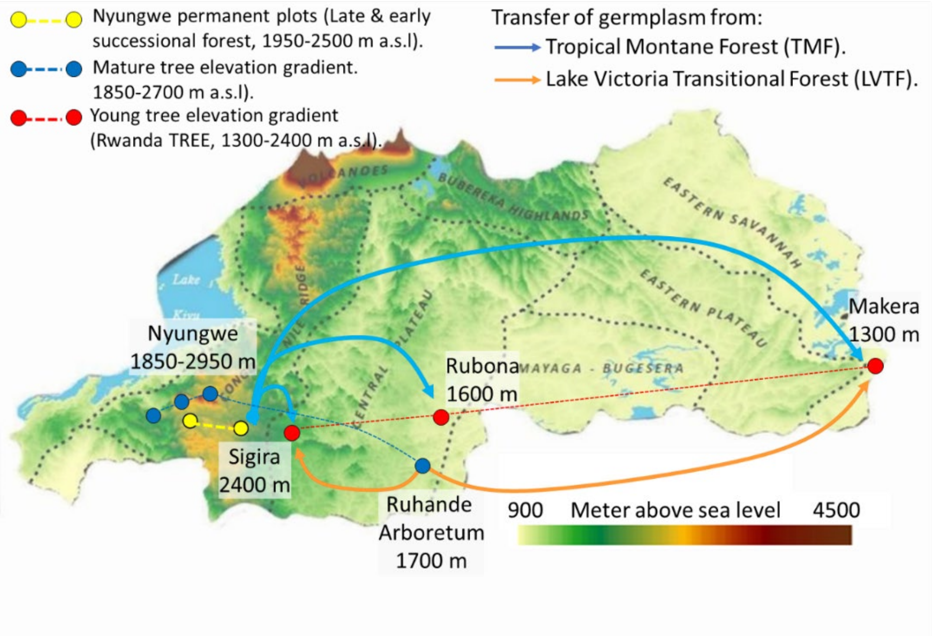


Figure 3. Topographic map of Rwanda indicating the sites' location of the young tree elevation gradient (red, Rwanda TREE: https://www.rwandatree.com/), the Mature tree elevation gradient (blue) and the Nyungwe permanent plots studies (yellow) and the site origin of the TMF and LVTF tree species in the Rwanda TREE. Map modified from REMA, 2011.

5.1. Tree species

All studies included ES and LS species from TMF, but only the young tree study included ES and LS species from LVTF. The species are native to TMF or LVTF of Eastern and Southern Africa, some are also from Pantropical Africa and a few endemics to the Albertine rift. The study on young trees was conducted on 18 species (5 ES/TMF, 5 LS/TMF, 5 ES/LVTF and 3 LS/LVTF) among 20 planted within Rwanda TRopical Elevation Experiment (Rwanda TREE). Two of the species had not enough leaves at the sampling period to be included in this study. The Mature tree elevation gradient included 4 species common to all three studies. These were two ES species: *Macaranga kilimandscharica*, *Polyscias fulva; and two LS species, Carapa grandiflora, Syzygium guineense,* which were among the 13 most common species in Nyungwe

TMF (Plumptre et al., 2002). The Nyungwe permanent plots study focused on 20 mature species (9 ES and 11 LS) among which 7 are common to Rwanda TREE (including *Faurea saligna, Harungana montana, Prunus Africana* in addition to the four above). In total 31 native tree species from 19 families were included (Table 3). The leaf type and shape of these species are dominated by simple leaves with alternate or opposite position and elliptic, obovate, ovate, or oblong shapes. They also include few species with pinnately compound elliptic or oblong leaves (Table 3).

5.2. Research sites

5.2.1. Young tree elevation gradient study

The study on young trees was conducted during 2018 and 2019 at the three sites of Rwanda TREE (see www.rwandatree.com): (i) Sigira, high-elevation (HE) site located in Nyamagabe district at the edges of Nyungwe National Park in TMF vegetation zone at 2400 m a.s.l; (ii) Rubona, mid-elevation (ME) site located in Huye district in the LVTF vegetation zone at 1600 m a.s.l; and (iii) Makera, low-elevation (LE) site located at Ibanda Makera in Kirehe district in the Evergreen and semi-evergreen bushland and thicket vegetation zone at 1300 m a.s.l (Table 2, Figure 3). At each site, 18 plots of 15 × 15 m, spaced by 2.5 m paths. Within each plot, 20 different tree species with a replication of 5 (i.e., 100 trees per plot) were planted at the turn of 2017 and 2018, using 1.5 × 1.5 m spacing and randomized position. Plot treatments was not applied before the sampling within this study was conducted.

Table 2. Weather and other characteristics of different sites in the order from low to high elevation in Rwanda where three complementary studies have been conducted. Weather data are annual mean ± SD. RTREE (Papers I & II), Tropical elevation experiment in Rwanda; Mat-Elev (Paper III), Elevation gradient on four tropical mature tree species; NPP, Nyungwe permanent plot study (Paper IV). ESBT, Evergreen and semi-evergreen bushland and thicket, LVTF, Lake Victoria transitional forest, TMF, Tropical montane forest. MAT, mean annual temperature, T day, temperature at daylight, MAP, mean annual precipitation (give at three time), VPD, vapor pressure deficit, PPFD, photosynthetic photon flux density.

	Makera	Rubona	Arboretum	Cyamu-dongo[5]	Nyungwe West	Sigira	Nyungwe central	Nyungwe East	Bigugu Mountain[5]
Studies	RTREE	RTREE	Mat-Elev	Mat-Elev	Mat-Elev, NPP	RTREE	NPP	Mat-Elev, NPP	Mat-Elev
Potental vegetation	ESBT	LVTF	LVTF	LVTF	TMF	TMF	TMF	TMF	TMF
East-West order	1	2	3	9	8	4	7	5	6
Latitude	S 2°6’31’’	S 2°28’30’’	S 2°36′55″	S 2°33′10″	S 2°28'38"	S 2°30’54’’	S 2°28'43"	S 2°31'54"	S 2°27'10"
Longitude	E 30°51’16’’	E 29°46’49’’	E 29°44′54″	E 28°59′20″	E 29°6'53"	E 29°23’44’’	E 29°12'00"	E 29°23'20"	E 29°14'50"
Elevation (m a.s.l.)	1300	1600	1700	1850	1950	2400	2465	2500	2700
MAT (°C)	20.6 ± 0.1	20.0 ± 0.0	19.5 ± 0.2	17.9	16.2 ± 0.4	15.2 ± 0.1	14.8 ± 0.2	14.3 ± 0.2	13.3
T day (°C)	24.0 ± 0.3	22.4 ± 0.1	21.1 ± 0.1	19.2	18.2 ± 0.5	17.1 ± 0.19	15.9 ± 0.2	15.6 ± 0.2	14.7
T 1%ile (°C)	10.9 ± 0.8	13.4 ± 0.2	16.0 ± 0.5		13.3 ± 0.4	10.9 ± 0.3	11.4 ± 0.3	11.7 ± 0.3	
T 99%ile (°C)	31.2 ± 0.4	28.4 ± 0.4	24.1 ± 0.2		20.4 ± 0.3	23.1 ± 0.4	20.3 ± 0.3	18.1 ± 0.2	
MAP (mm)[1]			1123 ± 124		3016 ± 63		1736 ± 85.4	1577 ± 75	
MAP (mm)[2]			1037 ± 172				1810 ± 301	1657 ± 163	
MAP (mm)[3]	1106 ± 33	1672 ± 136	1414 ± 346			2144 ± 61			
MAP (mm)[4]			1192 ± 207				1732 ± 234		
VPD - day (kPa)	1.14 ± 0.03	1.03 ± 0.01	1.02 ± 0.04		0.44 ± 0.06	0.51 ± 0.03	0.35 ± 0.04	0.39 ± 0.09	
PPFD day (μmol m^{-2} s^{-1})	740 ± 31	764 ± 62	733 ± 25		572 ± 17	611 ± 66	513 ± 50	633 ± 32	

[1]June 2013–May 2015; [2]June 2013–May 2017; [3]Feb 2018 –Jan 2020; [4]Average 2006 to 2021 (2-year gap) and 2007 to 2020 (4 years gap) at Arboretum and Nyungwe central, respectively. [5]Measured for 6 weeks and scale to annual values based on long term studies at neighbouring sites. Data from Paper I and II, III, IV (Manishimwe et al., 2022; Mujawamariya et al., 2018) and unpublished data from both Manishimwe et al. and Nyirambangutse et al.

Table 3. Taxonomy of species and their main forest type of origin, classification into successional group as well as their leaf type and shape. FT, forest type of origin; TMF, tropical montane forest (~ > 2000 m a. s. l.); LVTF, Lake Victoria transitional forest (~ 1500 - 2000 m a. s. l.); SG, successional group (ES, early LS, late); K, leaf area factor; *, species with mixed successional group features, A and Y represent adult and young trees.

Code	Scientific name and author[1]	Family name[1]	FT[2]	SG	Tree age	Distribution[3]	Leaf Type & shape[4]	Plant Functional Type[5]	Papers
Afa	*Afrocarpus falcatus* , (Thunb.) C.N.Page	Podocarpaceae	TMF	LS	A	Eastern and Southern Africa	Simple alternate, Oblong linear	Evergreen	IV
Asa	*Agauria salicifolia* , Hook.f. ex Oliv.	Ericaceae	TMF	ES	A		Simple alternate, elliptic-ovate	Evergreen	IV
Avo	*Afrocrania volkensii* , (Harms) Hutch.	Cornaceae	TMF	ES	A	Central-East Africa	Simple opposite, Elliptic	Evergreen	IV
Bbr	*Bridelia brideliifolia* , (Pax) Fedde	Euphorbiaceae/ Phyllanthaceae	TMF	ES	Y	Eastern and Southern Africa	Simple alternate, Elliptic	Semi-deciduous	I, II
Bmi	*Bridelia micrantha* , (Hochst.) Baill	Euphorbiaceae/ Phyllanthaceae	LVTF	ES	Y	Sub-Sahara Africa	Simple alternate, Elliptic	Semi-deciduous	I, II
Caf	*Chionanthus africanus* , (Knoblauch) Stearn	Oleaceae	TMF	LS	A		simple opposite, Obovate	Evergreen	IV
Cgo	*Chrysophyllum gorungosanum* , Engl	Sapotaceae	LVTF	LS	Y	Pantropical Africa mountains	Simple alternate, Obovate	Evergreen	I, II
Cgr	*Carapa grandiflora* , Sprague	Meliaceae	TMF	LS	Y, A	Albertine Rift and west Africa mountains	Pinnately Compound, Oblong	Evergreen	I, II, III, IV
Cme	*Croton megalocarpus* , Hutch.	Euphorbiaceae	LVTF	ES	Y	Pantropical Africa	Simple opposite, Ovate	Semi-deciduous	I, II
Cpo	*Cleistanthus polystachyus* , Hook.f. ex Planch.	Euphorbiaceae	TMF	LS	A		Simple alternate, Oblong-elliptic-ovate	Evergreen	IV
Dto	*Dombeya torrida* , (J.F.Gmel.) Bamps	Sterculiaceae/ Malvaceae	LVTF	ES	Y	East Africa mountains	Simple alternate, Elliptic lobate	Semi-deciduous	I, II
Eex	*Entandrophragma exselsum* , (Dawe & Sprague) Sprague	Meliaceae	LVTF	LS	Y	East Africa mountains	Pinnately Compound, Elliptic	Evergreen	I, II
Fla	*Ficalhoa laurifolia* , Hiern	Theaceae	TMF	LS	A	Sub-Sahara Africa	Simple alternate, Oblong-lanceolate	Evergreen	IV
Fsa	*Faurea saligna* , Harv	Proteaceae	TMF	LS	Y, A	Eastern and Southern Africa	Simple alternate, Oblong acute	Evergreen	I, II, IV
Fth	*Ficus thonninghii* , Blume	Moraceae	TMF	LS*	Y	Pantropical	Simple alternate, Elliptic	Semi-deciduous	I, II
Hma	*Harungana madagascariensis* , Lam. ex Poir	Hypericaceae	LVTF	ES	Y	Pantropical Africa	Simple opposite, Ovate	Semi-deciduous	I, II
Hmo	*Harungana montana* , Spirlet	Hypericaceae	TMF	ES	Y, A	Albertine Rift	Simple opposite, Ovate	Semi-deciduous	I, II, IV
Imi	*Ilex mitis* , Radlk.	Aquifoliaceae	TMF	ES	A		Simple alternate, Elliptic-oblong	Evergreen	IV
Mac	*Maytenus acuminata* , (L.f.) Loes.	Celastraceae	TMF	ES	A	Central to South Africa	Simple alternate, Ovate	Evergreen	IV
Mki	*Macaranga kilimandscharica* , Pax	Euphorbiaceae	TMF	ES	Y, A	East Africa mountains	Simple alternate, Ovate truncate	Semi-deciduous	I, II, III, IV
Mla	*Maesa lanceolate* , (Henriq.) F. White	Myrsinaceae/ Primulaceae	TMF	ES	Y	Sub-Sahara Africa and Madagascar	Simple alternate, Ovate	Semi-deciduous	I, II
Mlu	*Markhamia lutea* , (Benth.) K.Schum	Bignoniaceae	LVTF	ES*	Y	Paleotropical	Pinnately Compound,	Evergreen	I, II
Oke	*Ocotea kenyensis* , (Chiov.) Robyns & R.Wilczek	Lauraceae	TMF	LS	A		Simple alternate, Elliptic	Evergreen	IV
Oro	*Olinia rochetiana* , A.Juss.	Oliniaceae	TMF	LS	A	Sub-Sahara Africa	Simple opposite or ternate, Elliptic	Evergreen	IV
Osa	*Ocotea usambarensis* , Engl.	Lauraceae	TMF	LS	A		Simple opposite-alternate, Elliptic	Evergreen	IV
Paf	*Prunus Africana* , (Hook.f.) Kalkman	Rosaceae	TMF	LS	Y, A	Sub-Sahara Africa and Madagascar	Simple alternate, Oblong	Evergreen	I, II, IV
Pfu	*Polyscias fulva* , (Hiern) Harms	Araliaceae	TMF	ES	Y, A	Pantropical Africa	Pinnately Compound, Oblong	Semi-deciduous	I, II, III, IV
Pma	*Psychotria mahonii* , C.H.Wright	Rubiaceae	TMF	ES	A		Simple opposite, Obovate	Evergreen	IV
Rme	*Rapanea melanophloeos* , Mez	Myrsinaceae	TMF	ES	A	Tropical Africa	Simple alternate, Elliptic-oblong-ovate	Evergreen	IV
Sgu	*Syzygium guineense* , (Willd.) DC.	Myrtaceae	TMF	LS	Y, A	Sub-Sahara Africa and Madagascar	Simple opposite, Elliptic	Evergreen	I, II, III, IV
Ssc	*Strombosia scheffleri* , Engl.	Olacaceae	TMF	LS	A	Central Africa	Simple alternate, Oblong	Evergreen	IV

[1]Taxonomy information from The plant list at http://www.theplantlist.org and the World Flora Online WFO (2022) at http://www.worldfloraonline.org/. For family names, both classic and Angiosperm Phylogeny Group (APG III) system are given when applicable; [2]Forest type follows the Potential Natural Vegetation’s by Kindt et al. (Kindt et al., 2014); [3]www.gbif.org and WFO (2022) at http://www.worldfloraonline.org/ ; [4]Leaf type and shape classification follow Ellis et al.(Ellis et al., 2009), [5] Semideciduous species drop variable amounts of leaf depending the severity of drought, but are rarely completely defoliated. Decidousness information is from personal observation at experimental sites, or from various sources such as Fisher & Killmann (2008), website for useful tropical plants, world flora online and mozambique flora webpages. The classification of species into successional groups delivered from various studies conducted in Rwanda, Ethiopia, Tanzania and Uganda as indicated in supplementary table S4 of (Ntirugulirwa et al., 2023) and associated list of references.

5.2.2. Mature tree elevation gradient study

This study was conducted at 5 sites along an elevation gradient with Arboretum (A) planted forest site at Ruhande being at the lowest elevation (~1700 m a.s.l), followed by the Cyamudongo (C) forest patch adjacent to Nyungwe forest at 1800–1900 m a.s.l and three sites in Nyungwe forests, including Nyungwe West (N1) (~1950 m), Nyungwe East (N2; ~2500 m), and Bigugu Mountain in Nyungwe forest (N3; ~2700 m). The three highest sites are in Nyungwe National Park TMF while the Cyamudongo site is a natural forest a few km west of Nyungwe and Ruhande Arboretum, close to Huye town, in the LVTF vegetation zone (Table 2, Figure 3).

5.2.3. Nyungwe permanent plots study

Nyungwe permanent plots are in the Nyugwe TMF between 1600 and 2950 m a.s.l at 2º17´ - 2°50´S, 29°07´ - 29°26´E. In 2011/2012, 15 plots with a planimetric area of 100 m x 50 m (0.5 ha) along an east-west transect of 32 km at elevations of 1950 to 2550 m a.s.l., from Nyungwe east to west and the central in the middle (Table 2). Along this transect, species range from a dominance of ES to a dominance of LS forest stands, both having closed canopies interspersed with small open areas (Nyirambangutse et al., 2017).

5.3. Environmental conditions

At all sites, ambient air, relative humidity, precipitation, and solar radiation were recorded at a frequency of 30 minutes for at least two consecutive years except for two of the sites included in the mature tree elevation gradient. At these two sites only, temperature was measured for a 6-week period and scaled to an annual mean using neighbouring stations as a benchmark (Table 2). A reasonable accuracy in the scaling was possible because of the low seasonal variation in temperature (1-2°C difference between the coldest and warmest month). In the young tree study, the mean annual temperatures (MAT) ranged from 15.2 °C at highest elevation (Sigira) to 20.6 °C at lowest elevation (Makera), measured at 1.8 m above ground. In the mature tree study, the MAT ranged from 13.3 °C at highest elevation (Bigugu mountain) to 19.5 °C at the lowest elevation (Arboretum of Ruhande). The MAT of the sites for the Nyungwe permanent plots study with only about 550 m elevation difference, ranged from 14.3 to 16.2 °C. The extreme temperatures (expressed as 99% percentile) of all the sites in the three studies ranged from 3.8 to 10.6 °C above MAT, with the largest differences normally at lower elevation sites (Table 2).

The sites included in the three studies differed substantially in mean annual precipitation (MAP) ranging from 1037 mm to 3016 mm, mainly constrained by east-westerly position and elevation, with higher precipitation in the west and at higher elevations (Table 2). The interannual variation in MAP is also large and therefore differ between study periods. To compare different sites, MAP is given for 4 different time periods including data for a period above 10 years average for two sites. However, the relative seasonal distribution of precipitation was similar at all sites and years, with highest rainfall in March–May and a dry period in June–August. Day time mean solar radiation expressed as photosynthetic photon flux density (PPFD) ranged from 572 to 764 µmol m^{-2} s^{-1}, with lowest radiation in the west and at high elevation probably due to more frequency of clouds (Table 2).

Sites located in South-West region (Cyamudongo, Nyungwe and Sigira) are dominated by ultisols with patches of inceptisols (Nzeyimana et al., 2014) developed on quartzite schist, mica schist, schist, and granite as parent material (Verdoot and van Ranst 2003). Sites located in Central-South region (Rubona and Ruhande Arboretum) are dominated by oxisols (Nzeyimana et al., 2014) also known as ferralsols (Nsabimana et al., 2009; Rwibasira et al., 2021) developed from Precambrian phyllitic rocks seated in the roof of a granite batholith leading to red-brown ferrallitic soil (Moeyersons, 2003) while the site in South-East region (Ibanda Makera) is dominated by entisols with patches of oxisols, inceptisols and vertisols (Nzeyimana et al., 2014). The mineral topsoil consists of clay, sand and silt ranging between 2-71%, 9-61% and 5-61% with averages of 34, 43 and 23%, respectively (Gharahi Ghehi et al., 2014; Moeyersons, 2003).

5.4. Leaf sampling

Leaves or leaflets were collected from the young trees in Rwanda TREE study in two campaigns (August to December 2018 and June to August 2019), from the mature trees in the elevation gradient study in three campaigns (February-March 2017; September 2017; January-February 2018) and from mature trees in the Nyungwe permanent plots study in several campaigns in 2013 and 2015 (August to December 2013; February-April 2015). For species with compound leaves (Table 3), only leaflets were sampled but for simplicity they are denoted as leaves. Trees were selected through different random procedures in all studies. In the young tree study, most sampled trees had a basal diameter of less than 10 cm while sampled trees in two studies with mature trees had a breast height diameter (DBH) range of 7 to 93 cm with an average for all species between 22 to 53 cm.

In all studies, one to five mature and sun exposed leaves, mainly from the upper half of the tree crown, were sampled from each selected tree. In the two studies with mature trees, the leaves were collected up to a height of 20 m by cutting branches using a telescopic pole. In the young tree study, slightly more than 3100 leaves were collected from around 2000 individual trees (in total) in two campaigns (~2/3 in 2018 and 1/3 in 2019). In the mature tree elevation gradient study 1026 leaves (in total) were collected from six trees per species and site in three campaigns. In the permanent plot study, a total of 445 leaves were collected from 20 species and 3 trees per species per plot at 11 plots (five leaves from a total of 89 trees).

5.5. Leaf morphology measurements

In all studies, leaf length (LL), leaf width (LW) and leaf thickness (LT) were measured directly after leaf collection (LT only in 2019 campaign for the young tree study). Approximately three to five leaf discs of known diameter per leaf were sampled using punchers. First and second order veins were avoided for LT measurements and first order veins for sampling of discs. For species having narrow leaves in the young tree study (*A. falcatus* and *F. saligna*) photos were used instead of discs for leaf area determination. Both discs or photographed leaves and the remaining leaf material were brought in separate envelopes to the laboratory for later determination of LMA and nutrient contents, respectively.

5.5.1 Leaf shape and size estimation

Leaf shape was determined as LW to LL ratio (LW/LL or Leaf W/L ratio) and leaf size was expressed as leaf area (LA) and estimated from LW and LL using an allometric function:

$$\mathrm{LA} = \mathrm{LW} \cdot \mathrm{LL} \cdot \mathrm{K} \qquad (1)$$

where K is the leaf area factor, also known as the Montgomery parameter (Shi et al., 2019). Species-specific K-values were determined for the 18 species included in the young tree study based on 20 leaves of varied sizes collected from each species at the Rubona site in June 2020. Collected leaves were scanned in a flatbed scanner (CanonScan LiDE300, Tokyo, Japan) and thereafter their LA, LL, LW were analysed by using ImageJ software 1.50i (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA). Petioles and petiolules were excluded from the measurements.

The K-values were obtained by regression analysis of LA versus LW · LL, setting the intercept to zero. Independent of fixed or variable intercept, the R^2 values obtained were 0.97 – 0.99 except for the two species with the largest and lowest LW to LL ratio, where the R^2 values were 0.93 (*D. torrida*) and 0.89 (*A. falcatus*), respectively. The K-values among species varied between 0.66 and 0.85 (Table 2) and were used to estimate the LA of all sampled leaves in the three studies (Equation 1).

5.5.2. LMA, LD and LVA calculation

Each collected leaf disc or photographed leaf sample were oven dried at 70 °C for at least 48 hours and then weighed by using a laboratory balance with 0.1 mg resolution. The area, width and length of the photographed leaves were determined by using ImageJ software 1.50i. Total projected area of discs or photographed leaves were used to calculate LMA (Laurans et al., 2012). In the juvenile tree study, leaf area (LA) and leaf thickness (LT) were used to calculate leaf volume per area (LVA) while leaf mass (LM), LA and LT were used to calculate leaf density (LD) as follows:

$$\mathrm{LMA} = \frac{\mathrm{LM}}{\mathrm{LA}} \qquad (2)$$

$$\mathrm{LVA} = \frac{\mathrm{LA} \cdot \mathrm{LT}}{\mathrm{LA}} \qquad (3)$$

$$\mathrm{LD} = \frac{\mathrm{LM}}{\mathrm{LA} \cdot \mathrm{LT}} \qquad (4)$$

The following units were used to express Leaf size (LA), cm^{-2}; LMA, g m^{-2}; LVA, cm^{-3} m^{-2}; LD, g cm^{-3} in all papers.

5.6. Leaf nutrients analysis

In all studies, leaves sampled for nutrient analysis were oven dried at 70 ºC for at least 48 hours and thereafter ground into a fine powder with a ball mill grinder (MM 301, MM 200, Retsch, Germany). Mass-based leaf nitrogen (N_M) content was analysed by using an elemental analyser (EA 1108, Fison Instruments, Rodano, Italy). Ground samples were sent for analysis of 37 non-N elements using inductively coupled plasma mass spectrometry after digestion in HNO_3 and then aqua regia (Method VG101, Bureau Veritas Mineral laboratories, Vancouver, BC, Canada). The mass-based leaf nutrients were converted to area-based contents by multiplying with LMA.

5.7. Leaf nutrient resorption

To estimate the shrinkage of dry compared to fresh green leaves, 15 discs from fresh green leaves of known size were dried and measured for area using a scanner (Epson 1600+ scanner equipped with a transparency unit for dual scanning) and image analysing software (WinSEEDLE Pro 5.1a, Regent Instruments Inc., Canada). Leaf nutrient resorption (NuRE) was calculated from combined samples of collected leaves before abscission and litterfall during four consecutive three-month periods from January 2013 to December 2014 using ninety litter traps evenly distributed over all plots and oven-dried at 70ºC. A hole punch was used to obtain discs of a fixed area from each sample. The discs were weighted to calculate the litter leaf mass per unit area (LiMA).

Nutrient resorption efficiency was defined as the proportion of mature leaf nutrients that was withdrawn prior to abscission (Killingbeck, 1996; Li et al., 2016; Reed et al., 2012). During senescence the leaf area shrinks, and the leaf mass declines. These effects need to be accounted for when calculating resorption efficiency based on the nutrient content of green leaves and leaf litter (van Heerwaarden et al., 2003). Therefore, species-specific correction factors were established based on leaf shrinkage and leaf mass loss which were used when calculating the mineral resorption efficiency (NuRE) of the leaves.

The fraction of shrinkage in area of senesced ($A_{Shrinkage}$) leaves was estimated by:

$$A_{Shrinkage} = 1 - \left(\frac{L_{A,dry}}{L_{A,fresh}}\right) \qquad (5)$$

where $L_{A,fresh}$ and $L_{A,dry}$ are the areas of fresh green and dry leaf discs, respectively, assuming that the shrinkage during senescence mainly is due to drying. The fraction of mass loss (M_{Loss}) during senescence was estimated by:

$$M_{Loss} = \left(\frac{LiMA \times (1 - A_{Shrinkage})}{LMA}\right) \qquad (6)$$

where *LMA* and *LiMA* represent the leaf mass per unit area and the litter dry mass per unit area, respectively.

The NuRE in percent was then calculated as:

$$\text{NuRE} = \left(1 - \frac{[\text{Nu}]_{LT} \times (1 - M_{Loss})}{[\text{Nu}]_{L}}\right) \times 100 \qquad (7)$$

where $[\text{Nu}]_L$ and $[\text{Nu}]_{LT}$ are the nutrient concentration in the green leaves and litter, respectively.

5.8. Leaf nutrient limitation

Potential limiting leaf nutrient contents was defined as the ratio to N content below the optimal ratio (Sumner, 1978). The optimal ratios for each nutrient expressed in percentage (Table 4) were derived from (Ericsson & Kähr, 1993, 1995; Göransson, 1998; Göransson, 1993, 1994, 1999; Knecht & Göransson, 2004; Linder, 1995) and were compared to leaf nutrient ratios found in the different studies and calculated as follows:

$$\text{Nu}_{\text{ratio}}\ (\%) = \frac{[\text{Nu}]}{[\text{N}]} \cdot 100 \qquad (8)$$

where [Nu] stands for non-nitrogen leaf nutrient concentration and [N] for leaf nitrogen concentration.

Table 4: Documented nutrients optimal ratios (Table S2 in Paper II) based on the assumption that Terrestrial plants require nutrients in similar proportions (Knecht & Göransson, 2004). For nutrients with different literature values, both a lower and upper optimal ratio is given.

Optimal ratios	P/N	K/N	Ca/N	Mg/N	S/N	Fe/N	Mn/N	Zn/N	B/N	Cu/N	Mo/N
Lower	8	30	2	3.3	4	0.13	0.03	0.04	0.05	0.01	no data
Upper	10	35	2.5	3.5	4	0.2	0.05	0.05	0.05	0.03	no data

5.9. Statistical analyses

5.9.1. Leaf morphology and nutrients data analysis in Paper I and II

Mixed effect two-way between-groups analysis of variance (ANOVA) was used to test the site, species and year effect on leaf morphological traits (with tree height as a covariate) (Table 4 in Paper I). Standard two-way ANOVA was used to analyse the site and species effect for different macro- and micro-nutrients using soil nutrient content as a covariate (Table 7). A one-way ANOVA was used to analyse the site effect on different species individually when the interaction between site x species were significant (Tables 6, 8, 9); to test the site effect on soil parameters (Table 3 in Paper II) and to test for the differences between successional groups (Figure 4). To separate from possible confounding influences of non-temperature site differences, elevation gradient site's effects potentially caused by warming was defined as a significant change in the same direction (↗ increasing or ↘ declining effect) at both mid (ME) and low elevation (LE) compared to high elevation sites, or a significant change at low elevation compared to high elevation (HE) sites but no difference between ME and HE. For species with a decline or increase at ME but not at LE (→ no systematic temperature effect) compared to HE, the change was not attributed to warming (Tables 6 to 9).

Bivariate regression analysis was used to test the relationship between leaf morphological and chemical traits and multiple linear regression was used to test the relationship between LMA and its components (Figure 5). The relationship between amounts of different leaf nutrients (Table 5) and between nutrient contents in leaf and soil concentrations (Table 9, Paper II) were tested by using Pearson correlation. The effects of successional group (Table 4) and the effect of different LMA responses on leaf nutrient contents (Figure 8) was analysed by a t-test. The effect of tree age (juvenile trees in Rwanda TREE study compared to mature trees from other studies) on LA and LMA was tested for a common elevation of 2000 m a.s.l using a GLM with elevation as covariate and tree age as a fixed factor (Figure 11). Test of normality (Shapiro-Wilk) was performed to test the distribution of data, and Levene's tested for homogeneity of variance. Homogeneity of variance were obtained for analysis of individual species, while not always when all species were analysed together. Effects were considered statistically significant at $P < 0.05$ if homogeneity of variance was obtained and at $P < 0.01$ for main effects when homogeneity was not obtained. All statistical tests were made by using the SPSS 27 software package.

5.9.2. Leaf morphology and nutrients data analysis in Paper III

Differences among sites and among species for the different leaf morphological and chemical traits were tested using two-way ANOVA with site and species as fixed factors. Pairwise multi-comparisons among individual sites and species were conducted with the Tukey HSD test. Averages of data from different leaves on the same tree were used in these tests to treat trees as the statistical unit (Figure 8). All data were analysed in SPSS 16 software and differences were considered statistically significant at $p < 0.05$.

5.9.3. Leaf nutrients data analysis in Paper IV

Differences in leaf nutrient concentrations and resorption efficiency among species were analysed by one-way ANOVA and differences between ES and LS species or stands were analysed using two-tailed independent-samples t-test using SPSS 22 software. Data that violated the assumption of normality or those contained outliers were log-transformed before the statistical analysis. The significance of the relationship between concentration of nutrients and resorption of nutrients were determined using the regression analysis tool in SigmaPlot 12.5 software (Table 10).

6. Results

6.1. Morphological and chemical leaf traits of young trees across species

Large variation of leaf morphological (Leaf size, leaf W/L ratio, LMA, LVA, LD) and chemical (mass- and area-based N (N_M & N_A) and P (P_M & P_A) respectively) traits among young trees was observed, both within and between successional species groups (see Figure 4 a-f for variations at the HE site, Sigira, Paper I). Only LMA of ES species differed significantly between years, but only to a small degree. (Figure 4d). ES species showed more acquisitive traits (e.g., high leaf N and P concentration, leaf N:P ratio, large average LA and low LMA) compared to LS species (e.g., low nitrogen, high leaf density).

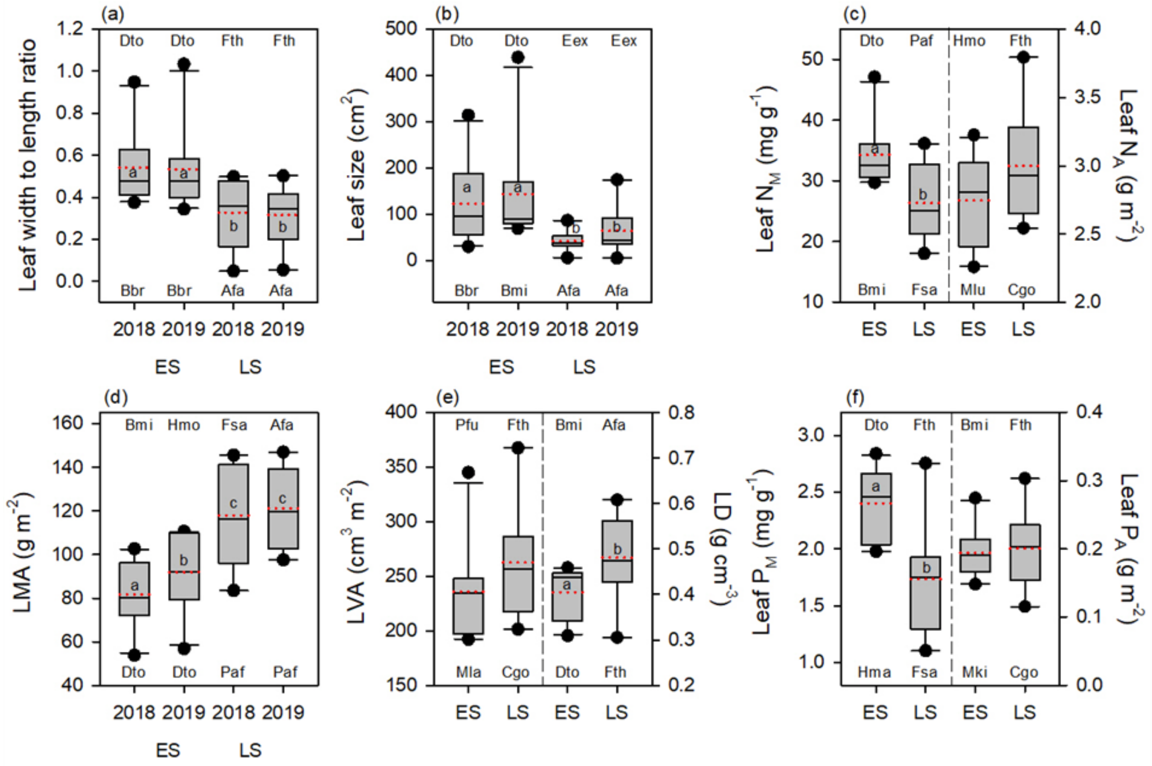


Figure 4 a-f. Leaf characteristics of 18 species at Sigira site (high elevation = control). (a) Leaf shape (i.e., leaf width to leaf length ratio: W/L), (b) leaf size, (c) leaf nitrogen (N) per unit mass (N_M) and area (N_A), (d) leaf mass per unit area (LMA), (e) leaf volume per area (LVA), leaf density (LD), and (f) leaf phosphorous (P) per unit mass (P_M) and area (P_A). Leaf shape, size and LMA were measured in both 2018 and 2019. Leaf N and P were measured in 2018 and LVA and LD in 2019 only. The boxplot boundary indicates the 25th and 75th percentile, the error bars indicate the 95th and 5thpercentiles, the black solid line and the red dotted lines inside the boxes indicate median and mean respectively. The black dots show the highest and lowest species means for which species codes (see Table 1) are indicated in the lower and upper part of the figure. Different letter (a - c) inside boxes indicate significant differences (P < 0.05) of the mean between early (ES) and late (LS) successional species and years when relevant (Paper I).

The leaf nutrient concentrations varied greatly across species, as shown for the HE (Sigira) site in Figure 5A. Specifically, the non-N and P nutrients varied more between species with greater variation in micronutrients than in macronutrients (Table S4 in Paper II). The successional strategy contributed to the variation as ES species had significantly higher mass-based concentrations of N, P, Mn, Zn and Cu compared to LS species, while no significant differences between successional groups were observed for other nutrients (Figure 5A). However, the variation was large also within a successional group especially for some nutrients (e.g., Mn, B). LS species (most commonly *F. saligna*, *P. africana* and *E. excelsum*) displayed the lowest concentrations of leaf macro and micronutrients, while the species with the highest concentrations were irrespective of successional strategy for both leaf macro and micronutrients.

The inter-specific variation of the area based nutrient content and N based nutrient ratios tend to be similar as for the mass-based contents (Figure 5 A-B). Only N_A had substantially lower inter-specific variation compared to N_M (14% compared to 22%, c.f. Table S5 & S4 in Paper II). However, the significant differences between successional groups disappeared due to the higher LMA of LS compared to ES species (Figure 4 d, c.f. Table S5 and S6 in Paper II). The species variation of the leaf C content was low (only 9% between max and min, Table S1 in Paper II), but this still indicate some species differences in the proportions of different types of organic compounds (carbohydrates, lignin, and proteins). For leaf nutrients to N ratio especially large variations were observed for S, Mn, and Zn in ES species and for Mn in LS species. A significant different ratio between ES and LS was observed only for P and S (Figure 5B).

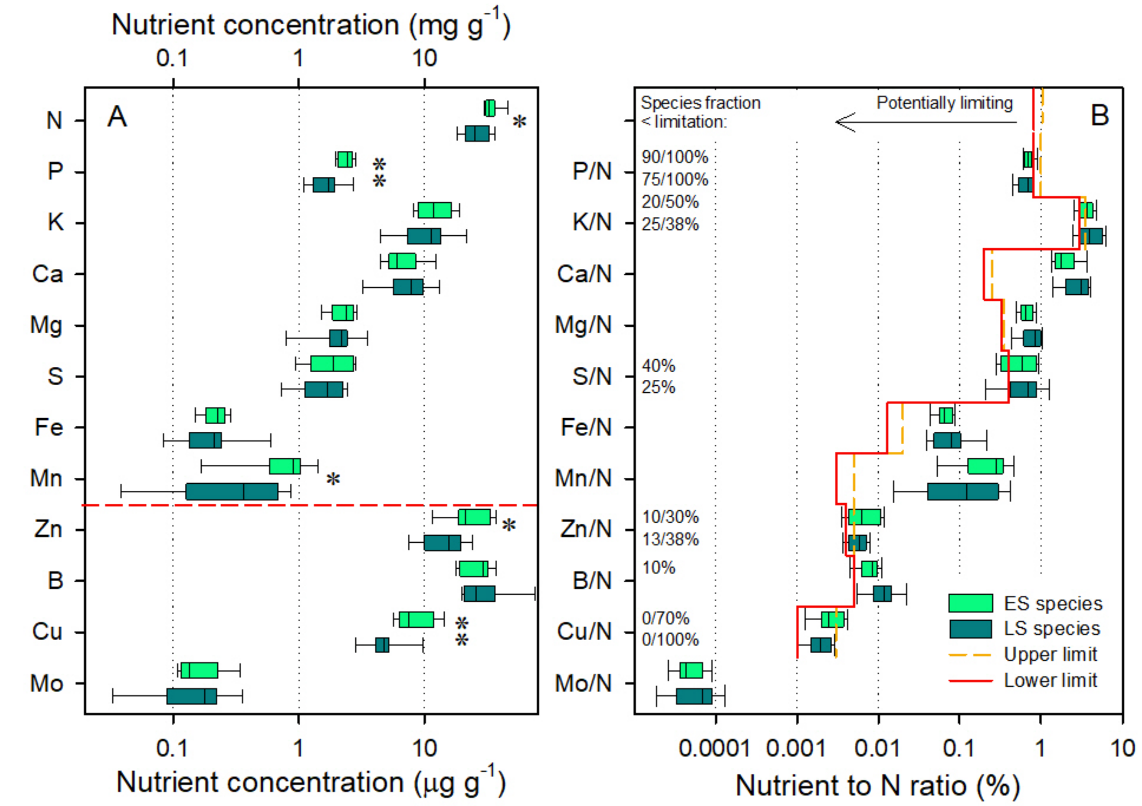


Figure 5. Leaf nutrient element concentrations (A) and ratios to N (B) of 18 species at the HE-site (Sigira), based on mean values of each species and their classification to early (ES; n = 10) or late (LS; n = 8) successional strategies (Table 1). Concentrations (A) above the red dashed line is in mg g^{-1} dry mass and below is in µg g^{-1} and ratios (B) is given as element to N ratio in percent. All x-axis scales are logarithmic. The boundary of the boxes indicates the 25th and 75th percentile, the error bars indicate the 90th and 10thpercentiles, the line within the box marks the median. The red solid (lower) and yellow (upper) dashed lines in B indicate literature values of the optimal nutrient ratios defined as those when all nutrients limit growth simultaneously. Values below these lines as potentially limiting. Note that no optimal values were found for Mo and Ni. The literature values are derived from: Ericsson and Kähr (1993, 1995), Göransson (1993), Göransson (1994), Göransson (1998), Göransson (1999), Knecht and Göransson (2004) and Linder (1995). Significant differences between successional groups are indicated by: * P <0.05; ** P<0.01.

When the nutrients ratio to N were compared to optimal ratios (Table 4), the limiting nutrient for most young species was P followed by S, K, Zn, Cu for some species in both ES and LS species (Figure 5B). B was only limiting for a few ES species. The ratio values of Ca, Mg, Fe and Mn were all well above the optimal values. Mo limiting potential could not be evaluated due to lack of information about optimal values for this element (Table 4).

6.2. Relationship between different morphological and chemical traits

6.2.1. Relationship between leaf morphology and nutrient content of young trees (Paper I)

Across species, within and between successional groups, and sites, LMA declined significantly with increasing leaf W/L ratio, leaf size, N_M, and P_M while LMA increased significantly with increasing N_A and P_A (Figure 6 a-f. The ES and LS species fall on the same line for most variables, but with a distinct co-separation of each trait by successional group, except in the cases of N_A and P_A. However, no significant correlation was observed between leaf size and N_M across LS species whereas leaf size increased significantly with increasing N_M across ES species (P<0.001; Figure S1 in Paper I).

6.2.2. Relationship between LMA and its components, LVA and LD (Paper I)

The degree to which LVA and LD contributed to the variation in LMA was investigated by plotting both the slopes and R^2 values of these relationships against each other (Figure 7). The contributions of LD and LVA to within-species LMA variation differed greatly among species; in some species LD contributed more than LVA, in other species LVA contributed more than LD, and in a third group of species LD and LVA equally contributed to the LMA variation (Figure 7). Species from both successional groups (ES and LS) and forest origin type (TMF and LVTF) were represented in the three LD & LVA vs LMA groups, indicating that the results are neither explained by successional strategy nor by the forest type of origin (Figure 7).

6.2.3. Relationship between different leaf macro- and micronutrients (Paper II)

Overall, both positive and negative relationships were observed within and between leaf macro- and micronutrients (Table 5). A strong positive correlation (Pearson correlation coefficient ≥ 0.5) was observed within the following three groups of elements: i) N, P, and K; ii) Ca, Mg and B; iii) Mn, Zn, and Cu. The elements of the second group also correlated relatively well to Fe and S (Pearson correlation 0.2-0.4). Most elements correlated positively with N, except for Mn, and B. The most substantial negative correlations were observed for Mn versus Ca, Mg, S, B, and Mo. Most elements had a weak negative correlation to C indicating a dilution effect by high C, probably caused by high lignification of cell walls.

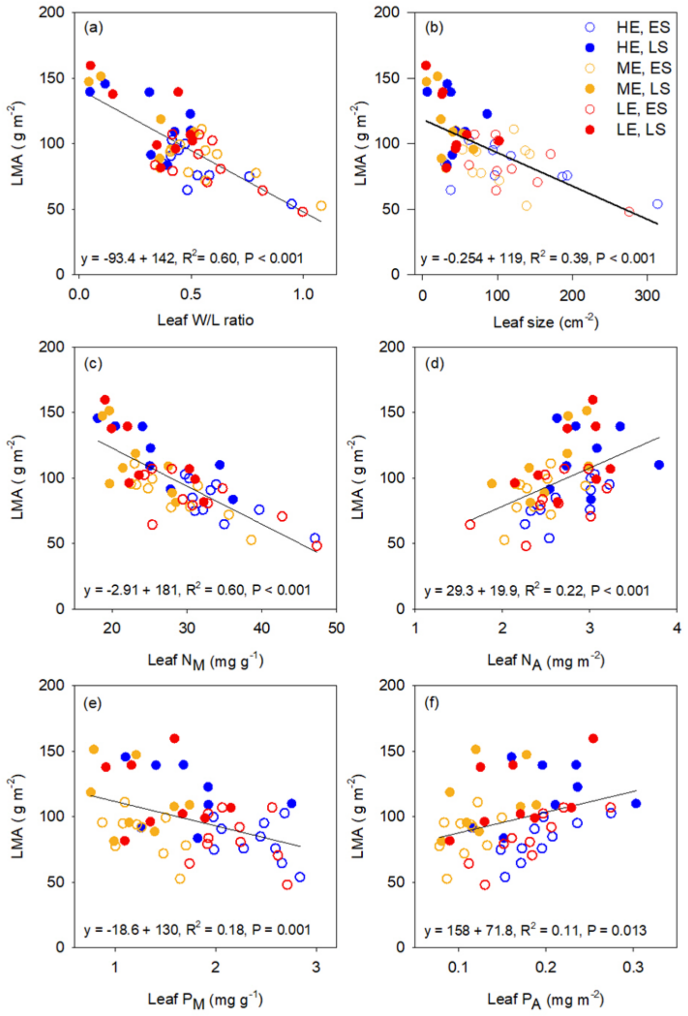


Figure 6 a-f. Leaf mass per unit area (LMA, y axis) in relation to (a) leaf width to length ratio (leaf W/L ratio); (b) leaf size; (c) leaf nitrogen per unit mass (Leaf N_M); (d) leaf nitrogen per unit area (Leaf N_A); (e) leaf phosphorus per unit mass (Leaf P_M); (f) leaf phosphorus per unit area (Leaf P_A). Each data point represent species mean at each site. HE, High elevation site (Sigira); ME, Medium elevation site (Rubona); LE, low elevation site (Makera); ES, early successional trees; LS, late successional trees. Lines, equation and R^2 represents the regression line for all sites and successional groups. P-value refers to the regression line (Paper I).

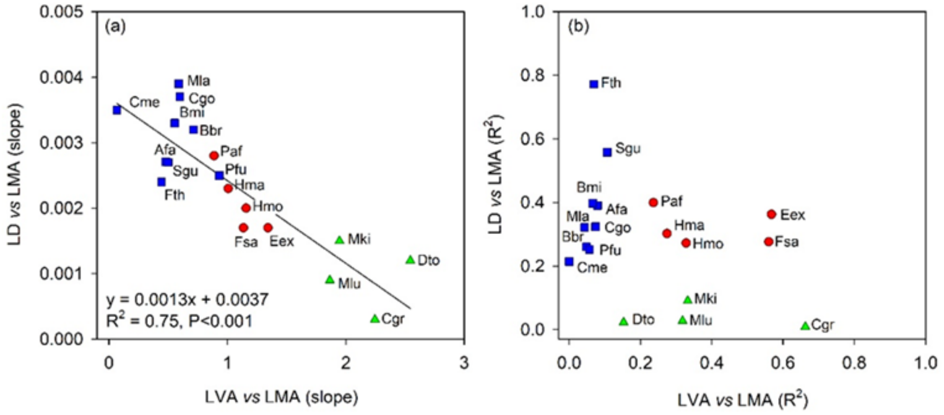
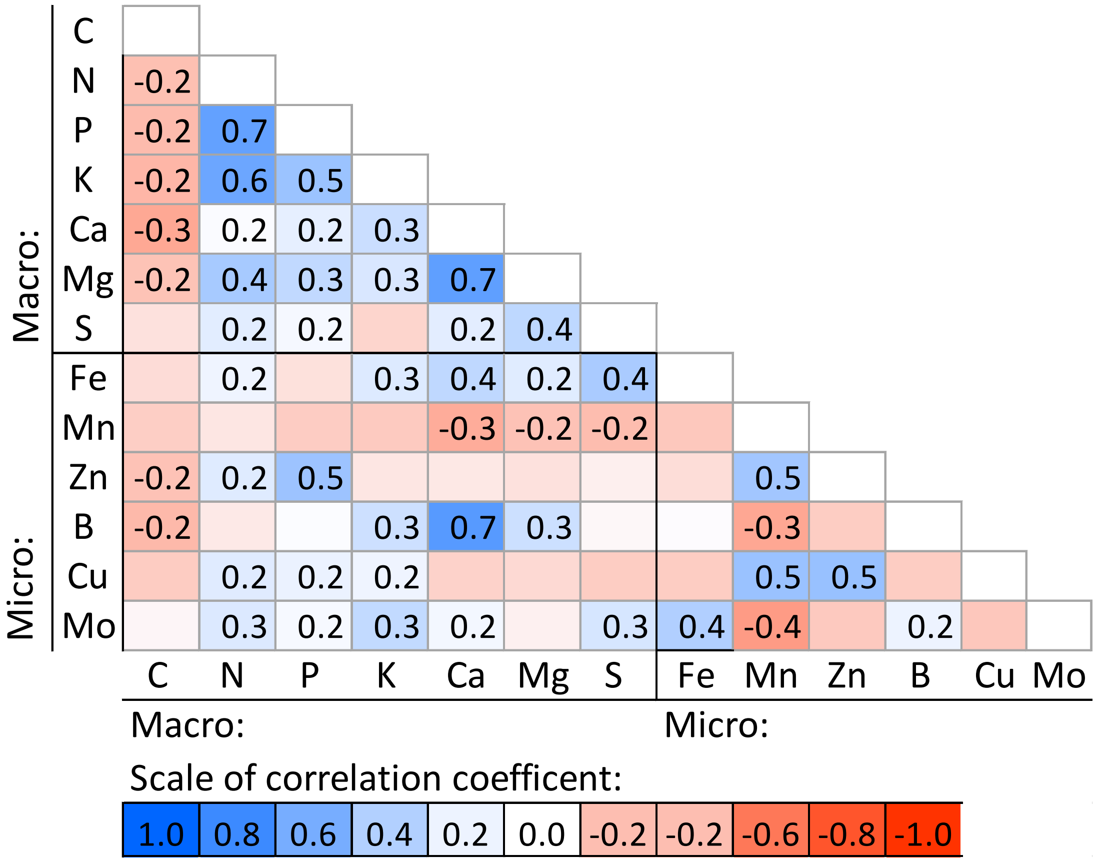


Figure 7 a-b. Slope (a) and R^2 (b) for leaf density (LD) versus leaf mass per unit area (LMA) in relation to slope and R^2 for leaf volume per unit area (LVA) versus LMA for 18 species (for species codes, see Table 1). Blue squares, LMA mainly influenced by LD; green triangles, LMA mainly influenced by LVA; red circles, LMA influenced by both LD and LVA. P-value refers to the regression line. Statistical results are in Figure 2, supplementary to Paper I.

Table 5. Pearson correlation coefficients between different nutrients across species and sites. Bluish to blue colours represent more positive correlations while reddish to red colours represent more negative correlations (Paper II).



		C	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
Macro:	C													
	N	-0.2												
	P	-0.2	0.7											
	K	-0.2	0.6	0.5										
	Ca	-0.3	0.2	0.2	0.3									
	Mg	-0.2	0.4	0.3	0.3	0.7								
	S		0.2	0.2		0.2	0.4							
Micro:	Fe		0.2		0.3	0.4	0.2	0.4						
	Mn					-0.3	-0.2	-0.2						
	Zn	-0.2	0.2	0.5						0.5				
	B	-0.2			0.3	0.7	0.3			-0.3				
	Cu		0.2	0.2	0.2					0.5	0.5			
	Mo		0.3	0.2	0.3	0.2		0.3	0.4	-0.4		0.2		
		Macro:							Micro:					

Scale of correlation coefficent:

1.0	0.8	0.6	0.4	0.2	0.0	-0.2	-0.2	-0.6	-0.8	-1.0

6.3. The effects of site and warming on leaf traits

6.3.1. Elevation gradient site effect on leaf morphology of young trees (Paper I)

Significant site*species interaction was observed on leaf W/L ratio, leaf size, leaf area and mass-based nitrogen and phosphorus, LMA, and LD but not on LVA indicating variable responses among species along the elevation gradient. These findings allowed analysis of site and warming effect on individual species (Table 6). The results show a decline in leaf size and LMA and an increase of leaf W/L ratio as result of warming effect only for approximately one third of the species while almost half of species were also affected by the no-temperature site effect. A significant site effect on leaf shape was observed, with leaf W/L ratio being on average 5.4% and 7.5% higher at the warmer ME and LE sites, respectively, compared to the HE (Table 4 in Paper I), but when analysed for individual species only 5 species were significantly affected including one species with negative warming response (Table 6).

The leaf size declined with warming for 7 species, mostly among ES species, while LMA declined independently of successional strategy with warming for 6 to 9 species, depending on year (Table 6). LD declined with warming for 6 species (3 ES and 3 LS) and 4 out of them were characterized by a greater contribution of LD to their LMA variation (Figure 7, Table 6).

Table 6. Significance of warming effect on leaf shape (width to length ratio, W/L) leaf size (leaf area) and leaf mass pear unit area (LMA) in 2018 and 2019 and on leaf volume per unit area (LVA), leaf density (LD) in 2019. Different letters (a, b, c) denote significant difference between sites. H, M and L represent high (Sigira), mid (Rubona) and low (Makera) elevation sites, respectively. Explanation of species codes is given in Table 1; SG, successional group (ES, early; LS, late); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest); CV, covariate used in statistical analysis when significant; TH, tree height. The arrows indicate the direction of warming effect of species with significant site effect (P < 0.05) (Paper I).

			Leaf W/L ratio 2018			Leaf W/L ratio 2019			Leaf size 2018			Leaf size 2019			LMA 2018			LMA 2019			LD 2019		
Spec	SG	FT	CV	P	H M L	CV	P	H M L	CV	P	H M L	CV	P	H M L ↘	CV	P	H M L	CV	P	H M L	CV	P	H M L
Bbr	ES	TMF	TH	**0.013**	ab a b →		0.101		TH	**<0.001**	a b a →		**0.028**	a a b ↘		**0.003**	a b a ↗→		0.468	↘		0.845	
Hmo	ES	TMF	TH	**<0.001**	a b ab ↗→		0.369			**0.001**	a b a ↘→	TH	**0.016**	ab a b ↘		**<0.001**	a b b ↘		**0.006**	a a b ↘		**0.528**	
Mki	ES	TMF		**0.013**	a ab b		0.124		TH	**<0.001**	a b b ↘		**<0.001**	a b b		**<0.001**	a a b		**<0.001**	a a b		**0.162**	↘
Mla	ES	TMF		0.077			**0.047**	a b ab ↗→		**<0.001**	a b b ↘		0.127	↘	TH	**<0.001**	a b ab ↘→		0.067	↘		<0.001	a b b
Pfu	ES	TMF		0.785			**0.003**	a b b ↗	TH	**<0.001**	a b b		**0.001**	a b b ↘		**<0.001**	a a b		**<0.001**	a a b	TH	**0.065**	
Bmi	ES	LVTF		0.143			**0.023**	a ab b	TH	0.107	↘		**0.009**	a a b	TH	0.137			0.606	↘		0.060	↘
Cme	ES	LVTF		0.522			0.366		TH	**0.036**	a ab b ↘	TH	0.563	↘		0.075	↘		**0.001**	a a b	TH	**0.003**	a a b ↘
Dto	ES	LVTF	TH	**<0.001**	a b ab →		0.164		TH	**<0.001**	a b b		**<0.001**	a b b		**0.011**	a a b	TH	0.230			0.008	a a b
Hma	ES	LVTF	TH	**0.001**	a b ab →	TH	0.920			**<0.001**	a b a →	TH	**0.023**	ab a b ↘→	TH	0.106		TH	0.096			0.797	
Mlu	ES	LVTF	TH	0.053			0.109		TH	0.896	↘	TH	**0.001**	a a b	TH	**0.002**	a b ab →		**0.009**	a b a →		**0.421**	
Afa	LS	TMF	TH	0.361			0.321			**<0.001**	a b b		0.056		TH	0.119	↘		**0.001**	a b a ↘→		**0.228**	
Cgr	LS	TMF	TH	0.820	↗		0.218		TH	**0.007**	ab a b →	TH	0.199			**0.006**	a b b	TH	**<0.001**	a a b ↘		**0.782**	
Fsa	LS	TMF		**<0.001**	a a b ↘		**0.002**	a b ab →	TH	**0.024**	a b ab →	TH	0.747			0.254		TH	**0.012**	a ab b		**0.485**	
Paf	LS	TMF		**0.023**	a ab b ↗		0.572	↗	TH	0.203	↘	TH	0.882		TH	0.056			0.480			0.195	
Sgu	LS	TMF	TH	<0.001	a b c ↗	TH	**<0.001**	a b c ↗	TH	**<0.001**	a b b		**0.039**	ab a b →	TH	**0.008**	a b ab ↘→	TH	**0.012**	a b ab ↘→	TH	**0.813**	↘
Cgo	LS	LVTF		**0.024**	a b b		**0.017**	a ab b	TH	0.434		TH	0.451		TH	**0.038**	* ↘	TH	**<0.001**	a a b ↘		**<0.001**	a a b ↘
Eex	LS	LVTF		0.788			0.313			**<0.001**	a b a →	TH	0.948		TH	**<0.001**	a b b		**<0.001**	a b c ↘		**<0.001**	a a b ↘
Fth	LS	LVTF		0.547			0.063		TH	0.093		TH	**<0.001**	a b ab →		0.735		TH	**0.023**	ab a b	TH	**0.027**	ab a b

↘ Decrease with warming (down at both M and L or no/small effect at M and down at L compared to H elevation sites).

↗ Increase with warming (up at both M and L or no/small effect at M and up at L compared to H elevation sites).

→ No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites).

* a statistically significant site effect was obtained in the ANOVA analysis, but not in the Bonferoni post-hoc test.

6.3.2. Elevation gradient site effect on leaf nutrients of young trees (Paper II)

Soil nutrients were included as covariate when significant in the statistical analysis of site and species effect on leaf nutrient contents (Table 7). The covariates reduced but did not eliminate the soil effects as a significant site, species and site*species effects were observed for all leaf nutrient contents expressed on mass, area and N based ratios, except mass and area-based C, and K, that were not affected by site (Table 7). Regardless of the soil nutrient effects on leaf nutrient contents, leaf mass-based content of Ca, Mg, Fe, B and Mo increased while N, P, Zn, and Cu declined with decreasing elevation and potentially with warming (Table 7, Figure 8). Similar trends were observed for both area based and N-based ratios for these nutrients, except P and Cu. The elevation trend of leaf N and P contents where a bit ambiguous as the ME sites had significantly lower contents compared to both HE and LE sites, but the LE site also had lower contents compared to the HE site (Figure 8). The site effect on leaf N and P content seem to be dominated by lower N and P availability at the ME site compared to the other two sites, possibly caused by the significantly lower soil NO3- and total P contents at the ME compared to the other sites (Paper II; Table 3). The change for leaf S was significantly negative at ME site and positive at LE site relative to HE site while opposite for Mn. A warming effect on these elements (i.e., S and Mn) is therefore not likely (Figure 8).

Table 7. Results from two-way ANOVA of the species and site effects on mass and area-based leaf nutrients content and leaf element ratios across all species (n=18) and sites. The analysis includes the covariate of soil nutrient (nut.) of each element and soil available nutrient (Av. Nut for N and P) but only when it is significant (P < 0.05). The main and interaction (inter.) effect is classified as significant at P<0.01 as the data did always pass the homogeneity test. The results from Bonferroni post hoc test are given in Figure 8. The arrows indicate the direction of warming effect of species with significant site effect (P < 0.05) indicated in the table footnote (Paper II).

	Mass based leaf content							Area based leaf content								Leaf element ratio						
		Main effect		Inter.	Covar				Main effect		Inter.	Covar					Main effect		Inter.	Covar		
Element	cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.		cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.		Element	cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.	
C		0.4	**<0.001**	0.03					0.56	**<0.001**	**<0.001**											
N	x	**<0.001**	**<0.001**	**<0.001**	**0.04**	**0.01**	↘		**<0.001**	**<0.001**	**<0.001**	1	0.15	↘	N/C	x	**<0.001**	**<0.001**	**<0.001**	**0.05**	**0.02**	↘
P		**<0.001**	**<0.001**	**<0.001**	0.53	0.060	↘	x	**<0.001**	**<0.001**	**<0.001**	0.11	**0.01**	→	P/N		**<0.001**	**<0.001**	**<0.001**	0.22	0.1	↘
K	x	0.06	**<0.001**	**<0.001**	**0**			x	0.09	**<0.001**	**<0.001**	**0.03**			K/N	x	**<0.001**	**<0.001**	**<0.001**	**0.05**		→
Ca		**<0.001**	**<0.001**	**<0.001**	0.94		↗		**<0.001**	**<0.001**	**<0.001**	0.87		↗	Ca/N		**<0.001**	**<0.001**	**<0.001**	0.51		↗
Mg	x	**<0.001**	**<0.001**	**<0.001**	**0.03**		↗	x	**<0.001**	**<0.001**	**<0.001**	**0.02**		↗	Mg/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↗
S		**<0.001**	**<0.001**	**<0.001**			→		**<0.001**	**<0.001**	**<0.001**			→	S/N		**<0.001**	**<0.001**	**<0.001**			↗
Fe		**<0.001**	**<0.001**	**<0.001**	0.17		↗		**<0.001**	**<0.001**	**<0.001**	0.19		↗	Fe/N		**<0.001**	**<0.001**	**<0.001**	0.10		↗
Mn		**<0.001**	**<0.001**	**<0.001**	0.08		→	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↘	Mn/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		→
Zn	x	**<0.001**	**<0.001**	**<0.001**	**0.03**		↘	x	**<0.001**	**<0.001**	**<0.001**	**0**		↘	Zn/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↘
B		**<0.001**	**<0.001**	**<0.001**			↗		**<0.001**	**<0.001**	**<0.001**			↗	B/N		**<0.001**	**<0.001**	**<0.001**			↗
Cu	x	**<0.001**	**<0.001**	**<0.001**	**0.02**		↘		**<0.001**	**<0.001**	**<0.001**	0.27		↘	Cu/N		**<0.001**	**<0.001**	**<0.001**	0.16		→
Mo		**<0.001**	**<0.001**	**<0.001**	0.44		↗		**<0.001**	**<0.001**	**<0.001**	0.47		↗	Mo/N		**<0.001**	**<0.001**	**<0.001**	0.59		↗

↘ Decrease with warming (down at both M and L or no/small effect at M and down at L compared to H elevation sites).

↗ Increase with warming (up at both M and L or no/small effect at M and up at L compared to H elevation sites).

→ No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites).

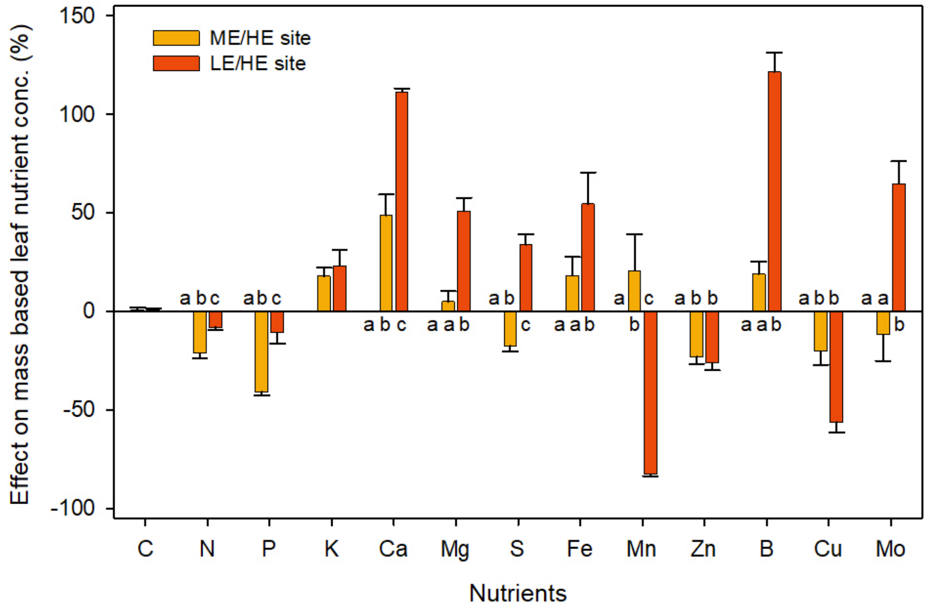


Figure 8. The effect on mass-based leaf nutrient concentration along the elevation gradient expressed as percent difference at ME and LE sites relative to the HE site, i.e., HE site is the zero line. Different letters (a, b, c, first letter for each nutrient represents the HE site) denote significant difference between sites. HE, high; ME, mid; LE, low elevation site. See Table 6 in Paper II for results from statistical analysis (Paper II).

The site*species interaction effects allowed for separate analysis of individual species (see Tables 8 & 9). The results show that most species responded similarly to the elevation gradient for most mass-based elements (and area-based, except C and Ca that differ substantially between mass- and area-based: results not shown) despite the large variation in nutrient content across species (Figure 5). Similar to the results across species, individual species were affected by declining elevation and potentially by warming for mass-based Ca, Mg, S, B (i.e., that increased), Zn, Mn, and Cu (i.e., that declined) while about 1/3 of species were affected by declining elevation for mass-based N, P (i.e., that declined), Fe and Mo (i.e., that increased) (Tables 8 & 9).

Table 8. Significance of site effects on leaf macronutrients in 18 tree species. SG, successional group (ES, early; LS, late); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest). Explanation of species codes is given in Table 1. H, M and L represent high (Sigira), mid (Rubona) and low (Makera) elevation sites, respectively. Different letters (a, b, c) denote significant difference between sites. The arrows indicate the direction of warming effect of species with significant site effect (P < 0.05), see Table footnote. * before P-value indicate a significant covariate and * after indicate that the post hoc was not significant (Paper II).

Mass based:			C					N					P					K					Ca					Mg					S				
SG	FT	Spec	P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L	
ES	TMF	Bbr	0.57					0.687					**<0.001**	a	c	b	↘	0.087					**<0.001**	a	a	b	↗	**<0.001**	a	a	b	↗	**0.005**	a	a	b	↗
		Hmo	0.74					**0.001**	a	c	b	↘	**<0.001**	a	c	b	↘	0.91					0.061					**0.005**	a	a	b	↗	**<0.001**	a	b	c	→
		Mki	**0.001**	a	a	b	↗	**0.006**	a	ab	b	↘	**0.001**	a	b	a	→	**0.024**	a	b	a	→	**0.005**	a	a	b	↗	**0.046**	a	ab	b	↗	0.37				
		Mla	0.19					**0.001**	a	b	a	→	**<0.001**	a	b	a	→	$^{+}$**0.044**					**0.001**	a	a	b	↗	**0.035**	a	ab	b	↗	**0.025**	a	b	b	↘
		Pfu	0.18					**<0.001**	a	b	a	→	**<0.001**	a	b	a	→	*0.009	a	ab	b	↗	**<0.001**	a	a	b	↗	**0.007**	a	a	b	↗	**0.003**	a	b	a	→
	LVTF	Bmi	**0.024**	a	b	ab	→	**0.019**	a	b	b	↘	**0.008**	a	b	a	→	0.30					**0.002**	a	a	b	↗	**0.003**	a	a	b	↗	**<0.001**	a	b	c	↗
		Cme	0.47					**0.038**	ab	b	a	→	**0.009**	a	b	a	→	0.79					**<0.001**	a	b	c	↗	**0.001**	a	b	c	↗	**<0.001**	a	b	c	↗
		Dto	0.11					**0.007**	a	b	a	→	**<0.001**	a	b	a	→	0.21					**<0.001**	a	b	c	↗	**0.002**	a	a	b	↗	**<0.001**	a	b	c	↗
		Hma	0.63					**0.003**	a	b	b	↘	**0.008**	a	b	a	→	0.072					**0.036**	a	b	b	↗	**0.012**	a	ab	b	↗	0.12				
		Mlu	0.34					**0.017**	a	b	a	→	**0.001**	a	c	b	↘	**0.011**	a	ab	b	↘	**0.001**	a	b	c	↗	**0.017**	a	ab	b	↗	**0.029**	a	ab	b	↗
LS	TMF	Afa	0.13					***0.006**	a	a	b	↘	**0.009**	a	b	a	→	**0.026**	ab	a	b	↘	**0.002**	a	b	b	↗	**0.001**	a	b	c	↗	**0.009**	a	ab	b	↗
		Cgr	0.93					**0.001**	a	c	b	↘	**<0.001**	a	b	b	↘	0.77					**0.006**	a	a	b	↗	0.10					**<0.001**	a	b	c	↗
		Fsa	0.87					0.010	a	b	b	↗	<0.001	a	c	b	↘	***0.005**	a	b	c	↗	0.19					**0.000**	a	b	b	↗	**<0.001**	a	b	b	↘
		Paf	**0.001**	a	b	b	↘	***0.013**	a	b	a	→	0.060					**0.020**	ab	a	b	→	**<0.001**	a	b	c	↗	**0.003**	a	a	b	↗	0.11				
		Sgu	0.34					**0.018**	a	ab	b	↘	**<0.001**	a	c	b	↘	0.40					**0.001**	a	c	b	→	**0.006**	ab	a	b	↗	**0.001**	a	b	a	→
	LVTF	Cgo	**0.003**	a	b	a	→	**0.032**	a	ab	b	↗	0.20					0.080					**0.001**	a	a	b	↗	**0.012**	a	a	b	↗	**0.003**	a	a	b	↗
		Eex	**<0.001**	a	a	b	↘	**0.017**	a	b	ab	→	**0.035**	a	b	ab	→	0.29					**<0.001**	a	b	b	↗	**0.002**	a	b	b	↗	0.79				
		Fth	0.26					**0.022**	a	b	ab	→	**<0.001**	a	c	b	↘	$^{*,+}$**0.021**					**0.003**	a	b	b	↗	0.21					**0.017**	ab	b	a	→

↘ Decrease with warming (down at both M and L or no/small effect at M and down at L compared to H elevation sites).

↗ Increase with warming (up at both M and L or no/small effect at M and up at L compared to H elevation sites).

→ No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites).

The decrease in leaf mass per unit area (LMA) at warmer sites in one third of the species, reported on the same trees in an earlier study (Paper I) contributed to significantly reducing the area-based leaf content of N, P, Zn, Mn and Cu of these species (#6) compared to species where LMA was not affected by elevation (#12, Figure 3). All these nutrients except Mn were also found to be potentially limiting (Figure 9).

6.3.3. Elevation gradient site effect on leaf nutrients of mature trees (Paper III)

For the four species (two ES species, *M. kilimandscharica* and *P. fulva and two LS species, S.guineense and C. grandiflora*) used in the mature elevation gradient study a significant site*species interaction was observed on both leaf area- and mass-based N as well as for N:P ratio, while not for P content. (Figure 10 a-f). However, no consistent trend in the leaf N and

N:P ratio with elevation was observed (Figure 10a). Thus, it seems not likely that downward warming affected the leaf N content of the mature trees. However, as no soil nutrient analyses was conducted a confounding soil effect cannot be excluded.

Table 9. Significance of site effects on leaf micronutrients in 18 tree species. SG, successional group (ES, early; LS, late); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest). Explanation of species codes is given in Table 1. H, M and L represent high (Sigira), mid (Rubona) and low (Makera) elevation sites, respectively. Different letters (a, b, c) denote significant difference between sites. The arrows indicate the direction of warming effect of species with significant site effect (P < 0.05), see Table footnote. * before P-value indicate a significant covariate. (Paper II).

Mass based:			Fe					Zn					Mn					Cu					B					Mo				
SG	FT	Spec	P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L	
ES	TMF	Bbr	0.093					**0.007**	a	b	b	↘	**0.001**	a	a	b	↘	0.059					**<0.001**	a	a	b	↗	**0.001**	a	b	b	↘
		Hmo	0.52					**0.000**	a	b	c	↘	**0.001**	a	a	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	0.080				
		Mki	0.37					**0.002**	a	b	b	↘	**0.006**	a	a	b	↘	**<0.001**	a	b	b	↘	**<0.001**	a	a	b	↗	0.055				
		Mla	**0.009**	a	b	b	↗	0.23					**0.027**	ab	a	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	**0.012**	a	a	b	↗
		Pfu	0.80					0.089					**<0.001**	a	a	b	↘	**0.002**	a	b	b	↘	**<0.001**	a	b	c	→	0.60				
	LVTF	Bmi	0.84					0.41					**0.003**	a	a	b	↘	**0.003**	ab	b	a	→	**<0.001**	a	a	b	↗	0.50				
		Cme	0.17					***0.004**	a	b	b	↘	**0.001**	a	b	a	→	**<0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	0.059				
		Dto	**0.002**	a	b	b	↗	0.11					**0.001**	a	a	b	↘	**0.001**	ab	b	a	→	**<0.001**	a	b	c	↗	**<0.001**	a	a	b	↗
		Hma	**0.015**	a	b	a	→	**0.034**	a	ab	b	↘	**<0.001**	a	b	c	↘	**<0.001**	a	b	c	→	**<0.001**	a	a	b	↗	**0.035**	ab	b	a	→
		Mlu	0.22					**0.005**	a	b	b	↘	**<0.001**	a	c	b	→	**<0.001**	a	b	c	→	**<0.001**	a	b	c	↗	**0.027**	ab	b	a	→
LS	TMF	Afa	0.082					***0.002**	a	b	c	→	**<0.001**	a	a	b	↘	0.073					0.18					**0.047**	*			↗
		Cgr	**0.023**	a	a	b	↗	0.087					**<0.001**	a	b	b	↘	**0.002**	a	a	b	↘	**0.003**	a	a	b	↗	0.10				
		Fsa	**0.034**	a	b	b	↗	0.74					***0.005**	a	ab	b	→	**0.004**	a	b	a	→	**0.002**	a	a	b	↗	**0.039**	*			↗
		Paf	**0.004**	a	ab	b	↗	0.30					**0.009**	ab	a	b	→	**0.011**	a	a	b	↘	**0.012**	a	ab	b	↗	0.16				
		Sgu	0.36					**0.001**	a	b	a	→	**<0.001**	a	a	b	↘	**0.002**	a	a	b	↘	**<0.001**	a	a	b	↗	0.86				
	LVTF	Cgo	**<0.001**	a	c	b	→	**0.003**	a	b	b	↘	**<0.001**	a	b	c	↘	0.62					0.30					**0.007**	a	b	ab	→
		Eex	**0.010**	a	b	b	↗	**0.032**	a	a	b	↘	***0.024**	ab	b	a	→	**0.003**	a	a	b	↘	**0.002**	a	a	b	↗	**0.028**	a	ab	b	→
		Fth	**0.004**	a	b	b	↗	**0.006**	a	b	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	**0.002**	a	a	b	↗

↘ Decrease with warming (down at both M and L or no/small effect at M and down at L compared to H elevation sites).

↗ Increase with warming (up at both M and L or no/small effect at M and up at L compared to H elevation sites).

→ No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites).

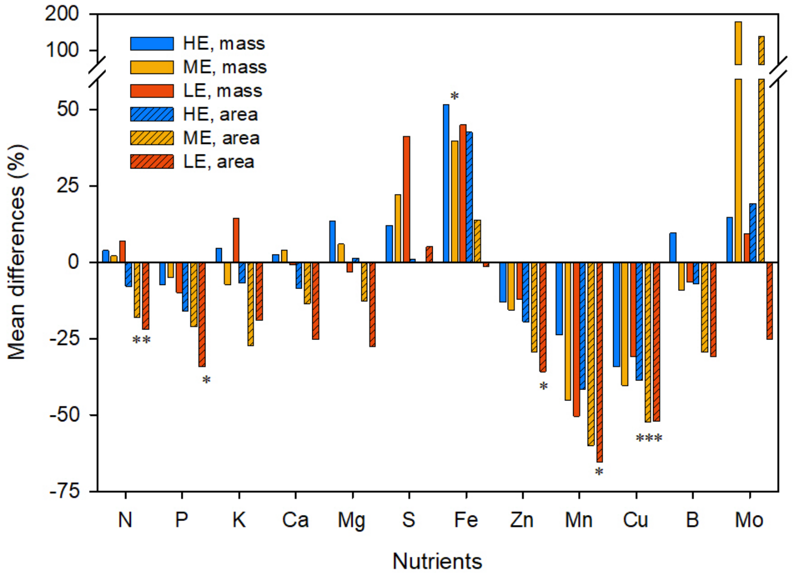


Figure 9. The mean difference in mass and area-based leaf nutrient contents between species with a significant warming decline in leaf mass per area unit (6#) compared to species with unaffected LMA (#12) along the elevation gradient. * show significant differences between the two groups of species (i.e., species with affected LMA versus non affected LMA at each site). HE, high; ME, mid; LE, low elevation site (Paper II).

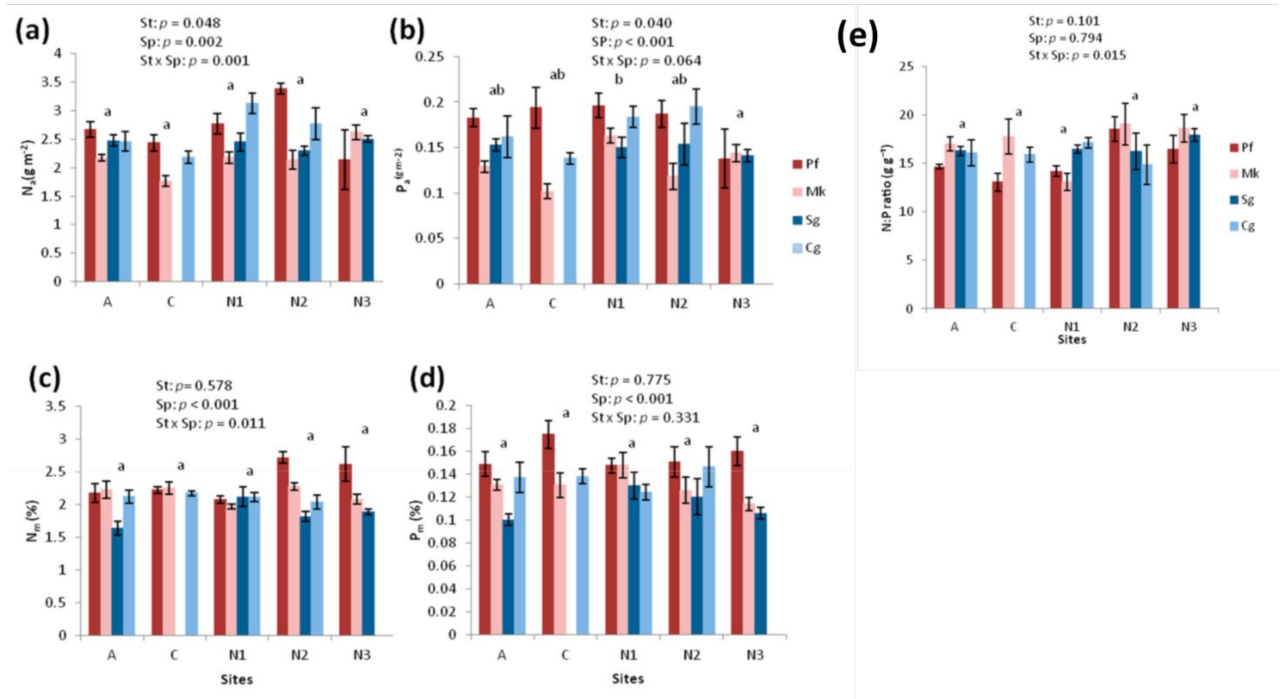


Figure 10 **a-e.** Area based Nitrogen and Phosphorus (N_a, P_a), mass based Nitrogen and Phosphorus (N_m, P_m), and Nitrogen to phosphorus ratio (N:P ratio), of four species (Sp), two ES species *M. kilimandscharica* and *P. fulva* and two LS species *S.guineense* and *C. grandiflora* at five sites (St) along an elevation gradient. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations are provided in part 5.2.2. of this thesis, the site order follows increasing elevation from left to right (Paper III). Corresponding statistical results are in Table S2 of Paper III.

6.3.4. Source of variance in leaf nutrients across all species and sites (Paper II, III, IV)

Analysis of source of variance in leaf nutrient content show that species is the main source when many species are included (18 to 20 species), among both young (~14-83 % of variance) and mature trees (~25-65 % of variance). Species is respectively followed by the residual (~18-80 % of variance) and plot/site in young trees (~3-40 % of variance), whereas, in mature trees, species is respectively followed by plot/site (~17-70 % of variance) and residual (~7-32 %). In the latter case, the effect of successional group is generally weak (<5 % of variance) in both young and mature trees, except for N, P, Mn, Zn, Cu in young trees and P in mature trees (Figure 11A-B). When including only the 4 common species to all three studies, within and between plots/sites become more important than species. This trend is more pronounced in Nyungwe permanent plots (with large within site effects and small residual effects) than in the rwanda TREE and mature elevation gradient studies (Figure 11 C, D & E). However, note that the within site variation is included in the residual effect for the mature tree elevation gradient. Overall largest contribution on species level were found for N > S > P > Fe (~70-40 % of variance) in mature trees (across 20 species) and in K > N > B > Mo > Zn > C > Ca > Cu > Mg (~85-40 % of variance) in young trees (across 18 species) (Figure 11 A-B). Across the 4 common species, only Fe, S in young and Sin mature trees had large variation on species level (>40 % of variance) (Figure 11 C, E).

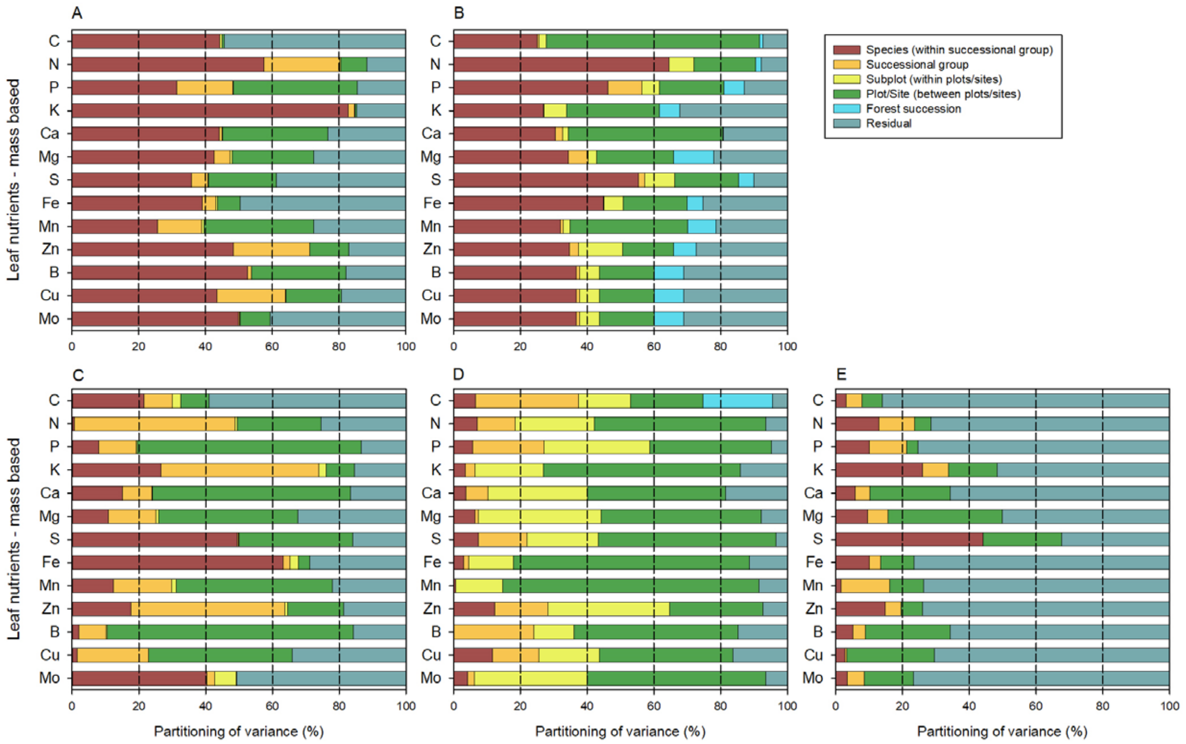


Figure 11 A-E. Partitioning of variance (%) of mass-based leaf nutrient content in relations to species, successional group, subplot/site (within sites), plots/sites (between plots/sites), forest stand successional type (only in B and D) and the residual not explained by other factors. A) RwandaTREE with 18 species; B) permanent plots in Nyungwe with 20 species; C) RwandaTREE with four species; D) permanent plots in Nyungwe with four species; E) mature tree elevation gradient with four species. The four species panels (C, D, E) always contain the same species (Figure A is from Paper II and the others are made of data from papers II, III, IV).

6.4. Comparison of young-planted trees with mature naturally regenerated trees

6.4.1. Leaf morphology of young versus mature trees (Paper I, III, IV)

A comparison of leaf morphology (LMA and leaf size) in young planted trees and mature naturally regenerated trees reveals that both tree age classes respond almost equally along the elevation gradient (i.e. warming) in three of four species, when data from all three studies were compiled (Figure 12). A significant main age (young vs mature) effect on leaf size or LMA in *M. kilimandscharica* was observed but not in the other three species (Table 5 in Paper I) when data points were standardized to an elevation of 2000 m a.s.l. Leaf size did not decline with rising elevation except in mature trees of *M. kilimandscharica* that slightly declined with elevation (Figure 12b). LMA significantly declined with declining elevation for *C. grandiflora*, *M. kilimandscharica* (mature data only) and *P. fulva* (only when data above 2500 m a.s.l. were excluded from linear regression as LMA to elevation relationship resembles an optimum curve with a sharp decline above 2500 m a.s.l) but not for *S. guineense* (Figure 12 e-h).

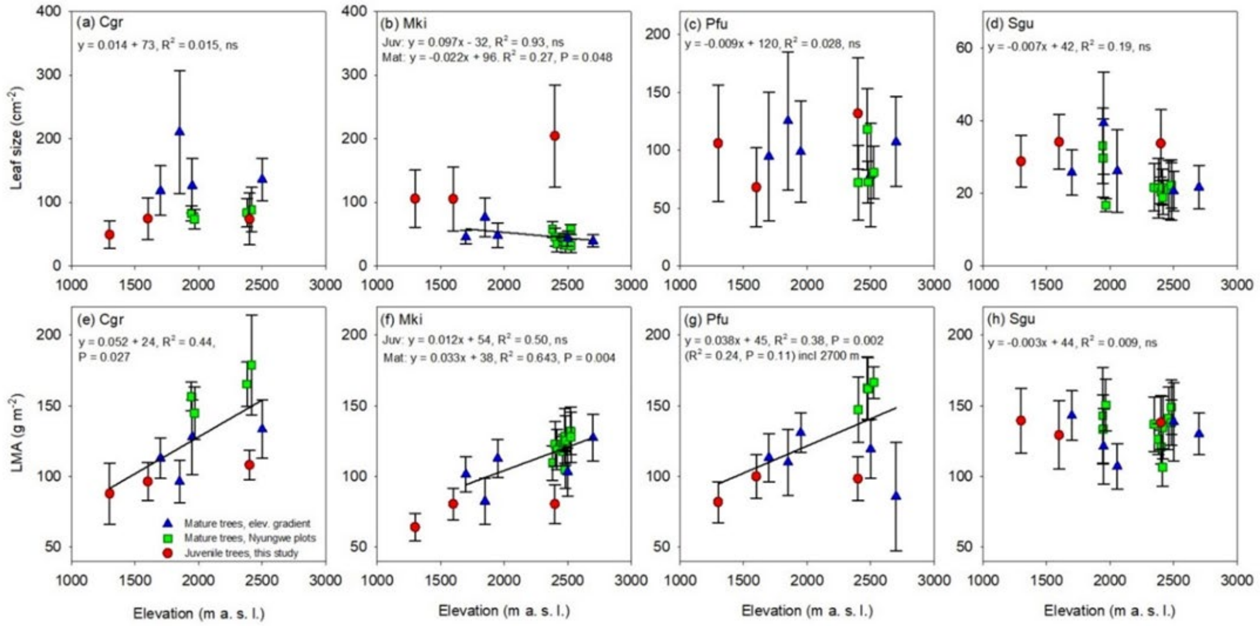


Figure 12 a-h. Leaf size and leaf mass per area unit (LMA) at different elevations for Cgr, *Carapa grandiflora* (a, e); Mki, *Macaranga kilimandscharica* (b, f); Pfu, *Polyscias fulva* (c,g); Sgu, *Syzygium guineense* (d, h) in three complementary studies in Rwanda. Each data point show mean±SD at each site included in the studies. Blue triangle, mature tree elevation gradient study; Green square, permanent monitoring plots in Nyungwe forest with mature trees; Red circle, Elevation gradient study with young trees (Rwanda TREE, this study); Lines, equation and R^2 represents the regression line for all tree studies. P-value refers to the slope coefficient. Corresponding statistics are in Table 5 of paper I.

6.4.2. Leaf nutrients and N based ratios of young versus mature trees (Paper II, III, IV)

A significant lower leaf N and P content was observed in the mature compared to the young trees. However, the N based ratios of most macro and micronutrients were higher in mature compared to young trees, except for K, Ca, Mn and B. (Table 10). The comparison of these N based ratios to the optimal ratios shows that the P/N ratio was under the optimum for both young and mature trees while Zn/N and Cu/N ratio on average were within or above the optimum ranges for mature trees but at the lower end or below for young trees (Table 10). These results inform that studied TMF trees are likely limited by P than N and they may also be limited by especially Cu and Zn for some species and potentially in the future as these elements declined with warming in young trees (Table 7). The results also show that ratios of Ca, Mg, S, Fe, Mn, and B to N are above the optimal ratios in both mature and young trees. The optimal ratios for Mo is absent from literature and the limitation by this element has therefore not been assessed in this study (Table 10).

6.5. Leaf nutrient resorption across species and successional groups

Limitation by nutrients may be potentially reduced by effective nutrient resorption (NuRE). In this study, NuRE differed significantly among species for three nutrients (N, P, K), but was independent of successional status (Table 11). NuRE for three nutrients (N, P, K) ranged between 12-79 % (Table 11), while the other macro- and micronutrients (see Paper IV) had rather highly variable NuRE (-90 ≤ 85 %) with non-significant difference between ES and

Table 10. Compilation of leaf N, P and ratios of macro and micronutrients to nitrogen content from the three complementary studies included in the thesis. The data are taken from the papers indicated by the roman number in the headings. The following data are compared using one-way ANOVA: Young and Mature trees of four common species from paper II, III and IV at a standardised elevation of 2000 m a.s.l using elevation as covariate; Red values indicate when mean nutrient rations are below optimum.

	Young (II)		Mature (III, IV)		Diff	P-	Optimal
Element	Mean	SD	Mean	SD	(%)	value	ratios
N (mg g^{-1})	26.1	± 2.8	20.1	± 2.7	-23	**<0.001**	
P (mg g^{-1})	1.5	± 0.3	1.3	± 0.2	-15	**0.012**	
N/P (g g^{-1})	18.8	± 3.3	16.0	± 3.1	-15	**0.013**	
P/N	5.7	± 1.0	6.4	± 0.9	13	**0.024**	8-10
K/N	34.8	± 12.5	38.5	± 12.1	11	0.37	30-35
Ca/N	30.5	± 12.7	37.0	± 12.2	22	0.12	2-2.5
Mg/N	8.9	± 4.8	14.2	± 4.6	61	**0.001**	3-3.5
S/N	7.1	± 3.6	12.0	± 3.5	68	**<0.001**	4
Fe/N	0.7	± 1.2	1.9	± 1.1	186	**0.002**	0.13-0.2
Mn/N	1.6	± 1.8	2.5	± 1.7	58	0.12	0.03-0.05
Zn/N	0.04	± 0.04	0.09	± 0.04	93	**0.002**	0.04-0.05
B/N	0.15	± 0.06	0.11	± 0.06	-31	**0.019**	0.05
Cu/N	0.017	± 0.017	0.038	± 0.017	124	**0.001**	0.01-0.03
Mo/N	0.0002	± 0.0005	0.0010	± 0.0005	318	**0.007**	

LS species (Table 2 in Paper IV). Species-specific values of nitrogen resorption (NRE) ranged between 12 – 60 % (mean = 37 %) while values of phosphorus (PRE) were higher and ranged between 31 – 74 % (mean = 48 %) and potassium resorption (KRE) between 9 – 79 % (mean 46 %). Three species had very low NRE (≤ 16 %; *C. africanus, I. mitis* and *M. acuminata)*, of which one was classified as LS and two as ES. *C. africanus (LS)* had also relatively low PRE and KRE while *P. Africana* (LS) had very low KRE (<10 %).

Across species, resorption efficiencies and leaf concentrations were not significantly related for N, P, and K, suggesting that mature leaf concentration was not a good predictor of the interspecific variation in NuRE (Figure S3 in Paper IV). However, analysis of plot-data for the dominant species *M. kilimandscharica* and *S. guineense* only, showed significant positive relationships between leaf N concentration and NRE for both species and for leaf K concentration and KRE in *S. guineense* (Fig. S4; Table S4 in Paper IV). The interspecific variation in PRE, KRE and SRE was significantly related to variation in NRE ($R^2 = 0.34$-0.73; $P \leq 0.0067$; Figure 1b, d, f in Paper IV). However, both PRE ($P < 0.0001$) and KRE ($P = 0.064$) were generally higher than NRE at low levels of NRE (Figure 1b, d in Paper IV & Table 10) and these 3 were higher than other macro and micronutrients resorption (i.e., KRE>PRE>NRE>MoRE>SRE>MgRE>ZnRE>CuRE>CaRE>BRE) across species (Table 2 in Paper IV).

Table 11. Leaf resorption efficiency of N, P, K (NRE, PRE, KRE) in senesced and shed leaves in relation to the element content of attached green leaves in 10 early successional (ES) and 10 late successional (LS) tree species. SG, successional group; #, number of replicates; Diff, the difference between LS and ES species; P values (SG), the results of t-test between ES and LS species; P-values (species), the results of one-way ANOVA testing differences among species. The replicated unit (#) for *M. kilimandscharica* and *S. guineense* is plots and for other species sub-plots. Bold value, P < 0.05 (Paper IV).

Species	SG	#	NRE	PRE	KRE
Afrocrania volkensii	ES	3	59.4	59.4	45.1
Agauria salicifolia	ES	2	34.8	42.7	20.2
Harungana montana	ES	4	36.9	48.5	68.1
Ilex mitis	ES	4	15.5	31.0	38.3
Macaranga kilimandscharica	ES	11	51.6	63.7	69.3
Maytenus acuminata	ES	3	12.4	31.9	21.4
Polyscias fulva	ES	3	53.8	58.9	51.8
Prunus africana	ES	2	26.0	48.9	9.2
Psychotria mahonii	ES	3	46.8	59.2	78.9
Rapanea melanophloeos	ES	3	60.4	74.5	51.2
Carapa grandiflora	LS	4	37.6	48.9	42.8
Chionanthus africanus	LS	3	4.0	29.2	19.3
Cleistanthus polystachyus	LS	3	40.2	33.6	37.5
Faurea saligna	LS	4	40.1	49.5	56.9
Ficalhoa laurifolia	LS	2	44.5	49.3	60.3
Ocotea kenyensis	LS	3	35.4	48.2	53.7
Ocotea usambarensis	LS	3	24.7	36.6	55.1
Olinia rochetiana	LS	3	48.4	54.2	54.4
Strombosia scheffleri	LS	3	35.5	33.7	29.1
Syzygium guineense	LS	11	40.5	56.4	58.9
Mean (All species)			39.9	48.9	49.6
Mean (ES)			41.3	52.2	49.4
Mean (LS)			38.5	45.6	49.9
Diff (%)			-7.2	-14.6	1.0
P-value (SG)			0.8	0.9	0.9
P-value (Species)			**<0.001**	**<0.001**	**<0.001**

7. Discussion

It was suggested that short-term inter-annual responses can predict long-term responses to warming in intact non-disturbed tropical forests (Sullivan et al., 2020). Therefore, I will discuss my results from the one to two years investigation of warming responses of leaf morphological and chemical traits of ATMFs tree species in relation to previous findings that mostly focussed on interspecific responses and were conducted on other continents.

7.1. Morphological and chemical leaf traits across tree species

The relatively large interspecific variation of morphological leaf traits observed in this study has also been found elsewhere in the tropics (Poorter et al., 2009; van de Weg et al., 2009). Traits were generally more acquisitive in ES species compared to LS species, as also reported elsewhere (Hernández-Vargas et al., 2019; Lohbeck et al., 2015; Prado-Junior et al., 2016; Sterck et al., 2011). However, interspecific variation was also large within the successional groups. Overall, the range of leaf morphology variation in the studied species seems to be typical for humid tropical evergreen and deciduous trees, although the extreme values that sometimes can be observed elsewhere (Poorter et al. 2009) are lacking in my results.

The leaf nutrient concentrations and N based ratios also varied greatly across species. Specifically, the non-N and P nutrients varied even more between species with greater variation in micronutrients than in macronutrients. These results are supported by previous findings of wide concentration ranges for most mineral nutrients between species (van den Driessche & Rieche, 1974; Wu et al., 2007) which supports the suggestion that each species is unique and has an optimum range as well as a minimum requirement level of nutrients (Chapin et al., 1986; Uchida, 2000) although some of the inter-specific variation may also be a result of different uptake mechanisms resulting in excess accumulation. Theoretical and experimental laboratory studies suggest that most plants require nutrients in similar proportions based on nutrients to N ratios (Knecht & Göransson, 2004), although this may vary for plants grown under natural conditions (Ågren, 2008; Drechsel & Zech, 1991).

Furthermore, successional groups and sites differently affected the leaf nutrient accumulation across species but the variation between species were more important. These results are in line with a study along an elevation gradient in Amazon forest that found large contribution of phylogenetic (i.e., family, genus, species) to the variance in leaf nutrients (e.g. N, P, Ca,) followed respectively by residual (unknown) and sites (Asner et al., 2014). Large variation between species has also been found among other leaf traits as reviewed by Messier et al., (2017). Other factors contributing to the large variance were sites, individual trees, strata, leaf and residuals. Combined, these results confirm the last part of hypothesis #6.

Other studies found that climatic, edaphic, geologic and phylogenetic factors influence the nutrient uptake by plants (Neugebauer et al., 2018; Poorter, 2009; Salinas et al., 2021). However, the plasticity of within-species patterns challenges traits covariation with environmental gradient (Anderegg et al., 2018). The significantly higher leaf N, P, Mn, Zn and Cu content in ES compared to LS species is supported by the stronger acquisitive traits in ES species compared to conservative traits in LS species observed in previous studies (Poorter et al., 2004; Schönbeck et al., 2015). These results confirm the first part of hypothesis #6 (Paper II, III, IV) for some nutrients and species but not for others. This study shows large variation between species also when grown in the same soil and climate, while most other studies compared trees grown in different soils and climate.

7.2. Correlation between different morphological and chemical traits

The observed negative correlation between LMA and leaf W/L ratio, leaf size, and mass based N and P across species (Paper I) is broadly in agreement with other studies (Lin et al., 2020; Wright et al., 2004). Similarly, the positive relationship found between LMA and area based N (Paper I) is in accordance with previous research (Bussotti, 2008; Castro-Diez et al., 2000; Onoda et al., 2017). These results confirm the hypothesis #3 (Paper I). LVA and LD differently contributed to within and between species LMA variation (Paper I) as it was found in previous studies (Coble & Cavaleri, 2017; Poorter et al., 2009; Villar et al., 2013; Zhang et al., 2020). In some species, LD contributed more than LVA to the LMA variation between sites, in other species LVA contributed more than LD, and in a third group of species, LD and LVA contributed equally (Figure 7). These findings support the part of hypothesis # (Paper I and III) saying that variation in LMA is more due to shift in LD than to shift in LVA but only for some species and not for others.

Overall, both positive and negative relationships were observed between leaf macro- and micronutrients (Table 4). This is in line with previous research that showed both positive (i.e., synergetic) and negative (i.e., antagonist) relationships between leaf macro and micro nutrients (Neugebauer et al., 2018; Smith, 1962). However these may vary depending on plant species, tree age, leaf age (Ji et al., 2021; Xie et al., 2020) and competitive exclusions or coexistence mechanisms, stoichiometry adjustments among trees of different species (Danger et al., 2008; Marastoni et al., 2019; Zhang et al., 2021). In my study, I found the same relationships between leaf nutrients like in previous research where positive relationships were found between N and P; N, P and Fe, B, Zn; Ca and Mg (Neugebauer et al., 2018). Negative relationships between Mg and K (Xie et al., 2020); Mn, and Cu (Marastoni et al., 2019); K and N, P, Mg, Ca, Cu, Zn, Mn, B (Smith, 1962) have also been found before, contrary to my results.

My results are supported by studies relating soil and shoot nutrients that found the same positive relationships between P and S, Zn, K; between Ca and P, S, Mg; between B and K, Mo, Ca, Mg; between Mo and P, S, K; and between Cu and Mn, Zn and negative relationships between Mn and Ca, Mg (Heineman et al., 2016; Jones et al., 2019; Ordoñez et al., 2009) as I found between leaf nutrients (Paper II) which implies the existence of positive and negative relationships between nutrients from the soil to the leaf continuum. These findings support hypothesis #4 saying that except for N/P ratio, leaf nutrients balance is maintained along the elevation gradient (Paper II). However, the coupling between nutrients varied compared to previous studies (Bai et al., 2019; Sobrado, 2014). This emphasizes the importance of examining all essential nutrients when studying their relationships to one another as they play different roles in the functioning of a plant and therefore may express different affinities from the soil to the leaves and within leaves.

7.3. The effects of site and warming on leaf traits.

7.3.1. Elevation gradient site effect on leaf morphology (Paper I, III)

LMA significantly declined with declining elevation in mature trees of three out of four species studied (Figure 12), as well as for young trees of half of the 18 species studied in Rwanda TREE (Table 6). This agrees with other studies that found an increase in LMA with increasing elevation (Neyret et al., 2016; van de Weg et al., 2009; Zhang et al., 2020) and decline with warming in both field and controlled experiments in the tropics (Doughty et al., 2018; Neyret et al., 2016) and several other biomes (Poorter et al., 2009; Zhang et al., 2020). Thus, the result in these studies confirms the second part of hypothesis #1 and agree with previous studies. However, LMA responses to warming were independent of successional strategy as opposed to the first part of hypothesis #1 and the findings by Cheeseman, Winter and Slot (Cheesman & Winter, 2013; Slot & Winter, 2018). The large variation between species may explain the differences between observed effects in this study and the literature regarding responses of different successional groups.

The contribution of shifts in LD and LVA to changes in LMA at warmer sites were less consistent. Sites effects on LMA versus site effects on LVA and LD respectively, showed a significant positive relationship between site effects on LMA and LVA, when both the ME and the LE sites were compared to the HE site, while no significant effects were observed for LD (Figure 6 in Paper I). A likely explanation is that the site effect on LD is strong but only for a few species, while the site driven effect on LVA is more subtle and has a more general influence on the site effects on LMA. The significant effect of tree height on LVA may also have contributed to conceal the site effect in accordance with other studies (Coble & Cavaleri, 2017; Nichlos et al., 2019). This implies that the part of hypothesis # saying that changes in LMA at warmer sites are more from shifts in LD than from shifts in LVA can be retained for few species but overall rejected for many species (Table 5).

The decrease in leaf size with warming for one out of four species in the study on mature trees and for almost half of studied young species disagree with the first part of hypothesis #2 (Paper I and III). This finding is likely a consequence of conflicting effects of temperature and water limitations on leaf size (I. J. Wright et al., 2017), with hydraulic constraints on water transport and leaf growth in the field (Coble & Cavaleri, 2017; Li, Zou, et al., 2020; Sun et al., 2019). It may be explained by high VPD at warmer sites (Table 2) that probably reduced the total number of epidermal cells per leaf and per leaf area according to Murphy et al. (2014) and which likely altered the relationships between LMA, LD and LVA with reference to Lin et al. (2020) and Wright et al. (2004).

Leaf size mostly declined with warming in ES species but not in LS species supporting the suggestion that ES trees have higher plasticity in response to warming compared to LS trees (Cheesman & Winter, 2013; Slot & Winter, 2018). This result confirms the last part of hypothesis #2. However, the leaf W/L ratio increased with warming as opposed to Li et al. (2020). Overall, this study reveals the importance of considering a broad range of species when studying the responses of tropical montane species to warming and elevation gradient to accurately capture response of leaf functional traits along environmental gradients.

7.3.2. Elevation gradient site effect on species leaf nutrients (Paper II, III, IV)

The decline in elevation trends between sites were observed for N_M and P_M similarly to studies that found a decline in N_M with increasing elevation (Tanner et al., 1998; van de Weg et al., 2009), a decline in P_M with increasing elevation (Vitousek et al., 1988). Area-based nutrients showed the same trend as mass-based nutrients contrary to other studies that found an increase in both N_A and P_A with increasing elevation (Kitayama & Aiba, 2002; Körner, 1989) and a study in the Andes that found an elevation trend only in N_M (van de Weg et al., 2009).

Based on the assumption that N limitation is increasing with increasing elevation in TMF due to reduced N-mineralization at high elevation (Benner et al., 2010). I hypothesized that N limitation decline while P limitation increase with declining elevation (second part of H#5). However, this was mostly not confirmed, but the P/N ratio in the young trees tended to decrease down the elevation gradient (Table 6, Paper II), so potential P limitation may increase with warming. However, the effect was small in the young trees and non-existing in the mature trees. (Table 5, Paper III). Thus the results do not support many studies from the neotropics that reported an increasing N limitation with increasing elevation (Austin & Vitousek, 1998; Dalling et al., 2016; Tanner et al., 1990, 1998; Tanner & Kapos, 1992; Vitousek, 1984; Wright et al., 2011). However, not all studies have observed this elevation trend in TMFs (Allen et al., 2020; Cárate-Tandalla et al., 2018) and Nyungwe TMF seem to be one of the forest without such trend. When results of N and P concentration and N:P ratio are taken together with low resorption efficiencies (in relation to global averages) of studied mature species in Nyungwe TMF (Paper IV) and compared to results reported for other TMFs together indicate high fertility and likely co-limitation by N and P, however progressively increasing towards P limitation during succession.

The site effect on area-based leaf P of young species in relation to both total and available soil P showed no elevation trend as it was found in some previous studies (Soethe et al., 2008; T. Wu et al., 2019), contrary to other studies that reported a decline in leaf P with declining elevation (Asner et al., 2014; Fyllas et al., 2009; Reich & Oleksyn, 2004; Šímová et al., 2019; Vitousek et al., 1988). The increase in leaf Ca and Mg with declining elevation in this study is in disagreement with earlier studies that found no elevation trends (Soethe et al., 2008; Wu et al., 2019), whereas Asner et al. (2014) found an increase in leaf Ca with declining elevation in Amazonian forest. Vitousek et al. (1988) found a decrease in leaf Mg with increasing elevation but no elevation trend for Ca in TMFs, similarly to my finding that the leaf Mg and ratio of Mg to N increase with declining elevation.

The findings that Cu decrease with declining elevation are supported by previous research that found the same trend (Litton et al., 2020; Martin et al., 2020). Overall, my results show no significant elevation gradient site effect on N, P, K These results are contradicting with findings that N, P, K increase with declining elevation or warming in tropical mountain forests (Soethe et al., 2008; Tanner et al., 1998; Vitousek et al., 1988) or a decline in N, P, K with increasing MAT in lowland forest (Fyllas et al., 2009) or global data set paterns (Reich & Oleksyn, 2004). However, the no elevation trend for N, P, K was found by (Soethe et al., 2008) in a wide range of sites, and by (Wu et al., 2019) for N, P. My results that leaf S/N increases with declining elevation was also found by Soethe et al. (2008). Litton et al. (2020) found a decline in Mn with warming or declining elevation which disagrees with my finding that Mn has no elevation gradient trend.

My findings partly confirm the first part of hypothesis #5 that leaf nutrients decreased with warming. The leaf content of the potentially most growth-limiting nutrients N and P, but also Zn and Cu declined in the young trees, but not in the mature trees, down the elevation gradient. Thus, the confirmation is valid only for some elements and some species at young age while rejected for others. These results are probably at least partly explained by soil pH effects that differently affected the availability of these elements at the warmer sites (Tables 3 and 5 in Paper II). The site effect was pronounced on area-based nutrient content of species with LMA that declined with warming and declining elevation with potential nutrient limitations, especially for P. This will probably have consequences for the photosynthetic capacity per leaf area unit of these species, , as especially N and P support photosynthesis (Osnas et al., 2013).

7.3.3. Source of variance in leaf nutrients across all species (Paper II, III, IV)

The findings that the partitioning of different nutrients in leaves across all species is controlled by different attributes with species occupying a central portion, followed by none explained factors (i.e., residual), site, and successional group effects are in agreement with a study that overall attributed canopy chemical and morphological traits partitioning of variances (%) to phylogenetic (species, genus, family) and unexplained components (residual) than to sites (within, between) in Amazonian tropical forests (Asner et al., 2014; Messier et al., 2017).

The fact that K partitioning show large variance in young trees is likely explained by the role played by K to N ratio in balancing C and N assimilation in physiological processes (Ali et al., 2020) because K play a role in stomatal regulation and functioning of enzymes responsible of C fixation (Ericsson et al., 1996). The overall no site effect on leaf K in young trees across 18 species is in contradiction while the effect of site on K in mature trees agrees with other studies that found an increase in K with warming (Litton et al., 2020) or a decline in leaf K with increasing elevation (Soethe et al., 2008; Vitousek et al., 1988).

The effect of site on leaf Ca, Mg and Mo is explained by the overall phyto-availability of their counterpart in soils (Neugebauer et al., 2018) in relation to water flow in the plant (Knecht & Göransson, 2004) while effect of site on leaf N, P, K, Fe, Cu, Mn and Zn is explained by the negative correlation between them and corresponding soil nutrients or pH (Tables 3 and 5 in Paper II). These results are also supported by other studies that suggested deficiencies or lower values in Cu, and Zn in tropical acidic soils, especially in Africa (Davies, 1997; Kang & Osiname, 1985). Previous studies reported the excess in Mn and possibly in Fe in tropical soils (Davies, 1997; Kang & Osiname, 1985) and TMF (Chacon et al., 1998), contrary to Davies who reported deficiency in B in tropical soils. In addition, it was suggested that species grown on poor-nutrients soils allocate more nutrients to their leaves than to other organs and increase their nutrients use efficiency (Boerner, 1984; Tsujii et al., 2020). These results confirm the last part of hypothesis #6 that variation in leaf nutrients among trees are more specific to species than sites.

7.4. Comparison of young-planted trees with mature and natural regenerated trees

7.4.1. Leaf mass per unit area and leaf size of young versus mature trees (Paper II, III)

The results show that the leaf morphology of young and mature trees respond almost similarly to elevation (i.e., warming) in at least three out of four compared species (Figure 12). This agrees with the second part of hypotheses #1 and the first part of hypothesis #2 that predict similar elevation trend in LMA and LA of both young and mature species . When standardized to an elevation of 2000 m a.s.l., a significant age (mature vs mature) difference was observed for leaf size and LMA in *M. kilimandscharica* only, but not in the other three species (Table 5 in Paper I). This is in contrast with previous studies that found ontogenetic changes in leaf traits (Brito-Rocha et al., 2016; Ishida et al., 2005; Sean C. Thomas & Winner, 2002).

Leaf size did not decline with rising elevation, contrary to previous studies (Milla & Reich, 2011; Nichlos et al., 2019) except for leaves of mature trees of *M. kilimandscharica* that slightly declined with elevation (Figure 11b). LMA significantly declined with declining elevation for *C. grandiflora*, *M. kilimandscharica* (mature data only) and *P. fulva* (when data at 2700 m a.s.l. was excluded) in agreement with previous findings (van De Weg et al., 2009; Neyret et al., 2016;Zhang et al., 2020) but not for *S. guineense* (Figure 12e-h).

7.4.2. Leaf nutrients and N based ratios of young versus mature trees (Paper II, III, IV)

The observed lower N, P but higher P/N and other macro and micronutrients to N ratios in mature than in young trees are supported by previous studies on ontogenetic developmental nutrients changes (He & Yan, 2018; Houter & Pons, 2012;Ji et al., 2021; Mediavilla et al., 2014). These results agree with the second part of hypothesis #6 except for N and P that are limiting growth of most species.

The higher N, P, and N based ratios of P, Mn, Zn, Cu of ES trees compared to LS trees maybe explained by the acquisitive strategy of ES species by high uptake of resources for fast growth compared to LS species (Hernández-Vargas et al., 2019; Prado-Junior et al., 2016; Sorensen, 2000; Sterck et al., 2011). This confirms the first part of hypothesis #6 for only N, P, P/N, Mn/n, Zn/N, and Cu/N but not for other studied macro and micronutrients. This likely because these elements were found either limiting by deficiency (i.e., N, P, Zn, and Cu) or excess (i.e., Mg) and less affected by elevation (i.e., N, P, K, see paragraph 2 in section 7.4.2). P and N limit growth in tropical trees (Santiago et al., 2012) and soil phosphorous control C storage (Hofhansl et al., 2020), but it was demonstrated that species adapted to phosphorous depleted soils acclimate their photosynthesis through P allocation to different foliar fractions (Mo et al., 2019) at higher C costs (Allen et al., 2020) and maintain high tree growth, especially on clayey soils in tropical forests (Soong et al., 2020).

Other types of limitation than N and P were detected to possibly occur in the studied species across all sites. Results show that Zn and Cu to N are sometimes below the optimum ratios and that N based ratios of Mn, Ca, Mg, Fe, and B are mostly above optimum ratios. This implies a possible limitation of species growth by deficiency in Zn and Cu and excess in Mn, Ca, Mg, and Fe. This is supported by a study that defined a limiting nutrient ratio as a too low or too high ratio compared to the optimal value (Sumner, 1978) and can happen in case species become unable to maintain leaf nutrient balance under soil conditions with deficient or toxic levels of micronutrients (Sobrado, 2014).

The tendency in excessive levels of leaf nutrients (i.e., ratios of Mn, Ca, Mg, Fe, and B to N) in my study, inform a likely potential accumulation capacity of studied species for available soil nutrients only rich in Mn but poor in other nutrients especially B and S (Table 3 in Paper II). This is almost supported by studies that tested accumulation capacity of species grown on soils with nutrient deficiencies and high concentrations of toxic heavy metals (e.g., Ni) and found that species accumulated K, Cu, Mn, Zn in their leaves with respect to their affinity with other soil macro and micronutrients (Meindl et al., 2021; Nkrumah et al., 2018). Plants also accumulate metals in their leaves as a strategy against herbivores and pathogens (Leitenmaier & Küpper, 2013; Rascio & Navari-Izzo, 2011). Possible toxic levels were found by other studies for Mn (Chacon et al., 1998; Millaleo et al., 2010), and excessive uptake in Ca, Mg and K (Knecht & Göransson, 2004). Contrary to my finding, Marastoni et al.2019 found toxic levels for Cu instead of deficiency (Marastoni et al., 2019).

Overall, P and Mn show respectively lowest and highest ratio compared to the optimal values range which implies a likely potential growth limitation by P deficiency and Mn excess across all species (Table 10). These results are supported by some studies that suggested a possible simultaneous limitation by various nutrients in TMFs (Dalling et al., 2016) and a possible limitation by K, Ca and Mg because of increased availability of N, P in TMFs (Velescu et al., 2021; Wright, 2019).

7.5. Nutrient resorption across species and successional groups

The average N, P and K resorption efficiencies (37%, 48 %, 46%; Table 6) were lower than the global averages (62%, 65%, 70%) estimated by (Vergutz et al., 2012). The results in this study agree with the general observation that NuRE for nutrients other than N, P and K is more variable (Liu et al., 2014; Vergutz et al., 2012; Yan et al., 2016). The very large and significant inter-specific variation in NRE (4 – 60%), PRE (29-75%) and KRE (9-79%) found in the present study is also consistent with previous studies (Aerts & Chapin, 2000; Killingbeck, 1996; Wood et al., 2011). Since there were no systematic within-species differences in leaf nutrient concentrations and resorption efficiencies of N, P and K among plots at different successional stages and locations (Table S2 in Paper IV), this inter-specific variability was caused by inherent differences among species rather than by variation in environmental conditions. Thus, the first part of hypothesis #7 that resorption efficiency is higher for P and K compared to enzyme-bound N and S was not confirmed.

It has been proposed that NuRE is higher at lower nutrient availability (Chen et al., 2021; Li et al., 2016), supported by the observation that NuRE decreases with increasing leaf nutrient concentrations in a global meta-analysis (Vergutz et al., 2012). However, I found no significant relationships between the NuRE and leaf concentrations of N, P or K across all species (Figure S3 in Paper IV) which is consistent with findings by (Enoki & Kawaguchi, 1999; Kazakou et al., 2007).

Moreover, I observed significant positive within-species relationships between NuRE and leaf concentrations of N, P and K in the two dominant species *M. kilimandscharica* and *S. guineense* (Fig. S2, Table S3 in Paper IV). While the species dominating ES stands (*M. kilimandscharica*) had higher NuRE and leaf nutrient content than the species dominating LS stands (*S. guineense*, Table S2 in Paper IV), this does not reflect a general species difference between the two successional groups as ES and LS species mostly did not differ (Table 5 & Table 2 Paper IV). Thus, the last part of hypothesis #7, that LS species have higher nutrient resorption efficiency than ES species, was not supported.

Therefore, further investigations are warranted to be conclusive as my results contrasts previous studied that found opposing trend in NRE, PRE and KRE in ES compared to LS species (Chen & Chen, 2022; Fahey et al., 1998; Zeng et al., 2017). The large variability in NuRE of macro- and micronutrients ($-90 \leq 85\%$) in this study agree with some previous studies (Liu et al., 2014; Vergutz et al., 2012; Yan et al., 2016) in contradiction to general assumption that NuRE is higher for macro- compared to micronutrients. Thus, the second part of hypothesis #7 is not confirmed.

8. Conclusion and implication

This study demonstrated large variability in both leaf morphology and nutrient contents between tropical tree species. Responses along elevation gradients were highly species specific for leaf morphology, while most leaf nutrients exhibit similar elevation trends with a possibly contribution by soil element contents and soil pH. The between species variation was partly linked to successional groups, but the variability is also large within these groups. In some species, plant leaves acclimated to warmer climate by investing less into individual leaves (smaller, lower LMA). This response is contrary to that reported in most controlled warming experiments reported elsewhere, where plants produce larger leaves with lower LMA when grown in elevated temperature. This may be linked to water economy, with hydraulic constraints on plant water transport capacity acting to decrease leaf size in air with higher temperature and VPD in the field., but not in chamber experiments with small and constantly well-watered plants. Warming induced reduction in leaf size was more common in ES species than in LS species, indicating that ES are better at preventing overheating in a warmer climate. The decline in both LMA and leaf size with warming may help the trees to avoid water and heat stress but likely at the cost of reduced photosynthesis. Though, this is valid at leaf scale, future research should explore if it is also the case at tree scale.

The nutrient concentrations and ratios differed significantly between young vs mature and ES vs LS species depending on the considered nutrient element. Contrary, the NuRE was more variable and very large for nutrients other than N, P and K and significant inter-specific variation were observed in NRE, PRE and KRE with mostly no difference between ES and LS mature tree species. The source of variation in leaf nutrients was mainly on species level.

Contrary to the common suggestion that N limitation is increasing with increasing elevation in TMF, the N/P ratio did not decline with increasing elevation and most species are mostly limited by P than N (P/N ratio < the optimal value) independent of elevation. However, the P/N ratio was slightly decreased in the young trees with decreasing elevation which is in line with the general assumption of P limitation in low elevation forest. Moreover, Cu and Zn is in sub-optimal level and in the future, these may contribute to the nutrient limitation of some species while Mn is in supra-optimal levels and this may in the future become toxic for some species independent of the elevation.

Lower LMA in a warmer climate in some species will likely make their leaves less resistant to external forces such as wind and herbivory. Additionally, the potentially limiting nutrients N, P, Cu and Zn declined on area basis in leaves with reduced LMA down the elevation gradient which may substantially reduce the leaf metabolism, and, thus, plant growth. The variability in leaf morphological and chemical responses to a warmer climate may therefore shift the competitive balance among species and between successional groups, potentially affecting tree community composition and productivity of future tropical montane forests in a warming world. This would lead to a decline in functional diversity (Functional Diversity: large range of traits values for instance) and in functional redundancy (Functional Redundacy: wide set of species with similar functional characteristics) with ultimate less resilient and productive forests.

The larger variability in leaf nutrients with species identity than with site opens a new room for research to explore why the leaf nutrients are mainly affected by species than site. Future research should investigate if different species have different demands for macro and micronutrients because of metabolic differences or functional demands. It should also explore to what extent the environmental variation is caused by climatic rather than soil conditions.

In addition, this research shows the importance of considering a wide range of species, traits and different approaches when studying how leaf morphological and chemical traits respond to warming in TMFs. Future research should also explore why responses to warming are more controlled by species identity than climate and soil in young Afromontane species. This should investigate if these responses depend on the phenotypic plasticity of individual species rather than predefined group of species (e.g., ES vs LS groups; Deciduous/Semi-deciduous vs Evergreen groups) and if yes, identify characteristics of genotype for individual species that similarly respond to warming. Overall, the results show that warming responses of morphological and chemical leaf traits of ATMFs tree species are specific to species and trends are independent of physiological responses to warming. Therefore, it is recommended that when planning for future restoration and afforestation, selecting species with high acclimation capacity would contribute to future sustainable forest management under warming climate.

9. Study limitations

In the elevation gradient study with young planted trees at three sites (Paper I and II), I could not control some confounding effects like, precipitation and soil nutrients. However, during the time this study was conducted all trees were irrigated, except during the dry period in 2019, with little precipitation during approximately one and half month at all sites. Although, all sites were treated equally in terms of water, higher temperature at lower elevations sites resulted in higher vapor pressure deficit (VPD) compared to the high elevation which probably affected their water economy. Later in the experiment, water treatment, liming and other nutrient treatments were applied (starting in November 2019) . Therefore, repeating the same study after treatment were applied could give insights on how the water and soil nutrient factors will affect tropical tree species, and how they may adapt or resist to climate change impacts.

In the mature tree elevation gradient study with mainly naturally regenerated trees of four species grown at five sites (Paper III, and a small part of I), I could not cover many individual species because only 4 species were studied while 18 individual species in the young elevation gradient study and 20 individual species in the study on mature tree species in permanent monitoring plots were studied. In addition, in the mature elevation gradient study, the elevation gradient difference between sites was relatively small (about 1000 m range) and studied individual species were not present at each elevations.

In the study of mature tree species from 15 permanent plots in Nyungwe montane forests established by Nyirambangutse et al. 2017, established plots were not following a systematic elevation gradient and therefore the effect of warming on leaf nutrient resorption, important for the nutrient cycling, could not be addressed.

10. References

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Paper I

forests

MDPI

Article

Warming Responses of Leaf Morphology Are Highly Variable among Tropical Tree Species

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Abstract: Leaf morphological traits vary along climate gradients, but it is currently unclear to what extent this results from acclimation rather than adaptation. Knowing so is important for predicting the functioning of long-lived organisms, such as trees, in a rapidly changing climate. We investigated the leaf morphological warming responses of 18 tropical tree species with early (ES) abd late (LS) successional strategies, planted at three sites along an elevation gradient from 2400 m a.s.l. (15.2 °C mean temperature) to 1300 m a.s.l. (20.6 °C mean temperature) in Rwanda. Leaf size expressed as leaf area (LA) and leaf mass per area (LMA) decreased, while leaf width-to-length ratio (W/L) increased with warming, but only for one third to half of the species. While LA decreased in ES species, but mostly not in LS species, changes in LMA and leaf W/L were common in both successional groups. ES species had lower LMA and higher LA and leaf W/L compared to LS species. Values of LMA and LA of juvenile trees in this study were mostly similar to corresponding data on four mature tree species in another elevation-gradient study in Rwanda, indicating that our results are applicable also to mature forest trees. We conclude that leaf morphological responses to warming differ greatly between both successional groups and individual species, with potential consequences for species competitiveness and community composition in a warmer climate.

Keywords: leaf mass per area; leaf density; leaf thickness; leaf volume per unit area; leaf area; leaf shape; climate change; elevation gradient; tropical montane forest

1. Introduction

Structural, chemical and physiological leaf traits have a decisive influence on the productivity of plants and their ecosystems [1,2]. Leaf morphological traits, such as leaf mass per unit area (LMA), leaf size and shape, influence the interception of light and the exchange of gases and energy between plants and the atmosphere, thereby controlling plant photosynthesis, transpiration and leaf temperature [3–6]. While it is well known that leaf traits are adapted to the environment where the species occur, the possibility to acclimate these traits to altered environmental conditions is crucial to the success with which plants and their ecosystems will respond to climate change [7–9].

LMA is one of the key traits of the leaf economics spectrum [1,10], used to assess the adaptation and acclimation of plants to environmental conditions [11–14]. LMA responds to environmental factors, such as light, temperature, water and nutrient availability [15,16]. It is also associated with functional leaf traits, such as leaf nutrient contents, photosynthesis and leaf respiration [17–20]. In controlled experiments, it is generally observed that LMA decreases with rising temperature, while it increases with drought [21]. Thus, the two most important climate change factors will act on LMA in opposite directions.

LMA responds to environmental factors through changes in both leaf density (LD) and leaf volume per area (LVA), the latter being directly proportional to leaf thickness (LT) in broad-leaved taxa [21–24]. High LVA is associated with a large number of cell layers (palisade and spongy parenchyma) and a large volume of intercellular air spaces, while high LD is a result of leaf tissues with high mass density (i.e., palisade parenchyma, vascular) and low proportion of epidermis, air spaces and overall smaller cells [22]. The observed decrease in LMA under high temperature and water and nutrient availability was mostly attributed to changes in LD rather than in LVA [21], whereas the increase in LMA under drought conditions was mostly due to increased LVA [21,25]. Choong et al. [26] reported that LVA contributes more than LD to variations in the LMA of tropical tree species, regardless of which environmental factor is causing these changes. Moreover, it was reported that a given change in temperature has a larger effect on LMA in tropical species compared to boreal species [21]. However, due to general scarcity of data and large-scale manipulative warming experiments in the tropics, the climate change responses of LMA and its underlying components, LD and LVA, remain highly uncertain for tropical tree species [21,27,28].

Elevation gradients can be used to investigate climate change effects under ecologically realistic conditions [29]. In such studies, LMA has been found to increase with elevation [13]. However, LMA also increases with tree height [30], which in turn declines with increasing elevation [31]. Moreover, while LMA and area-based leaf nitrogen (N) and phosphorous (P) content are positively correlated in global datasets [1,18,32], elevational trends in leaf N and P content are mixed, showing increases or no change in spite of consistent LMA increases [33–37]. These inconsistent global patterns and elevational trends of LMA with other factors complicate causal interpretations. Furthermore, most past studies along elevation gradients have investigated different species at different elevations and thus cannot distinguish between plastic acclimation and inherent differences among species and ecotypes. This could be resolved in semi-experimental elevation-gradient approaches with the same plant material planted at different elevations.

Leaf size and shape influences leaf energy balance, and size correlates with stomatal conductance [8,38], carbon assimilation rates and productivity [3,39]. Small and narrow leaves have thinner leaf boundary layers, and thus, more efficient heat dissipation, leading to lower leaf temperatures and heat stress under sunny and hot conditions [40–43]. Leaf size varies with environmental factors, such as light, water availability and temperature, along latitudinal and altitudinal gradients. Large leaves are found in wet and hot environments towards the equator, while small leaves are common where arid and hot environments are combined, and at high latitudes and elevations [44]. These conflicting influences of temperature and drought on leaf size make it difficult to predict the net effect on leaf size in areas such as the tropics, where both factors are likely to increase in intensity in a changing climate. Generally, observed declines in leaf size with increasing elevation are driven by variation in both precipitation and temperature, but more so with the former [45,46]. In addition, there were significant spatial patterns in leaf shape, i.e., leaves became shorter and rounder towards cooler climates [44,46,47].

Tropical forests, including tropical montane forests (TMF), are characterized by dynamic mixed successional stages, including early (ES), intermediate and late (LS) successional groups [48–50]. The shift in strategies (acquisitive versus conservative) is driven by changes in environmental conditions (e.g., light and water availability) during succession [51]. The resource-acquisition strategy of ES species is characterized by resource acquisition, carbon gain and fast growth, while LS species typically grow slower and use available resources more efficiently under unfavourable conditions (e.g., drought, shade) [52–54]. Previous experiments with tropical trees have indicated that ES species respond more favourably (or less negatively) to warming than LS species. This has been shown for plant growth [55] as well as for photosynthesis [56]. The plasticity of leaf traits, including LMA, in changes in light environment is also thought to be greater in ES compared to LS species [57]. Whether or not leaf morphological traits also show larger acclimation capacity to warming in tropical ES compared to LS species remains unknown. Furthermore, leaf morphology may also change with ontogenetic development, i.e., between juvenile and mature trees. LMA is normally higher in mature compared to juvenile trees [21], while leaf size is more ontogenetically variable [58]. Whether there are differences in leaf morphological responses to warming between young and mature trees remains to be investigated.

The East African region, with high mountains and great lakes, located between the monsoon domain of West Africa and the Indian ocean [59], faces some of the largest interannual rainfall variations in the world [60]. It is projected that precipitation extremes will increase between 2030 and 2052 in Sub-Saharan Africa and that East Africa will experience increases in precipitation in some regions and decreases in others [61]. Warming projections for the region exceed the global projection of 2 °C above pre-industrial levels by the end of the century [61,62]. In the future, these shifts in seasonal variations in rainfall and temperature are likely to affect tree species composition, phenological patterns (i.e., deciduous vs. evergreen) and carbon balance of tropical forests [63].

Afromontane tropical forests are highly productive and store large amounts of carbon compared to tropical montane forests (TMF) worldwide and to central-east Amazonian tropical lowland forests [64–66]. However, they are severely understudied and very little is known on how leaf morphological traits are influenced by climate and how they vary among species and successional strategies. To address this knowledge gap, we explored how leaf morphological traits respond to a warmer climate in a broad range of tropical tree species native to central and east Africa. The main study was conducted on juvenile trees in three multi-species common garden plantations along an elevation gradient. The results from these trees were compared to results from mature trees of the same species growing at different elevations in another elevation gradient. The elevation gradients were used to simulate different global warming scenarios, and the following hypotheses were tested for well-watered trees: (i) LMA decreases at warmer sites, with larger contributions from shifts in LD than from shifts in LVA (leaf thickness); (ii) Leaf size increases at warmer sites; (iii) LMA and leaf size of early successional species are more responsive to warming compared to late successional species; (iv) LMA and leaf size differ between juvenile and mature trees, but the responses to warming are similar.

2. Materials and Methods

2.1. Experimental Sites

This study was conducted during 2018 and 2019 in the TRopical Elevation Experiment in Rwanda (Rwanda TREE; see www.rwandatree.com (9 December 2021)) where 20 tropical tree species native to central and east Africa were grown at three experimental sites located at different elevations to expose the trees to different climates. A step down in the gradient was meant to represent a possible future climate-warming scenario. The sites were: (i) Sigira, high-elevation (HE) site, located in Nyamagabe district at 2°30′54″ S, 29°23′44″ E at the edges of Nyungwe national park in the tropical montane forest (TMF) vegetation zone at 2400 m a.s.l.; (ii) Rubona, mid-elevation (ME) site located in Huye district at 2°28′30.2″ S, 29°46′49.0″ E in the Lake Victoria transitional rainforest (LVTF) vegetation zone at 1600 m a.s.l.; (iii) Makera, low-elevation (LE) site located at Ibanda Makera in Kirehe district at 2°6′31″ S, 30°51′16″ E in the evergreen and semi-evergreen bushland and thicket vegetation zone at 1300 m a.s.l. (Table 1).

Table 1. Site, weather and soil characteristics at Rwanda TREE experimental sites. Weather data are annual mean ± SD for the period 1 February 2018–31 January 2020, except for wind, which is given for 1 February 2019–31 January 2020. Soil data are mean ± SD of three blocks (1 block = 6 subplots). HE, high elevation; ME, mid elevation; LE, low elevation; MAT, mean annual temperature; MAP, mean annual precipitation; T air day and night, mean air temperature in light and darkness ($<$ and >2 µmol m^{-2} s^{-1} PPFD), respectively; VPD day, daytime mean vapour pressure deficit; PPFD day, daytime mean photosynthetic photon flux density; gust wind speed, average of maximum half-hourly wind speeds; T soil, mean soil temperature at 10–20 cm depth; SWC, soil water content at 10–20 cm depth; SBD, soil bulk density; P, phosphorus, N, nitrogen; Org C, organic carbon; All soil parameters from SBD and downwards are average between 0–30 cm depth.

Site:	**Sigira (HE)**	**Rubona (ME)**	**Makera (LE)**
Site characteristics			
Elevation (m a.s.l.)	2400	1600	1300
Latitude	S 2°30′54″	S 2°28′30″	S 2°6′31″
Longitude	E 29°23′44″	E 29°46′49″	E 30°51′16″
Potential Natural Vegetation [1]	Montane forest	Lake Victoria Transitional rain forest	Evergreen and semi-evergreen bushland and thicket
Weather			
MAP (mm $year^{-1}$)	2144 ± 61	1672 ± 136	1106 ± 33
MAT (°C)	15.2 ± 0.1	20.0 ± 0.0	20.6 ± 0.1
T air day (°C)	17.1 ± 0.2	22.4 ± 0.1	24.0 ± 0.3
T air night (°C)	13.3 ± 0.1	17.5 ± 0.1	16.9 ± 0.03
T air 99%ile (°C)	23.1 ± 0.4	28.4 ± 0.4	31.2 ± 0.4
T air 1%ile (°C)	10.9 ± 0.3	13.4 ± 0.2	10.9 ± 0.8
VPD day (kPa)	0.51 ± 0.03	1.03 ± 0.01	1.14 ± 0.03
VPD 99%ile (kPa)	1.5 ± 0.004	2.5 ± 0.11	3.0 ± 0.16
PPFD day (µmol m^{-2} s^{-1})	611 ± 66	764 ± 62	740 ± 31
PPFD 99%ile (µmol m^{-2} s^{-1})	1683 ± 134	1884 ± 135	1841 ± 104
Wind speed (m s^{-1})	0.6	0.7	0.3
Wind speed gusts (m s^{-1})	1.2	1.2	0.7
Wind speed gusts 99%ile (m s^{-1})	3.5	4.0	3.0
Soil properties			
T soil (°C)	16.7	22.4	22.4
SWC (m^3 m^{-3})	0.29	0.19	0.20
SWC 1%ile (m^3 m^{-3})	0.12	0.09	0.10
SBD (g cm^{-3})	1.0 ± 0.0	1.6 ± 0.1	1.4 ± 0.1
Texture	Clay	Sandy clay	Clay loam
pH (Water)	4.2 ± 0.2	5.0 ± 0.1	6.1 ± 0.1
pH (KCl)	3.4 ± 0.1	3.9 ± 0.0	5.3 ± 0.2
Available P (µg g^{-1})	12.1 ± 3.9	14.1 ± 0.7	8.2 ± 2.5
NH_4^+ (µg g^{-1})	11.3 ± 2.9	10.1 ± 2.3	7.1 ± 1.0
NO_3^- (µg g^{-1})	28.3 ± 1.9	7.2 ± 3.0	24.1 ± 4.6
Tot N (mg g^{-1})	2.73 ± 0.35	2.00 ± 0.39	1.26 ± 0.04
Tot P (mg g^{-1})	0.65 ± 0.02	0.14 ± 0.04	0.27 ± 0.02
Org C (mg g^{-1})	38.6 ± 1.1	27.1 ± 3.1	27.3 ± 2.4

[1] Classification according to Kindt et al. [67].

2.2. Planting Design and Plant Material

At each site, 18 plots of 15 × 15 m, spaced by 2.5 m paths, were established on a 50 × 102.5 m area where all vegetation was cleared before planting. The 18 plots allowed for a full factorial experimental design, with three water levels and two fertility levels and a replication of three plots for each of the six treatment combinations. However, the treatments started after this study was conducted. Within each plot, 20 different tree species with a replication of 5 (i.e., 100 trees per plot) were planted using 1.5 × 1.5 m spacing. The positions of trees and species inside plots were randomized.

In this study, 18 tree species were included, representing early (ES) and late (LS) successional strategies, as well as TMF and LVTF vegetation zone origins as follows: 5 ES/TMF, 5 LS/TMF, 5 ES/LVTF and 3 LS/LVTF species (Table 2). Note that, from literature, the classification of two of the species into distinct successional groups is ambiguous (Table 2), but according to our assessment *Ficus thonningii* Blume and *Markhamia lutea* (Benth.) K.Schum belong mostly to LS and ES groups, respectively, and therefore we will use that classification in this study.

Table 2. Taxonomy for species and their main forest type of origin, classification into successional group as well as their leaf type and shape. FT, forest type of origin; TMF, tropical montane forest (~>2000 m a.s.l.); LVTF, Lake Victoria Transitional Forest (~1500–2000 m a.s.l.); SG, successional group (ES, early LS, late); K, leaf area factor; *, species with mixed successional group features.

Code	**Scientific Name and Author** [1]	**Family Name** [1]	**FT** [2]	**SG**	**Distribution** [3]	**Plant Functional Type** [4]	**Leaf Type & Shape** [5]	**K**
Afa	*Afrocarpus falcatus*, (Thunb.) C.N.Page	Podocarpaceae	TMF	LS	Eastern and Southern Africa	Evergreen	Simple alternate, Oblong linear	0.85
Bbr	*Bridelia brideliifolia*, (Pax) Fedde	Euphorbiaceae/ Phyllanthaceae	TMF	ES	Eastern and Southern Africa	Semi-deciduous	Simple alternate, Elliptic	0.76
Bmi	*Bridelia micrantha*, (Hochst.) Baill	Euphorbiaceae/ Phyllanthaceae	LVTF	ES	Sub-Sahara Africa	Semi-deciduous	Simple alternate, Elliptic	0.75
Cgo	*Chrysophyllum gorungosanum*, Engl	Sapotaceae	LVTF	LS	Pantropical Africa mountains	Evergreen	Simple alternate, Obovate	0.67
Cgr	*Carapa grandiflora*, Sprague	Meliaceae	TMF	LS	Albertine Rift and west Africa mountains	Evergreen	Pinnately Compound, Oblong	0.75
Cme	*Croton megalocarpus*, Hutch.	Euphorbiaceae	LVTF	ES	Pantropical Africa	Semi-deciduous	Simple opposite, Ovate	0.71
Dto	*Dombeya torrida*, (J.F.Gmel.) Bamps	Sterculiaceae/ Malvaceae	LVTF	ES	East Africa mountains	Semi-deciduous	Simple alternate, Elliptic lobate	0.72
Eex	*Entandrophragma excelsum*, (Dawe & Sprague) Sprague	Meliaceae	LVTF	LS	East Africa mountains	Evergreen	Pinnately Compound, Elliptic	0.75
Fsa	*Faurea saligna*, Harv	Proteaceae	TMF	LS	Eastern and Southern Africa	Evergreen	Simple alternate, Oblong acute	0.69
Fth	*Ficus thonningii*, Blume	Moraceae	LVTF	LS *	Pantropical	Semi-deciduous	Simple alternate, Elliptic	0.71
Hma	*Harungana madagascariensis*, Lam. ex Poir	Hypericaceae	LVTF	ES	Pantropical Africa	Semi-deciduous	Simple opposite, Ovate	0.70
Hmo	*Harungana montana*, Spirlet	Hypericaceae	TMF	ES	Albertine Rift	Semi-deciduous	Simple opposite, Ovate	0.70
Mki	*Macaranga kilimandscharica*, Pax	Euphorbiaceae	TMF	ES	East Africa mountains	Semi-deciduous	Simple alternate, Ovate truncate	0.66
Mla	*Maesa lanceolata*, Forssk.	Myrsinaceae/ Primulaceae	TMF	ES	Sub-Sahara Africa and Madagascar	Semi-deciduous	Simple alternate, Ovate	0.67
Mlu	*Markhamia lutea*, (Benth.) K.Schum	Bignoniaceae	LVTF	ES *	Paleotropical	Evergreen	Pinnately Compound	0.71
Paf	*Prunus africana*, (Hook.f.) Kalkman	Rosaceae	TMF	LS	Sub-Sahara Africa and Madagascar	Evergreen	Simple alternate, Oblong	0.73
Pfu	*Polyscias fulva*, (Hiern) Harms	Araliaceae	TMF	ES	Pantropical Africa	Semi-deciduous	Pinnately Compound, Oblong	0.79
Sgu	*Syzygium guineense*, (Willd.) DC.	Myrtaceae	TMF	LS	Sub-Sahara Africa and Madagascar	Evergreen	Simple opposite, Elliptic	0.67

[1] Taxonomy information from The plant list (http://www.theplantlist.org (9 December 2021)) and for family names, both classic and Angiosperm Phylogeny Group (APG III) system are given when applicable; [2] Forest type follows the Potential Natural Vegetation's by Kindt et al. [67]; [3] www.gbif.org (1 December 2021); [4] Semi-deciduous species drop variable amounts of leaf depending the severity of drought, but are rarely completely defoliated; [5] Leaf type and shape classifications follow Ellis et al. [68], see also Figure S3.

The trees were propagated from seeds, cuttings or wildlings, depending on species-specific difficulties of propagation, in poly-pots in a nursery at Rubona research station during 2017. The germplasms were collected from Nyungwe TMF (high-elevation), or

Rubona research station located in the LVTF vegetation zone (mid-elevation), depending on main species distribution range. After six to twelve months in the nursery and having reached a height of ~10–75 cm, depending on species, each plant was randomly assigned to site, plot and plot position (as constrained by the experimental design) and transplanted to the experimental plots within a period of approximately one month at the turn of the year from 2017 to 2018. All tree species received water when needed, irrespective of the planned water treatment, especially during the first dry periods in 2018.

2.3. Environmental Conditions

Weather stations were installed at all three Rwanda TREE sites to record ambient air and soil temperature, relative humidity, precipitation, solar radiation, soil water content, and wind speed and direction at a frequency of 30 min. The recording of most parameters started late January 2018, while wind and soil measurements started late August 2018 (Table 1). Soil temperature and water content sensors were installed in the center of 6 selected plots at each site, while other parameters were measured in an open area next to the plantations. The mean air temperatures (MAT), measured at 1.8 m above ground during the study period at the HE-site (Sigira), ME-site (Rubona) and LE-site (Makera) were 15.2, 20.0 and 20.6 °C, respectively. The daytime and extreme temperatures (expressed as 99%ile) were 17.1/23.1 °C at HE-site, 22.4/28.4 °C at ME-site and 24.0/31.2 °C at LE-site. The main reason for the larger difference in the daytime and extreme temperatures compared to MAT between the ME and LE sites was the larger difference between daytime and nighttime temperatures at the LE site. The sites also differed substantially in mean annual precipitation (MAP), decreasing progressively from HE (Sigira, c. 2100 mm) to ME (Rubona, c. 1700 mm) and LE (Makera c. 1100 mm; Table 1) sites. However, the relative seasonal distribution of precipitation was similar at all sites, with the highest rainfall in March–May and a dry period in June–August. Solar radiation was similar at ME and LE sites while the HE-site received less radiation, probably due to higher cloudiness. Soil temperatures were closely related to the site MAT, although it was probably also affected by radiation and canopy cover. Soil water content (SWC) was similar at LE and ME sites in spite of different MAP. The mean SWC at HE was substantially higher compared to the two other sites, probably because of both higher MAP and higher water-holding capacity due to higher soil clay content (Table 1).

In November 2017, previous to the planting of the trees, soil samples were collected at 0–10 cm and 20–30 cm depth in each plot. Soil bulk density (SBD), NH_4^+, NO_3^- and available P were analysed from composite samples of paired plots (→ 9 samples per site), while the remaining soil parameters were analysed from composite samples of six plots (→ 3 samples per site). All values presented here are averages for 0–30 cm soil depth (Table 1). The main soil texture differences between sites were a larger proportion of sand and less silt at ME site (Rubona, 53–62% and 5–9%, respectively) compared to the soil at HE (Sigira) and LE (Makera) sites (35–45% and 15–27%, respectively) while the clay content was relatively high at all sites (30–50%). The soil pH (water) was 4.2 at the HE-site and increased with approximately one unit for each step down the elevation gradient. Soil fertility expressed as total N and P content tended to decrease with decreasing elevation.

2.4. Leaf Sampling and Morphological Traits Measurements

Leaves or leaflets were collected in two campaigns (August to December 2018 and June to August 2019). For species with compound leaves (Table 2), only leaflets were sampled but for simplicity, we will denote them as leaves. In the first campaign, two randomly selected trees per species and plot were sampled, while one tree per plot and species was generally selected in the second campaign. However, for some species and plots, one additional tree was sampled during the second campaign, but sampling was always balanced between sites. Only plot averages were used for statistical analysis of site effects. In each campaign, one or more (2–3 from species with small leaves) mature and sun-exposed leaves from the upper half of the tree crown were sampled from each selected tree, resulting in: 1 sample * 2 trees * 18 plots * 18 species * 3 sites = 1944 leaves in 2018 and 1 sample * 1 tree * 18 plots * 18 species * 3 sites + 190 extra samples = 1162 leaves in 2019, giving a total number of 3106 leaf samples.

Leaf length (LL), leaf width (LW) and leaf thickness (LT, only in 2019 campaign) were measured directly after leaf collection, using a ruler and an electronic calliper (resolution 0.01 mm). Three to five leaf discs of known diameter (18 mm or 10 mm, depending on the size of the leaf) per leaf and tree were sampled using punchers. First- and second-order veins were avoided for LT measurements, as well as first-order veins for sampling of discs. For species with narrow leaves (*Afrocarpus falcatus* (Thunb.) C.N.Page and *Faurea saligna* Harv.) discs were not taken; instead, a photo was taken of the whole leaf next to a ruler for subsequent leaf-area determination. Both discs and photographed leaves and the remaining leaf material were brought in separate envelopes to the laboratory for later determination of LMA and nutrient contents, respectively.

2.5. Leaf Shape and Size Estimation

Leaf shape was determined as LW to LL ratio (leaf W/L ratio) and leaf size was expressed as leaf area (LA) and estimated from LW and LL using an allometric function:

$$\mathrm{LA} = \mathrm{LW} \times \mathrm{LL} \times \mathrm{K} \quad (1)$$

where K is the leaf-area factor, also known as the Montgomery parameter [69]. Species-specific K-values were determined for the 18 species based on 20 leaves of varied sizes collected from each species at the ME site (Rubona) in June 2020. Collected leaves were scanned in a flatbed scanner (CanonScan LiDE300, Canon Inc., Tokyo, Japan) and thereafter the LA, LL and LW were analysed using ImageJ software 1.50i (Rasband, W.S., U. S. National Institutes of Health, Bethesda, MD, USA). Petioles and petiolules were excluded from the measurements. The K-values were obtained by regression analysis of LA versus LW*LL, setting the intercept to zero. Independent of fixed or variable intercept, the R^2 values obtained were 0.97–0.99, except for the two species with the largest and lowest LW to LL ratio, where the R^2 values were 0.93 (*Dombeya torrida* (J.F.Gmel.) Bamps) and 0.89 (*A. falcatus*), respectively. The K-values among species varied between 0.66 and 0.85 (Table 2) and were used to estimate the LA of all sampled leaves.

2.6. Determination of LMA, LD and LVA

Each collected leaf disc or photographed leaf sample was oven-dried at 70 °C for at least 48 h and then weighed using a laboratory balance with 0.1 mg resolution. The area, width and length of the photographed leaves were determined using ImageJ software 1.50i. Total projected areas of discs or photographed leaves were used to calculate LMA [70]. Leaf area (LA) and leaf thickness (LT) were used to calculate leaf volume per area (LVA), while leaf mass (LM), LA and LT were used to calculate leaf density (LD) as follows:

$$\mathrm{LMA} = \frac{\mathrm{LM}}{\mathrm{LA}} \quad (2)$$

$$\mathrm{LVA} = \frac{\mathrm{LA \cdot LT}}{\mathrm{LA}} \quad (3)$$

$$\mathrm{LD} = \frac{\mathrm{LM}}{\mathrm{LA \cdot LT}} \quad (4)$$

The following units are used throughout the paper: Leaf size (LA), cm^{-2}, LMA, $g\ m^{-2}$, LVA, $cm^{-3}\ m^{-2}$, LD $g\ cm^{-3}$).

2.7. Leaf Nutrients Analysis

Composite samples of leaves (from six plots into one sample per species and site, constituting one block) were oven-dried at 70 °C for at least 48 h, and thereafter ground into a fine powder with a ball mill grinder (MM 301, MM 200, Retsch, Germany). Mass-based leaf nitrogen (N_M) content was analysed using an elemental analyser (EA 1108, Fison Instruments, Rodano, Italy). Ground samples were sent for analysis of 37 non-N elements using inductively coupled plasma mass spectrometry after digestion in HNO_3 and then aqua regia (Method VG101, Bureau Veritas Mineral laboratories, Vancouver, BC, Canada). Out of these non-N elements, only data for mass-based leaf phosphorus (P_M) are reported. The mass-based leaf nutrients were converted to area-based contents (N_A and P_A) by multiplying with LMA.

2.8. Comparison of Juvenile Planted Trees with Mature and Natural Regenerated Trees

The leaf size and LMA of two ES species (*Macaranga Kilimandscharica* Pax and *Polyscias fulva* (Hiern) Harms) and two LS species (*Carapa grandiflora* Sprague and *Syzygium guineense* (Willd.) DC) in this study were compared to values obtained from two other studies on mature trees in south-west Rwanda: (i) an elevation gradient with five sites ranging 1700–2700 m a.s.l. [71] and (ii) 11 permanent monitoring plots in Nyungwe TMF [66].

In the mature-tree elevation-gradient study, leaves from trees with an average diameter at breast height (DBH) of 22–32 cm for all species and a total range from 8 to 82 cm were collected in three campaigns: (i) February–March 2017; (ii) September 2017 and (iii) January–February 2018. The leaves were collected at five sites along the elevation gradient with Nyungwe Bigugu Mountain (~2700 m) being the highest and coolest site, followed by Nyungwe East (~2500 m), Nyungwe West (~1950 m), Cyamudongo (1800–1900 m) and the Ruhande Arboretum (1700 m). The three highest sites are located in Nyungwe National Park (TMF) while the Cyamudongo site is located in the adjacent Cyamudongo forest (TMF/LVTF) and Ruhande Arboretum (LTVF), close to Huye town. In total, 1080 leaf samples were collected: 3 leaves * 6 trees * 4 species * 5 sites * 3 campaigns. For detailed information, see [71].

For the Nyungwe TMF permanent plots, leaves were collected from trees of all species having an average DBH of 31–51 cm for all species and a total range of 7 to 96 cm during September–December 2013 and February–April 2015. The leaves were collected from 11 out of 15 permanent plots along a 32 km east–west transect at an elevation ranging between 1950 and 2500 m a.s.l established during late 2011 and early 2012. In total, 445 leaf samples were collected: 5 leaves * 89 trees. For detailed information about the plots, see [66].

All leaf samples were collected from mature and sun-exposed leaves from the upper canopy, following the same protocol for determination of leaf size and LMA as in the Rwanda TREE project.

2.9. Statistics

The site and species effects on variables measured both in 2018 and 2019 (leaf W/L, LA and LMA) were analysed using a mixed linear model, using site and species as fixed between-subject factors and year as within-subject factor (repeated measure). The site and species effects on variables measured in only one year (LVA, LD, N_M, N_A, P_M and P_A) were analysed using a general linear model (GLM), using site and species as fixed factors. In both types of analysis, tree height was used as a covariate when it was significant. Since tree height changed with time (Table S3) we used a repeated covariance and applied a first-order autoregressive structure with heterogeneous variance in the mixed model analysis. For significant interactions of year by species and site by species, a one-way ANOVA was used to analyse the site effect on different species individually, with a Bonferroni test for post hoc comparison of individual sites. For species where the response variable was significantly correlated to tree height, a regression analysis with tree height as a covariate was used. The site effect was analysed by using plot means (18 per site) as replicates for morphological parameters, while block means (3 per site, i.e., 6 plots per block) for each species were used as replicates for nutrient parameters. To test for the differences between successional groups at the HE (control) site, a one-way ANOVA (repeated measure if both years was included) was used. Site means of each species were used as replicates within the early (10 species) and late (8 species) successional groups. The effect of tree age (juvenile trees in this study compared to mature trees from other studies) on LA and LMA was tested for a common elevation of 2000 m a.s.l., using a GLM with elevation as covariate and tree age as a fixed factor. The significance of the relationship between LMA or LA versus elevation was tested using regression analysis. A test of normality (Shapiro–Wilk) was performed to test the distribution of data, and Levene's tested for homogeneity of variance. Homogeneity of variance was obtained for analysis of individual species, but not always when all species were analysed together. Effects were considered statistically significant at $p < 0.05$ if homogeneity of variance was obtained and at $p < 0.01$ for main effects when homogeneity was not obtained. All statistical tests were made using the SPSS 27.0 software package (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Leaf Trait Interrelationships and Variation between Species

Leaf size, leaf W/L ratio, LMA, LVA, LD, N_M, N_A, P_M and P_A varied greatly across species, as shown for the HE (Sigira) site (Figure 1a–f, Table S1). The difference between the two years was small and only significant for a relatively small difference in LMA within the ES group (11% lower in 2018 compared to 2019). The successional strategy of the species contributed largely to the inter-specific variation, as a significant difference between ES and LS species was observed for most variables except LVA, N_A and P_A content. In general, ES species had significantly higher leaf W/L ratio, LA, N_M and P_M, and lower LMA and LD compared to the LS species. *D. torrida* was often in the extreme position within the ES group while *A. falcatus* and *F. saligna* (depending on variable) shared the extreme position within the LS group. The species within a successional group that overlapped with species within the other group were more variable, but *F. thonningii*, classified as LS species, often had ES features that sometimes even exceeded the range of the ES species (Figure 1a,c,e,f).

The relationships between LMA and LA and other leaf traits were characterized by plotting LMA versus leaf W/L ratio, LA, N_M, N_A, P_M and P_A (Figure 2a–f) and LA versus N_M (Figure S1). The relationship followed the same pattern at all sites, but here we only present the details from the HE (Sigira) site. LMA decreased significantly with increasing leaf W/L ratio ($p < 0.001$), LA ($p < 0.001$), N_M ($p < 0.001$) and P_M ($p < 0.05$), while it increased with increasing N_A ($p < 0.05$) and P_A ($p < 0.005$). These patterns were consistent both within and across successional groups (Figure 2a–f). Leaf size increased significantly with increasing N_M across ES species ($p < 0.001$), while no correlation was observed for LS species (Figure S1).

The degree to which LVA and LD contributed to the variation in LMA was investigated by plotting both the slopes and R^2 values of these relationships against each other (Figure 3, Figure S2 and Table S2). Based on these plots, three groups of species were identified where LMA was mostly affected by (i) LVA (4 species), (ii) LD (9 species) or (iii) equally by both LVA and LD (5 species). Species from both successional strategies and both forest types of origin were represented in all three identified groups, suggesting that neither successional strategy nor forest type are main factors for explaining the different influences of LVA and LD on LMA.

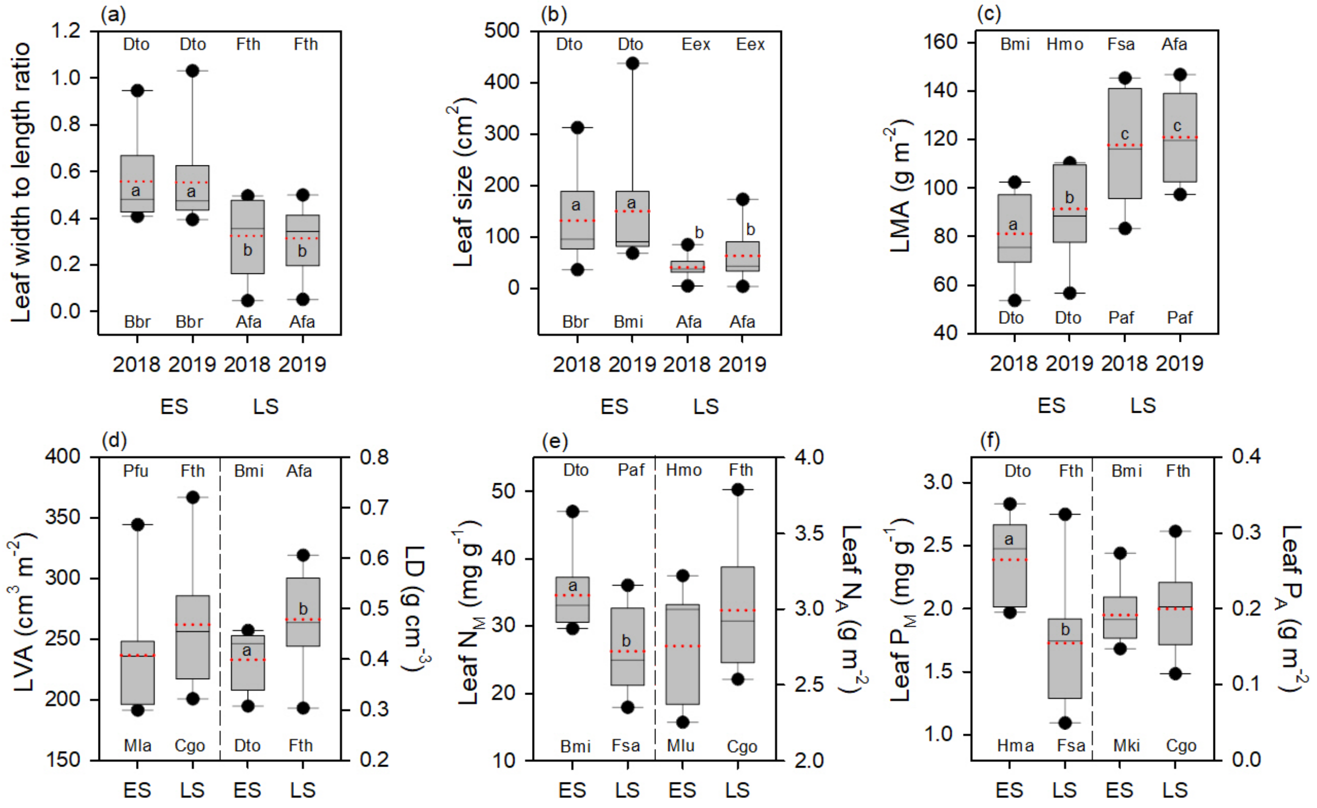

Figure 1. (**a**–**f**) Leaf characteristics of 18 species at high-elevation site (Sigira). (**a**) Leaf shape (i.e., leaf width to leaf length ratio: W/L), (**b**) leaf size, (**c**) leaf mass per unit area (LMA), (**d**) leaf volume per area (LVA), leaf density (LD), (**e**) leaf nitrogen (N) per unit mass (N_M) and area (N_A), and (**f**) leaf phosphorous (P) per unit mass (P_M) and area (P_A). Leaf shape, size and LMA (**a**–**c**) were measured in both 2018 and 2019. Leaf N and P were measured in 2018 and LVA and LD in 2019 only (**d**–**f**). The boxplot boundary indicates the 25th and 75th percentile, the error bars indicate the 95th and 5th percentiles, the black solid line and the red dotted lines inside the boxes indicate median and mean, respectively. The black dots show the highest and lowest species means, for which species codes (see Table 2) are indicated in the lower and upper part of the figure. Different letters (a–c) inside boxes indicate significant differences ($p < 0.05$) of the mean between early (ES) and late (LS) successional species and years when relevant.

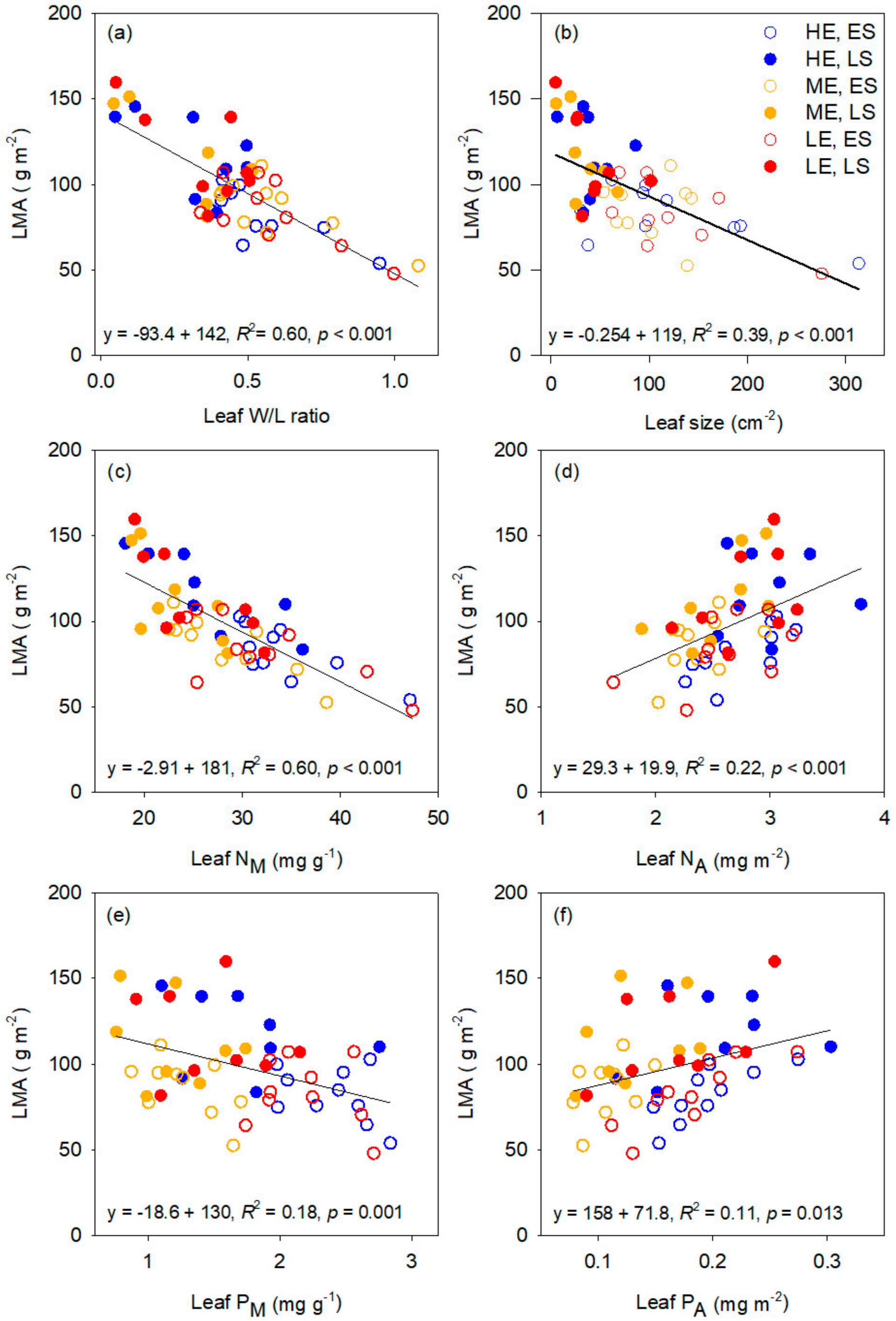

Figure 2. (**a**–**f**) Leaf mass per unit area (LMA, *y* axis) in relation to (**a**) leaf width-to-length ratio (leaf W/L ratio); (**b**) leaf size; (**c**) leaf nitrogen per unit mass (Leaf N_M); (**d**) leaf nitrogen per unit area (Leaf N_A); (**e**) leaf phosphorus per unit mass (Leaf P_M); (**f**) leaf phosphorus per unit area (Leaf P_A). Each data point represents species mean at each site. HE, High-elevation site (Sigira); ME, Medium elevation site (Rubona); LE, low elevation site (Makera); ES, early successional trees; LS, late successional trees. Lines, equation and R^2 represent the regression line for all sites and successional groups. *p*-value refers to the regression line.

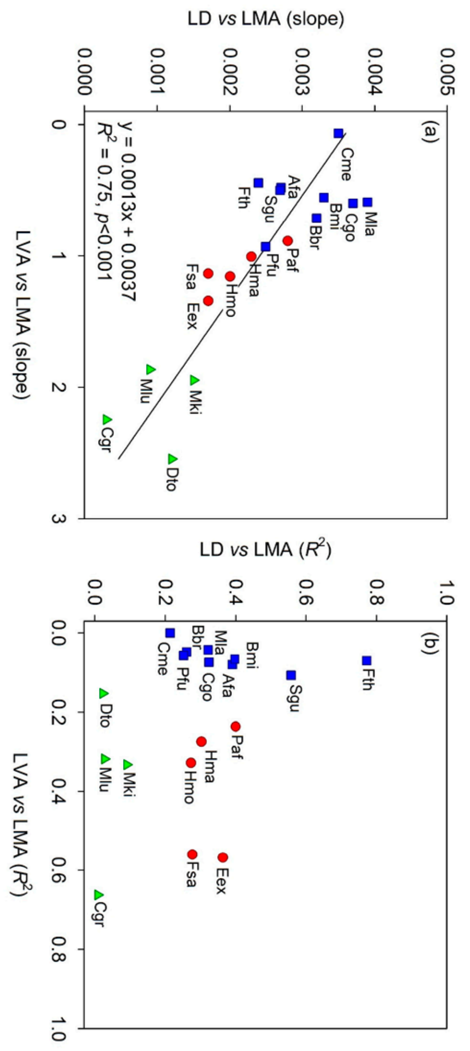

Figure 3. Slope (**a**) and R^2 (**b**) for leaf density (LD) versus leaf mass per unit area (LMA) in relation to slope and R^2 for leaf volume per unit area (LVA) versus LMA for 18 species (for species codes, see Table 2). Blue squares, LMA mainly influenced by LD; green triangles, LMA mainly influenced by LVA; red circles, LMA influenced by both LD and LVA. *p*-value refers to the regression line.

3.2. Leaf Shape, LMA and Leaf Size Responses to Sites and Warming

To separate from possible confounding influences of non-temperature site differences, we defined warming effects as a significant change in the same direction (↗ increasing or ↘ declining effect) at both ME and LE sites compared to HE, or a significant change at LE compared to HE sites but no difference between ME and HE (see Table 3). For species with a decline or increase at ME but not at LE (→ no systematic temperature effect) compared to HE, the change was not attributed to warming.

A significant site effect on leaf shape was observed (Table 4), with leaf W/L ratio being on average 5.4% and 7.5% higher at the warmer ME and LE sites, respectively, compared to the HE site. However, the site effect varied between species as well as between years (Table 4), and therefore the site effect of each species and year was analysed (Table 3, Figure 4a,b). Positive warming effects were observed for four species each year, but only *Chrysophyllum gorungosanum* Engl and *S. guineense* were similarly affected in both years. The only negative warming response of leaf W/L ratio was observed for *Prunus africana* (Hook.f.) Kalkman in 2018. No main site effects were observed for leaf size, LMA or LVA, but LD overall declined at warmer sites (Table 4). However, significant site * species, year * species and/or year * site * species * tree height interactions indicated variable site responses among species and years for LA, LMA, LD and leaf W/L ratio. Therefore, species-specific tests for individual years were conducted (Table 3), with tree height (TH) included as covariate for species where LA and LMA significantly correlated to tree height (Table 3 and Table S3). In 2018 and 2019, significant site effects on LA were observed for 13 and 10 species, respectively, of which seven species declined with warming in each year (Table 3, Figure 4c,d). Declines were more common in ES compared to LS species; five and seven out of the seven species with declines were ES species in 2018 and 2019, respectively. For LMA, significant site effects were observed for 11 and 12 species in 2018 and 2019, respectively, of which six and nine, respectively, declined with increasing warming (Table 3, Figure 4e,f). Contrary to the warming effects on LA, the effects on LMA were relatively evenly mixed between ES and LS species. A significant increase by warming was only observed for LMA of *Harungana montana* Spirlet in 2018 (Table 3). Although no significant main site effects were observed for LA and LMA, the across species average decreased with 23 and 35% for LA and 4.4 and 17.5% for LMA at the ME and LE sites, respectively, when compared to the HE site.

Table 3. Significance of warming effect on leaf shape (width-to-length ratio, W/L) leaf size (leaf area) and leaf mass per unit area (LMA) in 2018 and 2019, and on leaf volume per unit area (LVA) and leaf density (LD) in 2019. Different letters (a, b, c) denote significant differences between sites. H, M and L represent high- (Sigira), mid- (Rubona) and low- (Makera) elevation sites, respectively. Explanation of species codes is given in Table 2; SG, successional group (ES, early; LS, late); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest); C, covariate used in statistical analysis when significant; h, tree height. The arrows indicate the direction of warming effect of species with significant site effect ($p < 0.05$).

			Leaf L/W Ratio												Leaf Size												LMA												LD					
			2018						2019						2018						2019						2018						2019						2019					
Spec	**SG**	**FT**	**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**		**C**	**P**	**H**	**M**	**L**	
Bbr	ES	TMF	h	**0.013**	ab	a	b	→		0.101					h	**<0.001**	a	b	a	→		**0.028**	a	a	b	↘		**0.003**	a	b	a	→		0.468						0.845				
Hmo	ES	TMF	h	**<0.001**	a	b	ab	→		0.369						**0.001**	a	b	a	→	h	**0.016**	ab	a	b	↘		**<0.001**	a	b	b	↗		**0.006**	a	a	b	↘		0.528				
Mki	ES	TMF		**0.013**	a	ab	b	↗		0.124					h	**<0.001**	a	b	b	↘		**<0.001**	a	b	b	↘		**<0.001**	a	a	b	↘		**<0.001**	a	a	b	↘		0.162				
Mla	ES	TMF		0.077						**0.047**	a	b	ab	→		**<0.001**	a	b	b	↘		0.127					h	**<0.001**	a	b	ab	→		0.067						**<0.001**	a	b	b	↘
Pfu	ES	TMF		0.785						**0.003**	a	b	b	↗	h	**<0.001**	a	b	b	↘		**0.001**	a	b	b	↘		**<0.001**	a	a	b	↘		**<0.001**	a	a	b	↘	h	0.065				
Bmi	ES	LVTF		0.143						**0.023**	a	ab	b	↗	h	0.107						**0.009**	a	a	b	↘	h	0.137						0.606						0.060				
Cme	ES	LVTF		0.522						0.366					h	**0.036**	a	ab	b	↘	h	0.563						0.075						**0.001**	a	a	b	↘	h	**0.003**	a	a	b	↘
Dto	ES	LVTF	h	**<0.001**	a	b	ab	→		0.164					h	**<0.001**	a	b	b	↘		**<0.001**	a	b	b	↘		**0.011**	a	a	b	↘	h	0.230						**0.008**	a	a	b	↘
Hma	ES	LVTF	h	**0.001**	a	b	ab	→	h	0.920						**<0.001**	a	b	a	→	h	**0.023**	ab	a	b	→	h	0.106					h	0.096						0.797				
Mlu	ES	LVTF	h	0.053						0.109					h	0.896					h	**0.001**	a	a	b	↘	h	**0.002**	a	b	ab	→		**0.009**	a	b	a	→		0.421				
Afa	LS	TMF	h	0.361						0.321						**<0.001**	a	b	b	↘		0.056					h	0.119						**0.001**	a	b	a	→		0.228				
Cgr	LS	TMF	h	0.820						0.218					h	**0.007**	ab	a	b	→	h	0.199						**0.006**	a	b	b	↘	h	**<0.001**	a	a	b	↘		0.782				
Fsa	LS	TMF		**<0.001**	a	a	b	↗		**0.002**	a	b	ab	→	h	**0.024**	a	b	ab	→	h	0.747						0.254					h	**0.012**	a	ab	b	↘		0.485				
Paf	LS	TMF		**0.023**	a	ab	b	↘		0.572					h	0.203					h	0.882					h	0.056						0.480						0.195				
Sgu	LS	TMF	h	<0.001	a	b	c	↗	h	**<0.001**	a	b	c	↗	h	**<0.001**	a	b	b	↘		**0.039**	ab	a	b	→	h	**0.008**	a	b	ab	→	h	**0.012**	a	b	ab	→	h	0.813				
Cgo	LS	LVTF		**0.024**	a	b	b	↗		**0.017**	a	ab	b	↗	h	0.434					h	0.451					h	**0.038**	*			↘	h	**<0.001**	a	a	b	↘		**<0.001**	a	a	b	↘
Eex	LS	LVTF		0.788						0.313						**<0.001**	a	b	a	→	h	0.948					h	**<0.001**	a	b	b	↘		**<0.001**	a	b	c	↘		**<0.001**	a	a	b	↘
Fth	LS	LVTF		0.547						0.063					h	0.093					h	**<0.001**	a	b	ab	→		0.735					h	**0.023**	ab	a	b	↘	h	**0.027**	ab	a	b	↘

↘ Decrease with warming (down at both M and L or no/small effect at M and down at L compared to H elevation sites). ↗ Increase with warming (up at both M and L or no/small effect at M and up at L compared to H elevation sites). → No warming effect (up at one site and down at one site or down/up at M but no effect at L compared to H elevation sites). * a statistically significant site effect was obtained in the ANOVA analysis, but not in the Bonferoni post-hoc test.

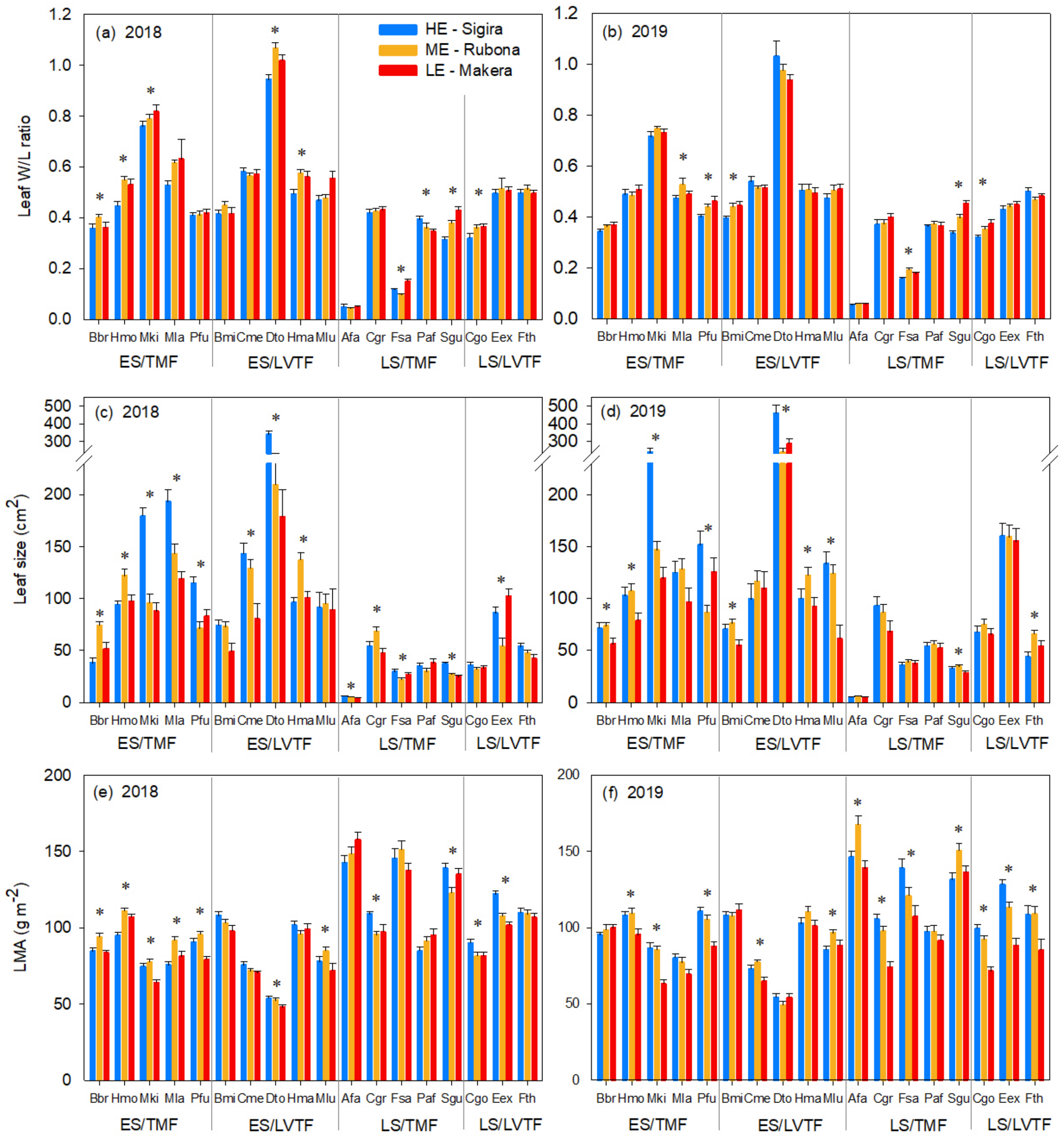

Figure 4. (**a**–**f**) Site (i.e., warming) effect on (**a**,**b**) leaf width-to-length ratio (W/L), (**c**,**d**) leaf size and (**e**,**f**) leaf mass per area unit (LMA) in 18 species (see Table 2 for full names) measured at three sites of different elevation (HE, ME and LE, high, mid and low elevations, respectively) in (**a**,**c**,**e**) 2018 and (**b**,**d**,**f**) 2019. The species are divided into four groups, depending on successional stage and forest type of origin. ES and LS, early and late successional, TMF, tropical montane forest and LVTF, Lake Victoria transitional forest. * indicate statistical differences between sites within species ($p < 0.05$); see Table 3 for detailed statistical results. Note the broken axis for leaf size.

The soil content of N and P (Table 1), as well as both mass- and area-based leaf N and P content, differed significantly between sites (Table 4). Linear regressions were used to test if these site differences of leaf N and P content could explain the site and 'warming' effect on LMA and leaf size (Figures S4 and S5). The only significant effect observed was a positive relationship between the LMA effect size and P_A effect size when the LE site (Makera) was compared to the HE site (Sigira). Since the P_A effect size was more negative at the ME site compared to LE site, while the opposite was true for LMA, it is not likely that variable leaf P_A could explain the warming effect.

Table 4. *p*-values for effects of year, site and species, and for leaf width-to-length ratio (W/L), leaf size, leaf mass per area unit (LMA) and effects of site, species for leaf volume per unit area (LVA), leaf density (LD) and leaf nitrogen per unit mass (N_M) and area (N_A), and leaf phosphorus per unit mass (P_M) and area (P_A). Tree height was used as covariate when significant (Table S3). df represents degree of freedom.

		***p*-Values for Two Years Data**			***p*-Values for One Year Data**					
Source	**df**	**Leaf W/L**	**Leaf Size**	**LMA**	**LVA**	**LD**	**N_M**	**P_M**	**N_A**	**P_A**
Tree height (covariate)	1	ns	**<0.001**	**<0.001**	**0.022**	ns	ns	ns	ns	ns
Year (repeated)	1	**<0.001**	**<0.001**	**0.039**						
Site	2	**<0.001**	0.596	0.249	0.116	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Species	17	**<0.001**	**0.007**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Year * Site	2	0.108	0.114	**0.007**						
Year * Species	17	**<0.001**	**<0.001**	**<0.001**						
Year * Tree height	1		**<0.001**	**0.011**						
Site * Species	34	**<0.001**	**0.033**	**<0.001**	0.217	**0.035**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Site * Tree height	2		0.815	0.717	0.203					
Species * Tree height	17		**<0.001**	**0.028**	**0.016**					
Year * Site * Species	34	**<0.001**	0.242	**<0.001**						
Year * Site * Tree height	2		0.517	0.313						
Year * Site * Tree height	17		**<0.001**	**<0.001**						
Site * Species * Tree height	34		**<0.001**	0.196	0.702					
Year * Site * Species * Tree height	34		**0.043**	**0.000**						

3.3. *Effect of Warming on LVA and LD, and Their Contribution to the Effect on LMA*

We observed significant main effects of both species and sites as well as an interaction between species and sites for LD (Table 4). The species-specific analysis showed a decline in LD with warming in six species (Table 3, Figure 5b). Most (four species) of these six species were included in the group where LMA was primarily affected by LD (Figure 3, Table S2). For LVA, a significant main effect was only found for species (Table 4, Figure 5a).

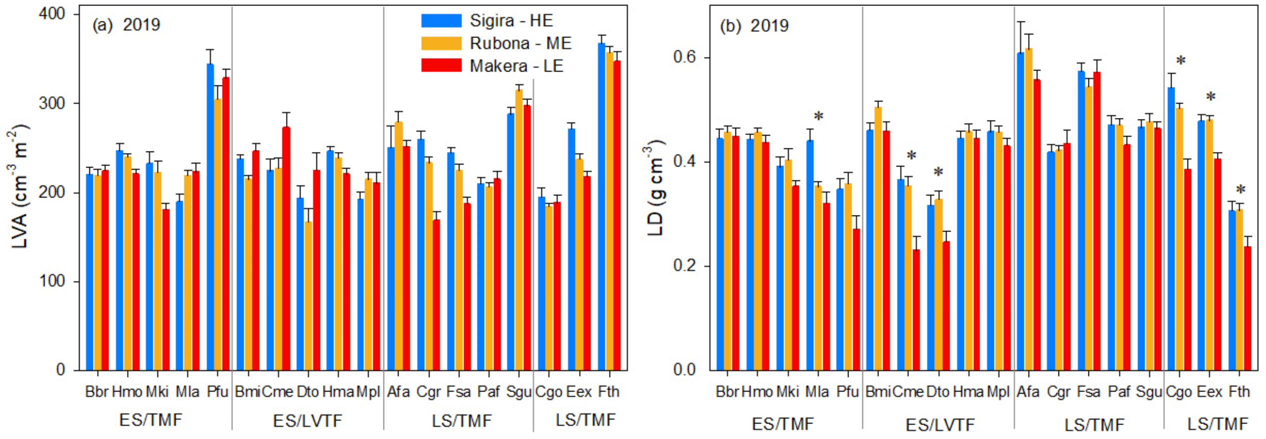

Figure 5. (**a**,**b**) Site (i.e., warming) effects on (**a**) leaf volume per area unit (LVA) and (**b**) leaf density (LD) in 18 species (see Table 2 for full names) measured at three sites of different elevation (HE, ME and LE, high, mid and low elevations, respectively) in 2019. The species are divided into four groups, depending on successional stage and forest type of origin. ES and LS, early and late successional, TMF, tropical montane forest and LVTF, Lake Victoria transitional forest. * indicates statistical differences between sites within species ($p < 0.05$); see Table 4 for detailed statistical results. Note that there was no significant species x site interaction for LVA, and therefore the site effect for individual species was not analysed.

To test if LD and/or LVA contributed significantly to the site effects on LMA, regression analyses between sites effects on LMA versus site effects on LVA and LD, respectively, were conducted (Figure 6). These analyses showed a significant positive relationship between site effects on LMA and LVA, when both the ME and the LE sites were compared to the HE site, while no significant effects were observed for LD. These results partly contradict the results from analysis of the main site effect using ANOVA (Table 4). A likely explanation is that the site effect on LD is strong but only for a few species, while the site driven effect on LVA is more subtle and has a more general influence on the site effects on LMA. The significant effect of tree height on LVA may also have contributed to conceal the site effect.

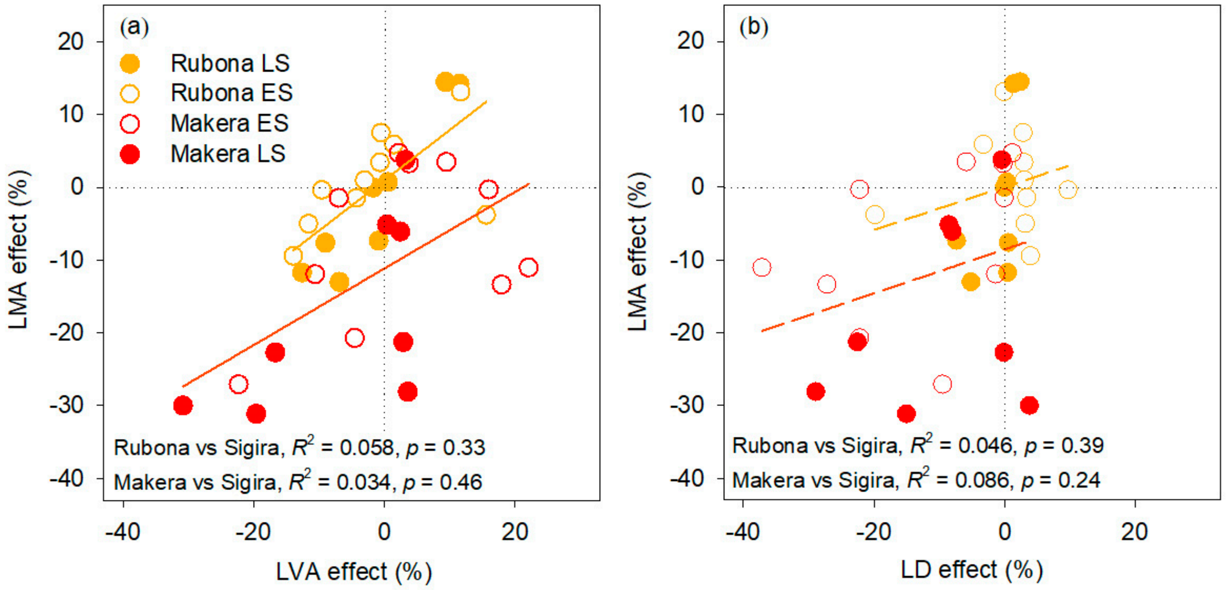

Figure 6. (**a**,**b**) The effect of (**a**) leaf volume per unit area (LVA) and (**b**) leaf density on leaf mass per unit area (LMA) between the mid- (Rubona) and low- (Makera) elevation versus high-elevation sites (Sigira). Each symbol represents one species, yellow, Rubona; red, Makera. Open and closed symbols, early (ES) and late (LS) successional trees, respectively. R^2 and p-values are given for the regression line of all species at a site.

3.4. Leaf Size and LMA in Juvenile and Mature Trees at Different Elevations

We compared the average leaf size and LMA from 2018 and 2019 of the juvenile trees in present study with those from mature trees from other studies of four species (two ES and two LS) growing at different elevations (1700 to 2700 m a.s.l) (Figure 7a–h, Table 5). When standardised to an elevation of 2000 m a.s.l., a significant main age (juvenile vs. mature) effect on leaf size or LMA was observed in *M. kilimandscharica,* but not in the other three species (Table 5). The elevation effect was also analysed using regression analysis, separating age classes for *M. kilimandscharica,* but not for the other species (Figure 7). Similarly to the analysis of the juvenile trees alone (Table 3), there was a significant decline in LMA, with declining elevation for *C. grandiflora*, *M. kilimandscharica* (mature data only) and *P. fulva,* but not for *S. guineense* (Figure 7e–h). However, the LMA-to-elevation relationship for *P. fulva* was only significant when the observation at 2700 m a.s.l. (Figure 7g, Table 5) was excluded. The reason to exclude the highest elevation is that the site was on the edge of the elevation range for that species. For leaf size, there was no significant elevation effect except for mature leaves of *M. kilimandscharica,* which declined slightly in size with increasing elevation (Figure 6b, Table 5).

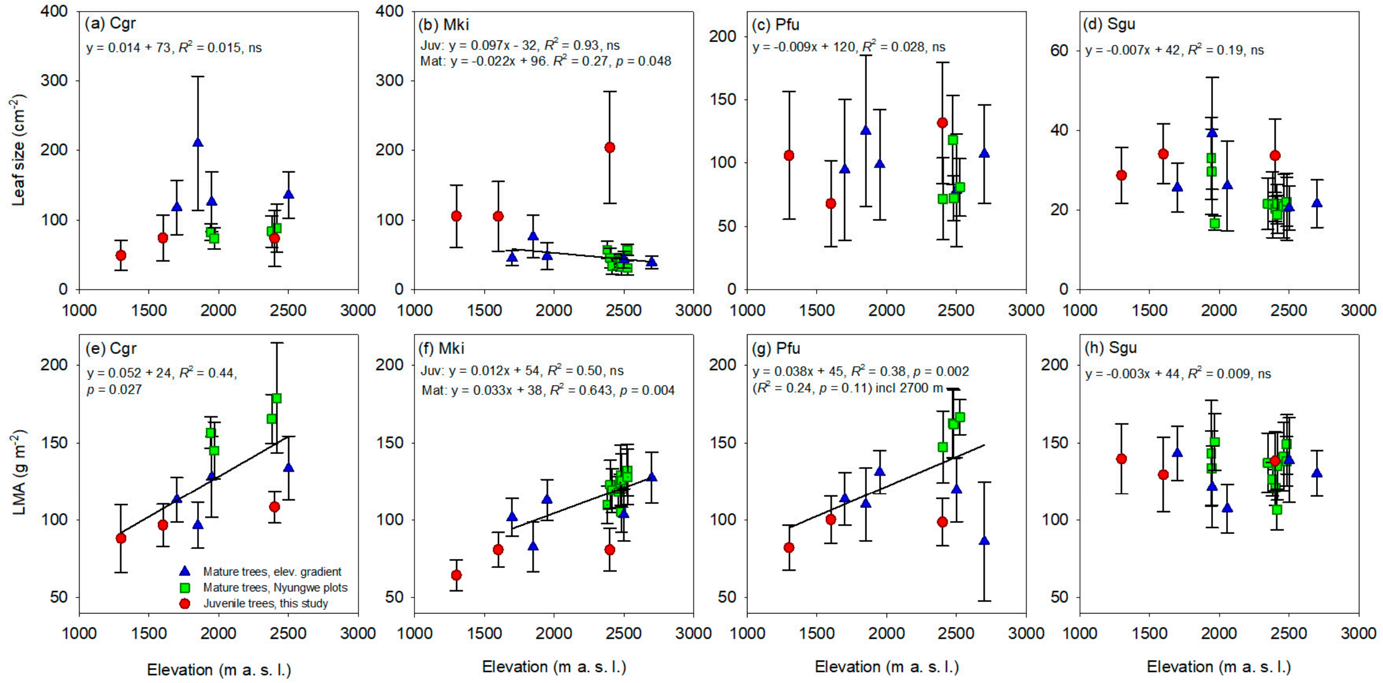

Figure 7. (**a**–**h**) Leaf size and leaf mass per area unit (LMA) at different elevations for Cgr, Carapa grandiflora (**a**,**e**); Mki, Macaranga kilimandscharica (**b**,**f**); Pfu, Polyscias fulva (**c**,**g**); Sgu, Syzygium guineense (**d**,**h**) in three independent studies in Rwanda. Each data point shows mean ± SD at each site included in the studies. Blue triangle, mature-tree elevation-gradient study; green square, permanent monitoring plots in Nyungwe forest with mature trees; red circle, elevation-gradient study with juvenile trees (Rwanda TREE, this study); lines, equation and R^2 represents the regression line for all tree studies. p-value refers to the slope coefficient.

Table 5. p-values for effect of tree age (juvenile and mature trees) on leaf size and leaf mass per area unit (LMA) of four species along elevation gradients using elevation as a covariate and standardised to 2000 m a.s.l (common for all gradients). The elevation effect was analysed by regression analysis, and for significant age * elevation interaction, a separate regression analysis was conducted for each tree age class (Figure 7a–h). For full name of species, see Table 2.

	***p*-Values for Species**			
Variable/Source	**Cgr**	**Mki**	**Pfu**	**Sgu**
Leaf size				
Age	0.20	**<0.001**	0.41	0.12
Elevation	0.72	**<0.001**	0.64	0.54
Age * Elevation	0.53	**<0.001**	0.14	**0.019**
LMA				
Age	0.070	**0.005**	0.21	0.83
Elevation	0.075	**0.013**	0.47	0.82
Age * Elevation	0.31	0.20	0.86	0.87

4. Discussion

We report results from an experimental elevation-gradient study where leaf morphological traits were collected from 18 Afrotropical tree species with different successional strategies and originating from different vegetation zones. All responses to a warmer climate were different among species and between early- and late-successional species groups. For species and traits that responded to warming, we generally observed decreased leaf size and LMA and increased leaf width-to-length ratio at warmer sites. Below, we discuss sources of variation in leaf morphology in tropical trees, including inter-trait relationships, acclimation to warming, and effects of species identity, successional strategy and ontogeny.

4.1. Characterisation of Leaf Traits in Different Species and Successional Groups

The LMA range (~50–150 g m^{-2}) observed across species in our study is comparable to those reported in studies of trees in tropical rain and deciduous forests reviewed by Poorter et al. [21] and in a study along an elevation gradient in the Neotropics [37]. The variation in species leaf width-to-length ratio and leaf size varied 20-fold (~0.05 to 1) and 15-fold (30–440 cm^2), respectively. The interspecific variation in LMA as a function of leaf width-to-length ratio, leaf size and leaf N and P per unit mass also broadly agreed with observation from other studies [1,72]. Furthermore, the ranges of LD and LVA in our study (see Figure S2) are comparable to those found in previous studies of evergreen and deciduous species [21]. Similarly to other studies [21–24], the contributions of LD and LVA to within-species LMA variation differed greatly among species; in some species, LD contributed more than LVA, in other species LVA contributed more than LD, and in a third group of species, LD and LVA equally contributed to the LMA variation (Figure 3). Our observed differences between successional groups are in agreement with previous studies, where ES species showed more acquisitive traits (higher leaf W/L ratio, LA, NM and PM, and lower LMA and LD) compared to LS species [51–54]. Overall, the range of leaf-morphology characteristics across the selected species in this study is typical for humid tropical evergreen and deciduous trees, although extreme values are lacking.

4.2. LMA Responses to Warming

Regardless of successional group, LMA declined with warming (i.e., increased with elevation; Table 3, Figure 4) for some species in our study, which confirms the first part of hypothesis #1. This is consistent with observations from both field studies and controlled experiments in the tropics as well as in several other biomes [1,13,15,21,24,36,73]. Within a given species, LMA usually correlates with photosynthesis, since thicker leaves with more palisade parenchyma tissue tend to have both higher LMA and photosynthetic capacity [21,74,75]. Indeed, the lower LMA at warm sites agrees with the decline in photosynthetic capacity observed in LS species in the present elevation gradient [76].

The results on the contribution by LD and LVA to warming-induced shifts in LMA are less consistent with previous research. We hypothesised that LD contributed more than LVA to shifts in LMA at higher temperatures, based on the global meta-analysis [21]. Indeed, there was a species-dependent effect of site on LD (declines in six species) but not on LVA (Tables 3 and 4). However, when the site effects on LVA and LD were compared with site effects on LMA using regression analysis, we found a significant positive correlation for LVA only (Figure 6). This suggests that shifts in both LVA and LD contributed to warming-induced changes in LMA, but that the contribution by shifts in LVA is consistent across species, while the contribution by LD is strong, but only for a few species. Therefore, the second part of hypothesis #1, that mostly LD affected the warming response of LMA, was not confirmed.

LVA shifts were found to be important for LMA changes in a study on 42 tropical tree species from different habitats in south-east Asia, regardless of environmental factors causing these changes [26], as well as in an elevation-gradient study on six tropical tree species in south China [73]. The conflicting results on LVA and LD contributions to warming-induced changes in LMA in these studies on tropical trees (including our study) compared to global datasets [21] may reflect mixed effects of warming and other factors, especially effects of VPD along elevation gradients. The effect of high VPD on leaf size (i.e., leaf size declines) by reducing the total number of epidermal cells per leaf and per leaf area [6] likely altered the relationships between LMA, LD and LVA at our warmer sites. Furthermore, de la Riva et al. [77] concluded that both LD and LVA varied in a study of 34 Mediterranean woody species along a water-availability gradient. Several studies have also attributed both LD and LVA to LMA variation as a result of several environmental factors, including warming, cooling, drought, aridity, light intensity and nutrients availability, which complicate the causal interpretation of LD and LVA contributions to LMA variation in response to warming for our three groups of species identified in Figure 3 [17,21,25,37,78,79].

4.3. Leaf Size and Leaf Width to Length Response to Warming

Leaf size significantly decreased with warming in several species, but not in all (Tables 3 and 5). This result was opposite to hypothesis #2. It is in contrast to a study on trees in a temperate rainforest along an elevation gradient in New Zealand [45] as well as an investigation of herbarium specimens from eight tree species in China sampled at different latitudes (~20 to 40° N) and prevailing temperatures [46]. The conflicting results on leaf size may be a result of interacting effects of temperature and water limitations on leaf size [44]. Although the plants in this study were watered at all sites, the VPD was higher at lower elevations (Table 1). High VPD causes declines in xylem and leaf water potential, production of abscisic acid and stomatal closure responses, even if soil water is abundant [80]. Our result is probably explained by hydraulic constraints on water transport and leaf growth [23,46,81]. This is also supported by Li et al. [46], who concluded that precipitation was more important than temperature for the effect on leaf size. Smaller leaf size in a warmer climate also implies thinner leaf boundary layer and more efficient heat dissipation, mitigating heat stress under sunny and hot conditions [40–43]. However, with the rather small changes in leaf size observed here, this effect was likely of less importance than that of hydraulic constraints.

The study by Li et al. [46] also observed that temperature was more important for leaf W/L ratio, and contrary to our observation, reported a decrease with increasing temperature. This suggests, together with the results from our 18 tree species, that the warming response of leaf W/L ratio is species-specific.

4.4. Responses to Warming in Species of Different Successional Groups

We found that leaf size typically declined with warming in ES species, but not in LS species, while the effects on both leaf W/L and LMA were fairly evenly distributed between ES and LS species (Table 3). Hypothesis #3 was therefore only confirmed for leaf size and not for LMA and leaf W/L ratio. However, there is much information supporting the theory that ES trees have higher plasticity in response to warming compared to LS trees [55,56]. The opposite effect (i.e., lower plasticity in ES compared to LS trees) has been found for changes in light conditions [82]. Whether the plasticity of leaf morphology in response to warming is higher in ES compared to LS species remains to be more extensively investigated.

4.5. LMA and Leaf Size in Juvenile and Mature Trees at Different Elevations

Mature and juvenile trees exhibited similar relationships between elevation (and thus temperature) and leaf size or LMA in three out of four species (Figure 7), thus mostly supporting hypothesis #4. When combining juvenile and mature tree data, LMA decreased with declining elevation, on average over the four species by 24 g m^{-2} per 1000 m. This is of similar magnitude to the decrease of 17 g m^{-2} per 1000 m observed for the community-weighted LMA of sun leaves down an Andes–Amazon elevation gradient [13]. This is a surprisingly low difference in elevation response when accounting for the variability in the LMA responses among the species in our study, and the differences in species compositions between the study sites.

When standardised at the same elevation (2000 m a.s.l.), no age effect was found on LMA and leaf size, except for *M. kilimandscharica*. Our findings do not support the common observation of ontogenic changes in leaf traits in other studies, with mature trees from both temperate and tropical biomes having higher LMA and thicker, larger leaves than juvenile trees [83–85]. Only our observation of lower LMA in juvenile compared to mature *M. kilimandscharica* trees is consistent with most previous research, while the larger leaf size in juvenile compared to mature trees of this species were instead opposite to the common observation. However, in a study of 51 tree species in south-east Asian rain forests, it was shown that juvenile trees could have larger, smaller and similar sized leaves compared to mature trees [58], which is more in line with our results. The larger variability of leaf size compared to LMA in our study, as evidenced by on average higher within-site coefficient of variation (33% compared to 15% for leaf size and LMA, respectively), could contribute to potential inconsistences. However, the overall similarity between the responses from young and mature trees provides support for using more frequently available data from young trees to predict climate change effects on leaf morphology of mature forest trees, but with caution, as the responses are very species-specific.

5. Conclusions

This elevation-gradient study demonstrated that effects of a warmer climate on leaf morphology are highly variable among species, and sometimes also between successional groups. Leaf size decreased at warm sites in ES species, but mostly not in LS species, while changes in LMA (decreases) and leaf W/L ratio (increases) were common in both groups. The similarity in the warming response of leaf morphology in juvenile and mature trees suggests that our results are also applicable to mature forest trees. The reduction in leaf size at warmer sites found in most ES species is contrary to results in most controlled warming experiments. This may be linked to water economy, with hydraulic constraints on plant water-transport capacity acting to decrease leaf size in air with higher temperatures and VPD in the field, but not in chamber experiments with small and constantly well-watered plants. The warming-induced reduction in leaf size in ES species only may imply that these are better than LS species in preventing overheating in a warmer climate. Lower LMA in a warmer climate in some species is likely to make their leaves less resistant to external forces, such as wind and herbivory. The large variability in leaf morphological responses to a warmer climate may therefore shift the competitive balance among species and between successional groups, potentially affecting the tree community composition of tropical montane forests in a warming world.

Supplementary Materials: The following supporting information can be downloaded online at: https://www.mdpi.com/article/10.3390/f13020219/s1, Table S1: Data of leaf morphology at the high-elevation site during 2018 and 2019; Table S2: Regression information for leaf volume per unit area (LVA) and leaf density (LD) versus leaf mass per unit area (LMA); Table S3: Tree height of sampled trees and the relationship of leaf morphology and nitrogen contents versus tree heights; Figure S1: Leaf size in relation to leaf nitrogen per unit mass; Figure S2: Leaf volume per area (LVA) and leaf density (LD) in relation to leaf mass per area unit (LMA); Figure S3: Scanned fresh leaves of the 18 studied species; Figure S4: The effect of leaf nitrogen and phosphorus on leaf size effects between sites; Figure S5: The effect of leaf nitrogen and phosphorus on LMA effect between sites.

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Supplementary figures and tables

Table S1. Mean and standard deviation of leaf shape (i.e. leaf width to leaf length ratio, W/L), leaf size, leaf mass per unit area (LMA), leaf volume per unit area (LVA) and leaf density (LD) of studied species at high elevation site (control/Sigira). Leaf W/L ration, leaf size and LMA were measured during both 2018 and 2019, while LVA and LD only in 2019. Explanation of species codes is given in Table 1; SG, successional group; ES and LS, early and late successional; FT, forest type; TMF, tropical montane forest; LVTF, Lake Victoria transitional forest.

			Leaf W/L ratio		Leaf size (cm^2)		LMA ($g\ m^{-2}$)		LVA ($cm^3\ m^{-2}$)	LD ($g\ cm^{-3}$)
Species	SG	FT	2018	2019	2018	2019	2018	2019	2019	2019
Bbr	ES	TMF	0.37 ± 0.07	0.34 ± 0.03	30 ± 13	72 ± 25	85 ± 13	96 ± 9	221 ± 33	0.44 ± 0.08
Hmo	ES	TMF	0.44 ± 0.07	0.49 ± 0.08	94 ± 20	89 ± 29	95 ± 11	111 ± 13	251 ± 33	0.44 ± 0.04
Mki	ES	TMF	0.76 ± 0.08	0.72 ± 0.08	187 ± 73	228 ± 86	75 ± 10	89 ± 14	239 ± 54	0.39 ± 0.09
Mla	ES	TMF	0.53 ± 0.07	0.47 ± 0.05	193 ± 66	129 ± 43	76 ± 14	81 ± 11	192 ± 38	0.43 ± 0.09
Pfu	ES	TMF	0.41 ± 0.04	0.40 ± 0.04	118 ± 36	152 ± 56	91 ± 13	110 ± 12	345 ± 75	0.34 ± 0.12
Bmi	ES	LVTF	0.41 ± 0.07	0.40 ± 0.04	62 ± 22	70 ± 21	103 ± 10	108 ± 10	237 ± 23	0.46 ± 0.06
Cme	ES	LVTF	0.58 ± 0.06	0.54 ± 0.09	97 ± 35	84 ± 33	76 ± 10	75 ± 13	233 ± 60	0.34 ± 0.09
Dto	ES	LVTF	0.95 ± 0.09	1.03 ± 0.24	314 ± 192	439 ± 204	54 ± 7	57 ± 7	198 ± 57	0.31 ± 0.08
Hma	ES	LVTF	0.47 ± 0.05	0.48 ± 0.07	97 ± 28	92 ± 26	100 ± 10	110 ± 15	247 ± 27	0.45 ± 0.06
Mlu	ES	LVTF	0.48 ± 0.08	0.47 ± 0.07	38 ± 19	82 ± 39	65 ± 9	85 ± 10	196 ± 37	0.45 ± 0.09
Afa	LS	TMF	0.05 ± 0.01	0.05 ± 0.01	6.2 ± 1.3	5.0 ± 1.3	142 ± 17	147 ± 18	250 ± 41	0.61 ± 0.14
Cgr	LS	TMF	0.43 ± 0.08	0.37 ± 0.08	57 ± 27	96 ± 46	109 ± 10	107 ± 10	263 ± 42	0.41 ± 0.06
Fsa	LS	TMF	0.12 ± 0.03	0.16 ± 0.02	33 ± 11	40 ± 12	146 ± 34	140 ± 26	245 ± 34	0.57 ± 0.08
Paf	LS	TMF	0.39 ± 0.05	0.36 ± 0.03	33 ± 14	49 ± 15	84 ± 19	98 ± 16	209 ± 29	0.47 ± 0.08
Sgu	LS	TMF	0.31 ± 0.06	0.33 ± 0.05	38 ± 10	34 ± 9	139 ± 19	137 ± 19	290 ± 35	0.48 ± 0.08
Cgo	LS	LVTF	0.32 ± 0.07	0.32 ± 0.03	40 ± 19	79 ± 29	91 ± 13	101 ± 11	202 ± 49	0.53 ± 0.12
Eex	LS	LVTF	0.50 ± 0.07	0.43 ± 0.05	86 ± 35	174 ± 66	123 ± 11	128 ± 14	276 ± 31	0.47 ± 0.06
Fth	LS	LVTF	0.50 ± 0.06	0.50 ± 0.06	44 ± 14	40 ± 13	110 ± 14	112 ± 18	368 ± 40	0.31 ± 0.05

Table S2. Slope, R^2 and regression p-values for leaf volume per unit area (LVA) and leaf density (LD) versus leaf mass per unit area (LMA), respectively, for each studied species in 2019. Data are grouped and sorted depending on the strength in the relationship of LVA and LD to LMA, respectively. See Table 1 for full name of species code. SG, successional group; ES and LS, early and late successional; FT, forest type; TMF, tropical montane forest; LVTF, Lake Victoria transitional forest; n = tree level replicates.

LMA relation	Species	SG	FT	n	LVA vs LMA			LD vs LMA		
					Slope	R2	P value	Slope	R2	P value
LD	Fth	LS	LVTF	59	0.16	0.07	0.043	318	0.77	**<0.001**
	Sgu	LS	TMF	73	0.22	0.11	0.005	205	0.56	**<0.001**
	Bmi	ES	LVTF	62	0.12	0.07	**0.044**	120	0.40	**<0.001**
	Afa	LS	TMF	48	0.16	0.08	0.052	146	0.39	**<0.001**
	Cgo	LS	LVTF	60	0.12	0.07	**0.036**	88	0.32	**<0.001**
	Mla	ES	TMF	58	0.07	0.04	0.118	83	0.32	**<0.001**
	Bbr	ES	TMF	64	0.07	0.05	0.081	83	0.26	**<0.001**
	Pfu	ES	TMF	75	0.06	0.06	0.039	99	0.25	**<0.001**
	Cme	ES	LVTF	62	0.00	<0.01	0.925	62	0.21	**<0.001**
LVA	Cgr	LS	TMF	66	0.30	0.66	**<0.001**	30	0.01	0.449
	Mki	ES	TMF	72	0.17	0.33	**<0.001**	60	0.09	0.010
	Mlu	ES	LVTF	62	0.17	0.32	**<0.001**	30	0.03	0.193
	Dto	ES	LVTF	63	0.06	0.15	**0.002**	19	0.02	0.235
LD & LVA	Eex	LS	LVTF	73	0.42	0.57	**<0.001**	213	0.36	**<0.001**
	Fsa	LS	TMF	75	0.49	0.56	**<0.001**	164	0.28	**<0.001**
	Paf	LS	TMF	64	0.27	0.24	**<0.001**	143	0.40	**<0.001**
	Hmo	ES	TMF	76	0.28	0.33	**<0.001**	137	0.27	**<0.001**
	Hma	ES	LVTF	75	0.27	0.27	**<0.001**	131	0.30	**<0.001**

Table S3. Tree height of sampled trees and the relationship of leaf size, leaf mass per unit area (LMA), leaf volume per unit area (LVA), Leaf density (LD) and Nitrogen per mass (N_M) versus tree heights (TH) at sampling time. See Table 1 for full name of species code 1.

		Tree height (cm) at sites:				Leaf size *vs* TH		LMA *vs* TH		LVA *vs* TH		LD *vs* TH		N_M *vs* TH	
Year	Species	Sigira	Rubona	Makera	P-value	R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-valu
2018	Bbr	65 ± 10	112 ± 32	226 ± 48	**<0.001**	0.214	**<0.001**	0.007	0.561					0.058	0.534
	Bmi	81 ± 17	96 ± 32	219 ± 39	**<0.001**	0.101	**0.019**	0.169	**0.002**					0.293	0.132
	Cgo	30 ± 11	25 ± 6	21 ± 6	**0.011**	0.314	**<0.001**	0.100	**0.022**					0.169	0.272
	Cgr	54 ± 9	51 ± 10	49 ± 10	0.201	0.154	**0.004**	0.011	0.448					0.177	0.260
	Cme	77 ± 13	95 ± 21	182 ± 21	**<0.001**	0.553	**<0.001**	0.047	0.117					0.316	0.115
	Dto	120 ± 27	101 ± 32	173 ± 27	**<0.001**	0.336	**<0.001**	0.027	0.236					0.413	0.062
	Eex	39 ± 5	36 ± 6	33 ± 7	**0.014**	0.000	0.995	0.150	**0.004**					0.201	0.226
	Fsa	105 ± 14	67 ± 13	76 ± 19	**<0.001**	0.380	**<0.001**	0.018	0.330					0.603	**0.014**
	Fth	89 ± 31	102 ± 42	232 ± 50	**<0.001**	0.516	**<0.001**	0.000	0.926					0.026	0.680
	Hma	97 ± 20	116 ± 25	200 ± 29	**<0.001**	0.002	0.732	0.076	**0.044**					0.222	0.200
	Hmo	95 ± 10	104 ± 20	173 ± 27	**<0.001**	0.001	0.801	0.017	0.354					0.013	0.770
	Mki	94 ± 13	68 ± 27	97 ± 26	**0.001**	0.279	**<0.001**	0.029	0.221					0.006	0.844
	Mla	134 ± 18	107 ± 29	199 ± 32	**<0.001**	0.011	0.447	0.074	**0.047**					0.476	**0.040**
	Mpl	30 ± 7	62 ± 28	196 ± 25	**<0.001**	0.816	**<0.001**	0.660	**<0.001**					0.059	0.528
	Paf	74 ± 15	61 ± 14	135 ± 27	**<0.001**	0.455	**<0.001**	0.235	**<0.001**					0.005	0.851
	Pfa	87 ± 16	70 ± 18	119 ± 19	**<0.001**	0.030	0.235	0.106	**0.022**					0.054	0.547
	Pfu	57 ± 10	40 ± 10	67 ± 20	**<0.001**	0.442	**<0.001**	0.019	0.323					0.475	**0.040**
	Sgu	90 ± 18	76 ± 18	103 ± 18	**<0.001**	0.147	**0.004**	0.265	**<0.001**					0.246	0.174
2019	Bbr	201 ± 31	281 ± 87	349 ± 80	**<0.001**	0.067	0.059	0.002	0.773	0.002	0.776	0.003	0.718		
	Bmi	184 ± 28	243 ± 52	334 ± 73	**<0.001**	0.006	0.565	0.070	0.052	0.022	0.283	0.006	0.563		
	Cgo	69 ± 41	50 ± 27	40 ± 16	**0.016**	0.463	**<0.001**	0.154	**0.003**	0.170	0.002	0.005	0.630		
	Cgr	83 ± 23	77 ± 26	63 ± 32	0.165	0.199	**0.002**	0.149	**0.007**	0.278	**<0.001**	0.075	0.062		
	Cme	135 ± 53	214 ± 50	320 ± 46	**<0.001**	0.193	**0.001**	0.060	0.076	0.052	0.101	0.102	0.020		
	Dto	234 ± 75	229 ± 63	326 ± 63	**<0.001**	0.041	0.152	0.171	**0.002**	0.002	0.765	0.071	0.056		
	Eex	49 ± 11	47 ± 10	48 ± 14	0.835	0.186	**0.001**	0.006	0.577	0.049	0.112	0.016	0.363		
	Fsa	186 ± 26	171 ± 39	111 ± 32	**<0.001**	0.379	**<0.001**	0.140	**0.006**	0.340	**<0.001**	0.032	0.200		
	Fth	165 ± 44	256 ± 82	380 ± 64	**<0.001**	0.222	**<0.001**	0.234	**<0.001**	0.096	0.027	0.113	0.016		
	Hma	202 ± 34	275 ± 39	295 ± 52	**<0.001**	0.086	**0.033**	0.170	**0.002**	0.118	0.012	0.017	0.348		
	Hmo	162 ± 28	245 ± 49	268 ± 63	**<0.001**	0.128	**0.008**	0.066	0.061	0.027	0.231	0.030	0.213		
	Mki	248 ± 30	231 ± 62	254 ± 67	0.436	0.028	0.229	0.001	0.868	0.001	0.822	0.011	0.455		
	Mla	272 ± 45	283 ± 50	292 ± 58	0.550	<0.001	0.932	0.010	0.488	0.002	0.761	0.008	0.534		
	Mpl	91 ± 36	201 ± 94	365 ± 82	**<0.001**	0.390	**<0.001**	0.015	0.389	0.031	0.207	0.018	0.336		
	Paf	122 ± 42	121 ± 60	267 ± 65	**<0.001**	0.221	**<0.001**	0.014	0.387	0.001	0.827	0.009	0.503		
	Pfa	162 ± 23	178 ± 48	246 ± 65	**<0.001**	0.017	0.369	0.034	0.193	0.071	0.100	0.000	0.912		
	Pfu	125 ± 31	112 ± 49	212 ± 60	**<0.001**	0.073	0.055	0.044	0.142	0.028	0.244	0.091	0.032		
	Sgu	169 ± 34	198 ± 62	181 ± 41	0.208	0.005	0.613	0.112	**0.014**	0.009	0.499	0.099	0.022		

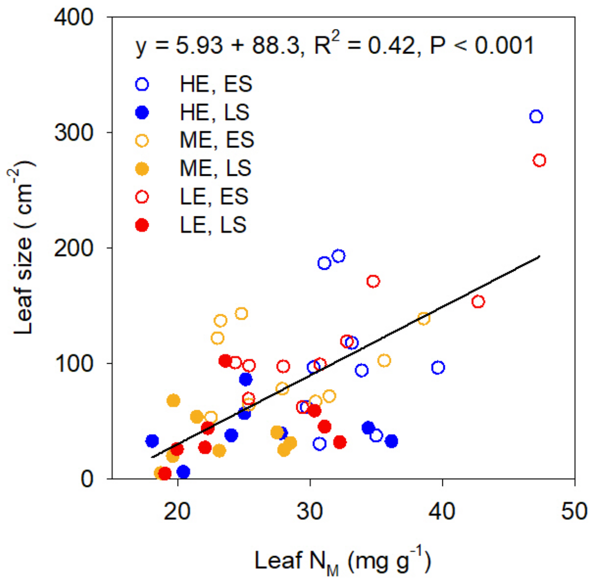

Figure S1: Leaf size in relation to leaf nitrogen per unit mass (Leaf N_M). HE, High elevation site (Sigira); ME, Medium elevation site (Rubona); LE, low elevation site (Makera); ES, early successional trees; LS, late successional trees. Lines, equation and R^2 represents the regression line for all sites and species. P-value refers to the regression line.

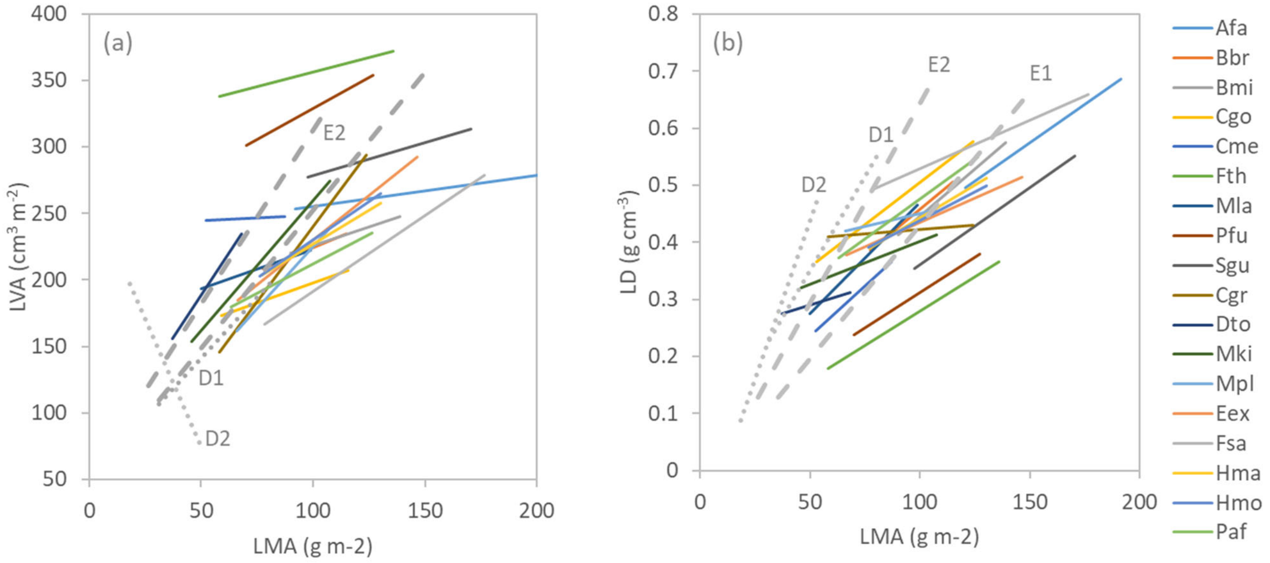

Figure S2ab. (a) Leaf volume per area (LVA) and (b) leaf density (LD) in relation to leaf mass per area unit (LMA). Lower and upper lines extremities indicate the minimum and maximum LVA and LD at 5%ile and 95%ile in a and b, respectively. Grey dotted lines (D1 and D2) and dashed lines (E1 and E2) is reference values of two datasets on deciduous and evergreen species, respectively, from Poorter et al (2009). Abbreviations on legend and corresponding line colors indicate studied species (See Table 2 for full name).

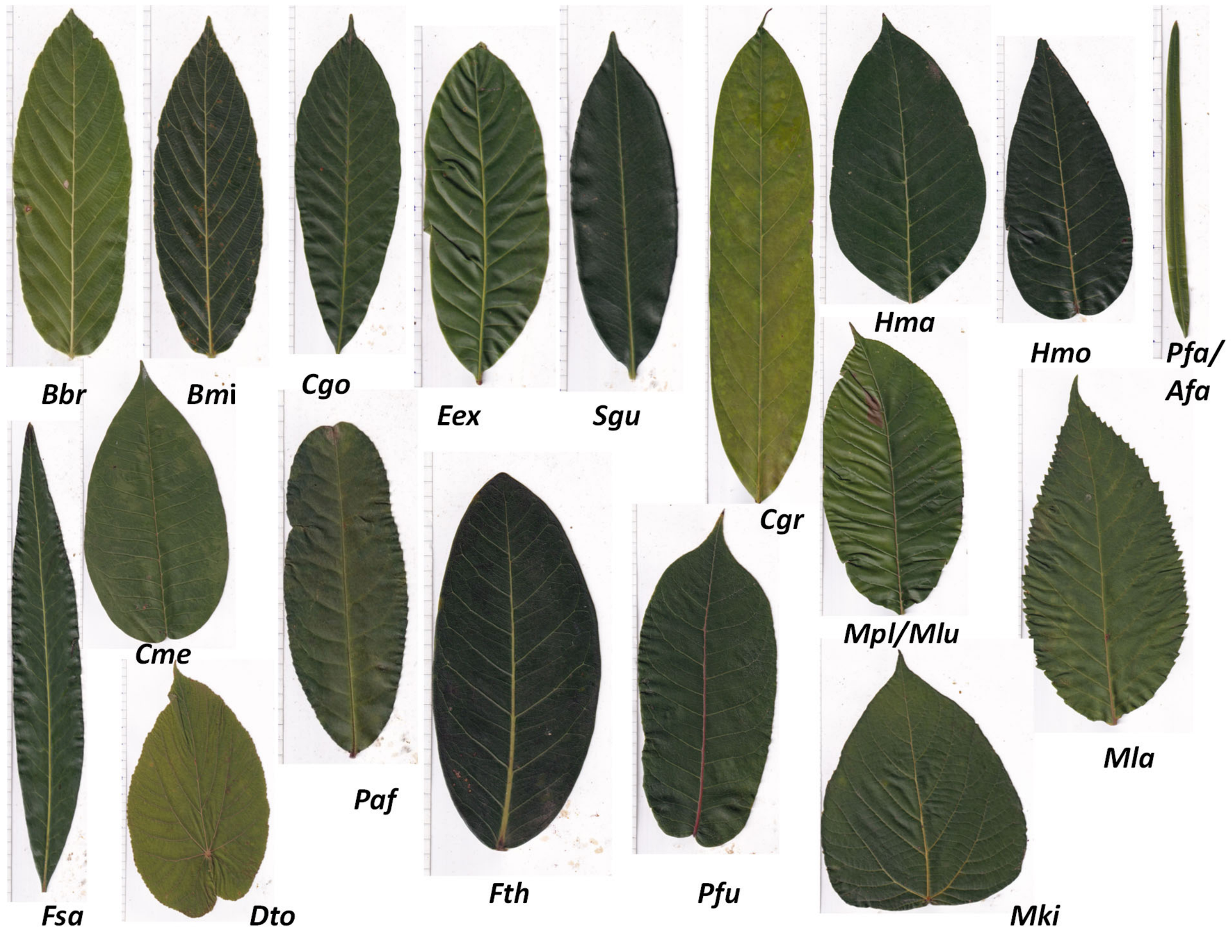

Figure S3: Scanned fresh leaves of the 18 studied species. Code under leaves represent the species name (See Table 2 for full name).

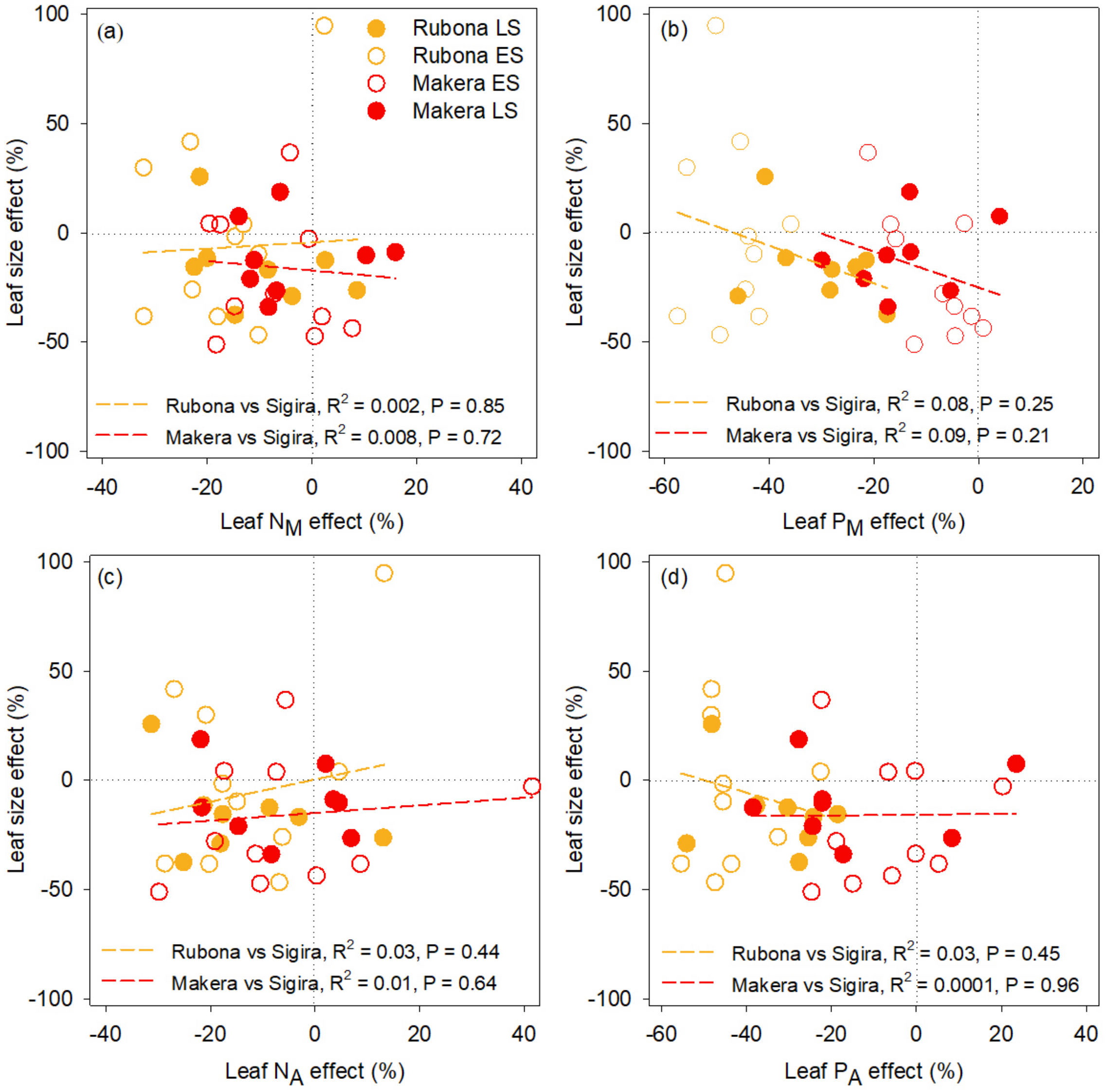

Figure S4 a-d. The site effect of leaf (a, c) nitrogen per unit mass (N_M) and area (N_A) and (b, d) phosphorous per unit mass (P_M) and area (P_A) on leaf size between the mid (Rubona) and low (Makera) elevation versus high elevation site (Sigira). Each symbol represents one species, yellow, Rubona; red, Makera. Open and closed symbols, early (ES) and late (LS) successional trees, respectively. R^2 and P-values are given for the regression line of all species at a site.

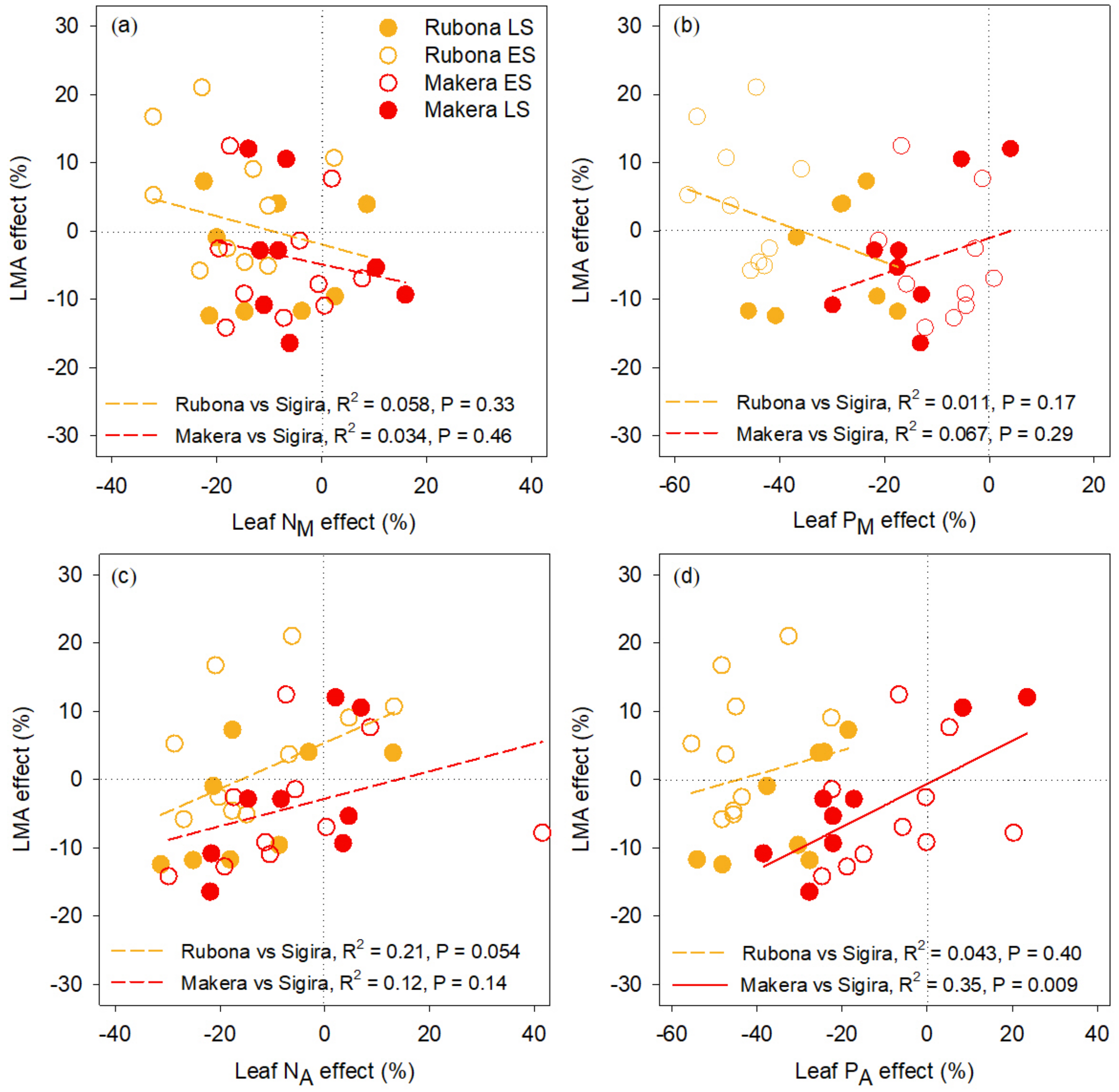

Figure S5 a-d. The site effect of leaf (a, c) nitrogen per unit mass (N_M) and area (N_A) and (b, d)) phosphorous per unit mass (P_M) and area (P_A) on leaf mass per unit area (LMA) between the mid (Rubona) and low (Makera) elevation versus high elevation site (Sigira). Each symbol represents one species, yellow, Rubona; red, Makera. Open and closed symbols, early (ES) and late (LS) successional trees, respectively. R^2 and P-values are given for the regression line of all species at a site.

Paper II

Leaf nutrient content in young Afromontane trees along an elevation gradient is more controlled by species identity than by soil and climate

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Abstract: Leaf nutrient content plays a critical role in plant productivity. While it is influenced by climatic, edaphic, geologic, and phylogenetic factors, it remains unclear to what extent leaf nutrient content is affected by a rapidly changing climate and how this varies among species. We investigated the warming responses of the leaf content of 13 nutrient elements in young trees of 18 Afromontane species planted at three sites along an elevation gradient from 2400 to 1300 m a.s.l (with a mean annual temperature of 15.2 °C to 20.6 °C, respectively) in Rwanda. Early (ES) and late successional (LS) species, from two different elevation origins (1600-2000 and > 2000 m a.s.l.) were included.

At the high elevation site, ES species had significantly higher leaf content of N, P, Mn, Zn and Cu than LS species, while LS species did not have higher contents of any element. The stochiometric ratios of elements to N indicated a strong P limitation in most species, although for some species also K, S, Zn, Cu could potentially be co-limiting. Down the elevation gradient, mass-based leaf contents of Ca, Mg, Fe, B and Mo increased while Zn, and Cu decreased with most species responding similarly. Leaf N and P also decreased downwards, but less consistently. The changes in leaf nutrient content along the elevation gradient are likely caused by a combination of warming and variation in soil nutrient availability with these influences varying among nutrients. However, warming is likely the main cause of the reduction in area-based nutrient content of N, P, Mn, Zn, Cu in species where LMA decreased downwards.

In general, leaf nutrient contents and ratios varied much more strongly between species than between sites, despite large site differences in both soil and climate conditions. This means that biogeochemical cycling of Afromontane forests in a changing climate depends more strongly on shifts in tree community composition than on intraspecific warming-induced shifts in leaf nutrient content.

Key words: Carbon, Nutrient limitation, Elevation gradient, Warming, Tropical montane forest

1. Introduction

Leaf nutrient contents and stoichiometries play a great role in tree growth and forests productivity (Ågren, 2008; Šímová et al., 2019; van der Sande et al., 2018) through their functions in leaf physiological processes (Hänsch & Mendel, 2009; Maathuis, 2009; Santiago, 2015; Uchida, 2000) and partitioning of photosynthate to different organs (Dickson et al., 2000; Marschner et al., 1996). Plants require at least 17 nutrient elements including: i) structural non-mineral nutrients (C, H and O) incorporated from carbon dioxide and oxygen in the atmosphere and from soil water; ii) mineral macro nutrients (N, P, K, Ca, Mg, S) and micro nutrients (Fe, Zn, Mn, Cu, B, Mo, Cl and Ni) from either minerals or organic matter in the soil (Karthika et al., 2018; Mitra, 2017; Uchida, 2000).

Leaf nutrient uptake is regulated by both plant and soil processes which are strongly influenced by species-specific and environmental factors (climatic, edaphic, geologic; Dalling, et al., 2016a; Neugebauer et al., 2018; Poorter, 2009; Salinas et al., 2021; Uchida, 2000; van den Driessche & Rieche, 1974; Wu et al., 2007). Tropical montane forests (TMFs) have particularly high variability in these factors due to their complex topography and sharp elevation gradients in precipitation and temperature. This, in turn, may affect leaf nutrient contents through climatic influences on soil processes, soil-plant interactions and tree community composition (Dalling et al., 2016a). Future increases in temperature and changed precipitation regimes may therefore alter nutrient cycling in TMFs (Dalling et al., 2016a; Dantas de Paula et al., 2021; Gay et al., 2022; Litton et al., 2020).

TMFs are generally characterized by increased amounts of soil organic carbon (C) and total nitrogen (N) with increasing elevation, while N availability declines due to lower C and N mineralisation associated with low temperature and high soil water content at higher elevations (Gay et al., 2022) Consequently, it has long been assumed that N increasingly limits the productivity of TMFs upwards, as also indicated by many observations of increasing ratio of other nutrient elements to N (Austin & Vitousek, 1998; Fisher et al., 2013; Tanner et al., 1990; Tanner et al., 1992; Vitousek, 1984; Wright et al., 2011). However, the elevational trend in leaf nutrient contents and ratios is not always consistent and there is growing evidence that other essential elements, notably P, may play important roles in co-limiting productivity in many TMFs (Allen et al., 2020; Dalling et al., 2016b; Cárate-Tandalla et al., 2018; Mujawamariya et al., 2018; Soethe et al., 2008; Tanner et al., 1998; van de Weg et al., 2009). In addition, at a broad range of sites, both the trend direction and the quantitative change in leaf nutrient content and ratios with elevation and temperature have been observed to vary among species and elements (Crous et al., 2022; van de Weg et al., 2009; John et al., 2007; Litton et al., 2020; Soethe et al., 2008; Tanner et al., 1998; Wu et al., 2019). The large variation in results among studies may be a result of varying effects of warming on nutrient source-sink relationships. Increased temperature is likely to increase both soil nutrient availability (source) and plant demand for growth (sink), but to different degrees for different nutrients, species, and environments (De Mello Prado, 2021). For elements where warming stimulates demand more than supply, the net effect would be reduced leaf nutrient content. This may be more likely for growth-limiting nutrients, while effects could be more variable for nutrients in surplus. When studying the effects of climate change on leaf nutrient contents and how they may limit tree growth, it is therefore imperative to consider all essential nutrients, a broad range of species, and the possible influences of variation in soil factors.

It is well known that leaf nutrient contents differ both between tree species (van den Driessche & Rieche, 1974; Wu et al., 2007) and within tree species depending on the environment (Kitayama & Aiba, 2002; Tanner et al., 1990). One of the most striking differences between species are linked to successional strategy. TMFs are characterised by a dynamic mix of early (ES), intermediate and late successional (LS) species (Carson et al., 2008; Coelho et al., 2016; Fan et al., 2019) as both natural and anthropogenically generated disturbances are common (e.g. Cayuela et al., 2006; Freund & Silman, 2023). The shift in strategies (acquisitive versus conservative) is driven by changes in resource availability (e.g., light, water, nutrients, etc.) during succession (Lohbeck et al., 2015). The resource-acquisition strategy of ES species is characterised by high uptake of resources and fast growth while the conservative strategy of LS species is characterised by typically slow growth and efficient use of available resources under unfavourable conditions (e.g., drought, shade, etc.) (Hernández-Vargas et al., 2019; Prado-Junior et al., 2016; Sterck et al., 2011). In line with this, LS species typically have lower leaf nutrient contents compared to ES species in tropical forest (Poorter et al., 2004; Fonseca et al., 2018). Warming experiments with tropical trees have shown that both growth and photosynthesis of ES species respond more favourably (or less negatively) to warming than LS species (Cheesman & Winter, 2013; Mujawamariya et al., 2023; Ntirugulirwa et al., 2023; Slot & Winter, 2018). Whether leaf nutrient contents of tropical ES and LS species respond differently to warming remains unknown.

Species distributions in tropical forests are influenced by the availability of individual nutrients (e.g., P, K, Ca and Mg) and soil moisture (Condit et al., 2013). A study of various neotropical forests including TMF found that more than one third of the tree species distributions were explained by soil nutrient distributions (John et al., 2007). Positive correlation between leaf and soil nutrients are commonly found (Koukoulakis et al., 2013; Ordoñez et al., 2009), but not always for all nutrients (N, S, K, Fe; (Bai et al., 2019). The variable direction and strength of the correlations are likely due to other factors affecting the availability of the nutrient elements (Augusto et al., 2017; Ipek & Eşitken, 2017). The phyto-availability of nutrients is typically lower in acidic (e.g., P, Mo, Mg, Ca, K), alkaline and calcareous soils (P, Fe, Zn, Mn and Cu) (Neugebauer et al., 2018). Furthermore, inputs of one or two nutrients (usually N and P) results in increased in demand of other nutrients (e.g., K, Ca, Mg) (Ågren et al., 2012; Boardman &

McGuire, 1990; Marklein & Houlton, 2012; Tian et al., 2019) that may then potentially become limiting (Velescu et al., 2021).

Although leaf nutrient contents vary depending on species and soil factors, theoretical and experimental laboratory studies suggest that most plants require nutrients in similar proportions compared to N (Knecht & Göransson, 2004). Although N based ratios may vary considerably for plants grown under natural conditions due to element availability and the possibility of excess uptake (Ågren, 2008; Drechsel & Zech, 1991) leaf nutrient ratios have commonly been used as an indicator to determine the limiting element for plant growth (Aerts & Chapin, 2000; Fisher et al., 2013). Most studies on nutrient limitations in forests use leaf N:P ratios of > 16 and < 14 as indications of P and N limitation, respectively (Koerselman, & Meuleman, 1996). However, these indicators are highly uncertain if other elements than N or P are co-limiting (Yan et al., 2017). This uncertainty is substantially reduced if a full set of N based nutrient ratios is used (Linder, 1995).

African TMFs were reported to be highly productive and with high C stocks compared to other TMFs worldwide (Cuni-Sanchez et al., 2021; Nyirambangutse et al., 2017; Spracklen & Righelato, 2014) but warming responses of traits, including nutrients, controlling these forests are less studied. Manipulative warming experiments are therefore urgently needed in tropical forests to accurately predict how tropical tree species will respond to warming (Cavaleri et al., 2015), especially how nutrient cycling is affected in TMFs.

In this study we investigate how an almost full set of essential leaf nutrients of 18 tropical tree species (Table 1) grown at three sites within the Rwanda TRopical Elevation Experiment (Rwanda TREE) respond to warming, and if responses differ between successional groups. We hypothesize that:

H#1. Leaf nutrient content is influenced by soil nutrient content and the soil pH mediated nutrient availability.

H#2. Different leaf nutrients respond differently to a warmer climate (after accounting for soil nutrient content and pH), with decreases in the potentially more growth limiting nutrients N and P and increases in the ratios of most other nutrients to N.

H#3. ES species have higher mass-based leaf nutrient content compared to LS species, while the area-based leaf nutrient contents are similar due to higher LMA in LS species.

H#4. With strongly contrasting leaf nutrient contents of species with different growth strategies, species identity explains a higher fraction of the variation in leaf nutrient content than site, despite large variation in soil conditions and climate.

2. Materials and methods

Experimental setup and sites

This study was conducted in the TRopical Elevation Experiment in Rwanda (Rwanda TREE; see www.rwandatree.com). Mixed plantations with 20 tropical tree species native to different elevations of upland forests in East and Central Africa were established at three sites along an elevation gradient from 2400 to 1300 m a.s.l, with 170 km distance between highest and lowest sites (Table 2). The mean annual temperature (MAT) increased by 5.4 °C down the elevation gradient. The high elevation (HE; 2400 m a.s.l.) site is located at Sigira in Nyamagabe district at 2°30'54" S, 29°23'44" E near Nyungwe national park in the Tropical montane rainforest (TMF) zone of the Albertine rift ecoregion. The mid-elevation site (ME; 1595 m a.s.l.) is located at Rubona site in Huye district at 2°28'30.2" S; 29°46'49.0" E in the Lake Victoria transitional rainforest (LVTF) vegetation zone. The low elevation (LE; 1300 m a.s.l.) site is located at Ibanda Makera in Kirehe district at 2°6'31" S; 30°51'16" E in the Evergreen and semi-evergreen bushland and thicket vegetation zone. The HE site (Sigira) is considered as the control site in this experiment since today's remaining natural forests are predominantly montane and all species except one can be found at >2000 m elevation (Ntirugulirwa et al., 2023). Most of the species used in this experiment naturally grow in the neighbouring NNP, ranging from 2950 down to 1600 m a.s.l. (Fisher and Killman, 2008; Nyirambangutse et al., 2017). With the HE site as control, the ME (Rubona) and LE (Makera) sites represent two different warming scenarios.

Plot design and plant material

At each site, 18 plots of 15 x 15 m, spaced by 2.5 m paths, were established on a 50 x 102.5 m area. Within each plot at each site, 20 different species with a replication of 5 (i.e., 100 trees per plot) were planted using 1.5 x 1.5 m spacing at the turn of 2017 and 2018. The within-plot position of species and individuals were randomized, resulting in mixed multispecies plantations. The experiment was prepared for a full factorial design, with three water levels and two fertility levels at each site, but this study was conducted before the treatments started. During the establishment, all trees received water when needed, to compensate for site differences in precipitation and to avoid drought stress during the dry period in June to August 2018. Before planting at sites, the germplasms were propagated in a nursery close to the ME site during 2017. The present study used 18 species representing early- (ES) and late successional (LS) strategies and originating from LVTF (~1600-2000 m a.s.l.) and TMF (~2000-3000 m a.s.l.) in the following combinations: 5 ES/TMF, 5 LS/TMF, 5ES/LVTF and 3 LS/LVTF species (Table 1, S1).

Table 1. Species characteristics. Forest type (FT) of origin: Tropical montane forest (TMF), > 2000 m a.s.l.; and Lake Victoria transitional forest (LVTF), 1600-2000 m a.s.l., and successional strategy (SG): early successional (ES); late successional (LS). See Table S2 for detailed descriptions of the species.

Species code	Scientific name and author	FT	SG
Bbr	*Bridelia brideliifolia,* (Pax) Fedde	TMF	ES
Hmo	*Harungana montana,* Spirlet	TMF	ES
Mki	*Macaranga kilimandscharica,* Pax	TMF	ES
Mla	*Maesa lanceolate,* (Henriq.) F. White	TMF	ES
Pfu	*Polyscias fulva,* (Hiern) Harms	TMF	ES
Cgr	*Carapa grandiflora,* Sprague	TMF	LS
Paf	*Prunus Africana,* (Hook.f.) Kalkman	TMF	LS
Fsa	*Faurea saligna, Harv*	TMF	LS
Afa	*Afrocarpus falcatus+,* (Thunb.) C.N.Page	TMF	LS
Sgu	*Syzygium guineense,* (Willd.) DC.	TMF	LS
Bmi	*Bridelia micrantha,* (Hochst.) Baill	LVTF	ES
Cme	*Croton megalocarpus,* Hutch.	LVTF	ES
Dto	*Dombeya torrida,* (J.F.Gmel.) Bamps	LVTF	ES
Hma	*Harungana madagascariensis,* Lam. ex Poir	LVTF	ES
Mlu	*Markhamia lutea+,* (Benth.) K.Schum	LVTF	ES
Cgo	*Chrysophyllum gorungosanum,* Engl	LVTF	LS
Eex	*Entandrophragma exselsum,* (Dawe & Sprague) Sprague	LVTF	LS
Fth	*Ficus thonninghii,* Blume	LVTF	LS

Weather and soil conditions

In January 2018, weather stations were installed at all sites to record ambient air temperature, precipitation, wind speed and direction, solar radiation, and relative humidity. Soil sensors were also installed to record the soil temperature and water content. The MAT, mean daytime temperature and extreme temperatures (expressed as 99%ile) were 14.6/17.1/23.1 °C at HE-site, 20.0/22.4/28.4 °C at ME-site, and 20.6/24.0/31.2 °C at LE-site (Table 2). The sites differed substantially in mean annual precipitation (MAP), decreasing progressively from HE (Sigira, c. 2100 mm) to ME (Rubona, c. 1700 mm) and LE (Makera, c. 1100 mm) sites. However, the relative seasonal distribution of precipitation was similar at all sites, with highest rainfall in March–May and a dry period of about two months occurring in June to August. Solar radiation was similar at ME and LE sites while the HE-site received somewhat less radiation, probably due to higher cloudiness. Soil temperatures were closely related to the site MAT, although they were probably also affected by radiation and canopy cover. Soil water content (SWC) was similar at LE and ME sites despite different MAP, but substantially higher at HE compared to the two other sites. The higher SWC at HE is probably because of both higher MAP and higher water holding capacity due to higher soil clay content (Table 2).

Table 2. Site, weather and soil water and temperature at Rwanda TREE from 2018 to 2020. Weather data are annual mean ± SD for the period 1 Feb 2018 – 31 Jan 2020 except for wind, which is given for 1 Feb 2019 – 31 Jan 2020. HE, high elevation; ME, mid elevation; LE, low elevation; PNV, potential natural vegetation; MAT, mean annual temperature; MAP, mean annual precipitation; T air day and night, mean air temperature in light and darkness, respectively; VPD day, daytime mean vapour pressure deficit; PPFD day, daytime mean photosynthetic photon flux density; gust wind speed, average of maximum half-hourly wind speeds; T soil, mean soil temperature at 10-20 cm depth; SWC, soil water content at 10-20 cm depth.

	Site		
	HE - Sigira	ME - Rubona	LE - Makera
Site characteristics			
Elevation (m a.s.l.)	2400	1600	1300
Latitude	S 2° 30′ 54′′	S 2° 28′ 30′′	S 2° 6′ 31′′
Longitude	E 29° 23′ 44′′	E 29° 46′ 49′′	E 30° 51′ 16′′
Potential natural vegetation	Tropical montane rainforest	Lake Victoria transitional rain forest	Evergreen & semi-evergreen bushland and thicket
Climate			
MAP (mm yr^{-1})	2144 ± 61	1672 ± 136	1106 ± 33
MAT (°C)	15.2 ± 0.1	20.0 ± 0.0	20.6 ± 0.1
T air day mean (°C)	17.1 ± 0.2	22.4 ± 0.1	24.0 ± 0.3
T air night mean (°C)	13.3 ± 0.1	17.5 ± 0.1	16.9 ± 0.03
T air 99%ile (°C)	23.1 ± 0.4	28.4 ± 0.4	31.2 ± 0.4
T air 1%ile (°C)	10.9 ± 0.3	13.4 ± 0.2	10.9 ± 0.8
VPD day mean (kPa)	0.51 ± 0.03	1.03 ± 0.01	1.14 ± 0.03
VPD 99%ile (kPa)	1.45 ± 0.004	2.47 ± 0.11	2.98 ± 0.16
PPFD day mean (µmol m^{-2} s^{-1})	611 ± 66	764 ± 62	740 ± 31
PPFD 99%ile (µmol m^{-2} s^{-1})	1683 ± 134	1884 ± 135	1841 ± 104
Wind speed (m s^{-1})	0.6	0.7	0.3
Wind speed gusts (m s^{-1})	1.2	1.2	0.7
Wind speed gusts 99%ile (m s^{-1})	3.5	4.0	3.0
Soil T and water			
T soil (°C)	16.7	22.4	22.4
SWC (m^3 m^{-3})	0.29	0.19	0.20
SWC 99%ile (m^3 m^{-3})	0.45	0.32	0.35
SWC 1%ile (m^3 m^{-3})	0.12	0.09	0.10

In November 2017, before planting of the trees, soil samples were collected at 0-10 cm and 20-30 cm depth in the centre of each plot (18 x 3 sites) using soil sample rings (Ø 53 mm, Ejkelkamp soil & water, Giesbeek, the Netherlands). Soil bulk density (SBD), NH_4+, NO_3- and available P (Bray 1 method) were analysed from composite samples of paired plots (→ 9 samples per site), while the remaining soil parameters (see Table 3) were analysed from composite samples of six adjacent plots (→ 3 samples per site). All values presented here are averages for the 0-10 and 20-30 cm soil depths (Table 3). Oven dried (70 °C) soil samples of known volume were used to determine SBD. Samples for analysis of total soil nutrient concentrations were pre-grinded using a ceramic mortar and pestle and further grinded to a fine powder using ball mill (model MM 301, Retsch, Haan, Germany) equipped with grinding jars and balls made of stainless steel. Soil N was determined by dry combustion using an elemental analyser (EA 1108, Fison Instruments, Rodano, Italy) and all other elements by inductively coupled plasma mass spectrometry (procedure AQ250; Bureau Veritas Mineral Laboratories, Vancouver, BC, Canada). For all other soil parameters, air dried soil samples were sent for analysis to the Soil & Plant Analytical Laboratory at Rwanda Agriculture and Animal Resource Development Board (RAB) at Rubona research station, Rwanda.

The main soil texture differences between sites were a larger proportion of sand and less silt at Rubona (53-62% and 5-9%, respectively) compared to the soil at Sigira and Makera sites (35-45% and 15-27%, respectively) while the clay content was relatively high at all sites (30-50%; Table 3). The soil pH (water) was 4.2 at the HE-site and increased with approximately one unit for each step down the elevation gradient, which explain the decline in Al^{3+} content with decreasing elevation but did not affect the cation exchange capacity (CEC) which was similar at HE and LE sites. The NH_4^+ and available P soil content were not significantly affected by site, while NO_3^- was significantly lower at ME site compared to HE and LE sites. However, total N tended to decline while K, Ca, Mg and Mn contents tend to increase with decreasing elevation. Total P content was significantly lower at the ME site compared to both HE and LE sites. Significant site effects were also observed for S, Fe, Cu and Mo contents, but the patterns for these elements were more variable. Note that B, Ca, and S content were below the measurement detection limit at some (Ca and S) or all sites (B, Table 3) when analysing total soil content.

Plant material, leaf sampling and nutrient analysis

Leaves or leaflets from two (out of five) randomly selected trees per species and plot were collected during September to November 2018. For species with compound leaves, only leaflets were sampled but for simplicity, we will denote these as leaves in the following. To determine leaf mass per unit leaf area (LMA), discs of known diameter were sampled from each sampled leaf (for details, see Manishimwe et al., 2022). The remaining part of the leaves were grouped into 3 composite samples made of 2 leaves x 6 plots (forming a block) = 12 leaves per block and species, i.e., 162 samples (3 sites x 18 species x 3 replicates per site). The composite samples were brought to the lab and oven dried at 70 °C for at least 48 hours. Subsequently, the leaves were split into smaller pieces by a quick pre-grinding in a coffee grinder and thereafter grinded into fin particles using ball mill (model MM 301, Retsch, Haan, Germany) equipped with grinding jars and balls

Table 3. Soil physics and chemistry at the Rwanda TREE sites. SBD, soil bulk density; CEC, Cation exchange capacity; Org C, organic carbon concentration. Wind, soil T and SWC were only measured during the 2019/20 period. HE, high elevation; ME, mid elevation; LE, Low elevation.

	Site			
	HE - Sigira	ME - Rubona	LE - Makera	P-values
Soil characteristics				
SBD (g cm^{-3})	1.0 ± 0.03a	1.6 ± 0.1b	1.4 ± 0.1c	**<0.001**
Sand (%)	36.4 ± 2.0a	56.4 ± 1.4b	41.4 ± 1.6c	**<0.001**
Silt (%)	19.5 ± 1.7a	6.5 ± 0.5b	26.1 ± 0.6c	**<0.001**
Clay (%)	44.1 ± 3.4a	37.1 ± 1.1b	32.6 ± 1.1b	**0.002**
Texture	Clay	Sandy clay	Clay loam	
pH (Water)	4.2 ± 0.2a	5.0 ± 0.1b	6.1 ± 0.1c	**<0.001**
pH (KCl)	3.4 ± 0.1a	3.9 ± 0.04b	5.3 ± 0.2c	**<0.001**
Al^{3+} (meq/100g)	4.7 ± 0.4a	1.4 ± 0.4b	0.0 ± 0.0c	**<0.001**
Ca^{2+} (meq/100g)	0.31 ± 0.21a	0.87 ± 0.23a	5.44 ± 0.89b	**<0.001**
Mg^{2+} (meq/100g)	0.11 ± 0.02a	0.23 ± 0.09a	1.68 ± 0.21b	**<0.001**
K^+ (meq/100g)	0.19 ± 0.09	0.26 ± 0.03	0.31 ± 0.06	0.16
Na^+ (meq/100g)	0.12 ± 0.03	0.13 ± 0.02	0.47 ± 0.26	**0.045**
CEC (meq/100g)	15.6 ± 2.1a	8.0 ± 1.0b	13.9 ± 1.1a	**0.002**
Available P (µg g^{-1})	12.1 ± 3.9	14.1 ± 0.7	8.2 ± 2.5	0.095
N-NH_4^+ (µg g^{-1})	11.3 ± 2.9	10.1 ± 2.3	7.1 ± 1.0	0.13
N-NO_3^- (µg g^{-1})	28.3 ± 1.9a	7.2 ± 3.0b	24.1 ± 4.6a	**0.001**
Org C (mg g^{-1})	38.6 ± 1.1a	27.1 ± 3.1b	27.3 ± 2.4b	**0.001**
Total soil nutrient content				
N (mg g^{-1})	2.73 ± 0.3 a	2.00 ± 0.4ab	1.26 ± 0.0b	**0.003**
P (mg g^{-1})	0.65 ± 0.02a	0.14 ± 0.04b	0.27 ± 0.02c	**<0.001**
K (mg g^{-1})	0.32 ± 0.03a	0.75 ± 0.2b	0.93 ± 0.1b	**0.001**
Ca (mg g^{-1})	0.30	0.33 ± 0.1	2.42 ± 0.4	*
Mg (mg g^{-1})	0.15 ± 0.00a	0.57 ± 0.2b	0.63 ± 0.1b	**0.006**
S (mg g^{-1})	0.30 ± 0.0	<MDL	0.20 ± 0.0	*
Fe (mg g^{-1})	43.0 ± 1.8 a	12.8 ± 3.5b	18.6 ± 1.5b	**<0.001**
Mn (µg g^{-1})	107 ± 7 ab	195 ± 36.5b	158 ± 31.2b	**0.024**
B (µg g^{-1})	<MDL	<MDL	<MDL	*
Zn (µg g^{-1})	8.3 ± 0.7 a	9.6 ± 2.3a	6.3 ± 0.7a	**0.08**
Cu (µg g^{-1})	36.9 ± 4.2 a	7.0 ± 1.7b	10.1 ± 0.7b	**<0.001**
Mo (µg g^{-1})	3.0 ± 0.4 a	0.6 ± 0.1b	1.3 ± 0.0c	**<0.001**

*No statistical test conducted due to values below measurement detection limit (<MDL)

made of wolfram carbide. Leaf C and N were determined by dry combustion using an elemental analyser (EA 1108, Fison Instruments, Rodano, Italy) and all other elements by inductively coupled plasma mass spectrometry (procedure VG101; ACME Analytical Laboratories, Vancouver, BC, Canada). In this paper, we present results for C and macro (N, P, K, Ca, Mg, S) and micro (Fe, Zn, Mn, Cu, B, Mo) nutrient elements. The leaf nutrient content will be expressed on leaf dry mass basis, leaf area basis using LMA as conversion factor, and N based ratios. The potential effect on the element concentration of the pre-grinding method was tested without finding any significant difference between splitting the leaves by hand using lab-gloves or using a coffee grinder, except for Ni which therefore was excluded from this study (Figure S1).

Leaf nutrient limitation

Potential limiting leaf nutrient contents were defined as the ratio of non-N element content compared to N content (in short this will be termed N based ratios in the following) below the optimal ratio. The optimal ratios for each nutrient expressed in percentage (Table S2) were derived from eight articles (Ericsson & Kähr, 1993, 1995; Göransson, 1993, 1994, 1998, 1999; Knecht & Göransson, 2004; Linder, 1995). For several elements (P, K, Ca, Mg, Fe, Mn, Zn, Cu), different levels of optimal ratios were determined in the different studies. For these elements, the potential limitations were evaluated against the lower and the upper values. The optimal ratio for Mo could not be assessed as we did not find convincing information about the limiting ratios to N for this element.

Statistical analysis

The site effect on soil variables were analysed by one-way ANOVA with site as the main factor. When a significant site effect was detected, Tukey's post hoc significance tests was used to evaluate differences among sites. The effects of successional group on leaf nutrient content were analysed by a t-test using species as replicates. The leaf internal relationship between different elements and between leaf nutrient concentration and soil conditions (element concentration and pH) were analysed across species and sites by Pearson correlation statistics. The site and species effects on each leaf nutrient content were analysed using a two-way ANOVA, using site and species as fixed factors and soil nutrient content as covariate. However, a covariate was only included for elements having a significant correlation between leaf and soil contents and when the covariate was significant ($P<0.05$). In case of significant interactions by site and species, a one-way ANOVA was used to analyse the site effect on different species individually, with a Bonferroni test for post hoc comparison of individual sites. The replication within site was on block level (3 per site) for both soil and leaf variables.

Systematic elevational trends were defined as a significant change in the same direction (↗ increasing or ↘ declining effect) at both ME and LE sites compared to HE, or a significant change at LE compared to HE sites but no difference between ME and HE. No elevational trend was thus determined if there was a change at ME but not at LE (compared to HE). Shapiro-Wilk's test was used to test for normal distribution of data, and Levene's test was used to test for homogeneity of variance. Homogeneity of variance were obtained for analysis of individual species, while not always when all species were analysed together. Effects were considered statistically significant at $P < 0.05$ if homogeneity of variance was obtained, and at $P < 0.01$ when homogeneity was not obtained. All statistical tests were made using the SPSS 27.0 software package (SPSS, Inc., Chicago, IL, USA).

3. Results

Leaf nutrient contents at the high elevation site

The leaf nutrient concentrations varied greatly among species, as shown for the HE (Sigira) site (Figure 1A, S3 and Table S4). The inter-specific coefficient of variation (CoV) varied for macronutrients between 22% (N) to 38% (K, S) and for micronutrients between 43% (Zn) to 66% (Mn) (Table S4). LS species usually had the lowest concentrations of both macro- and micronutrients compared to ES species, although only significant for N, P, Mn, Zn and Cu. The inter-specific CoV was similar for area and ratio based nutrient content as for mass-based nutrient contents, but the significant differences between successional groups disappeared (Tables S4, S5, S6) as an effect of the higher LMA in LS species. Only N had substantially lower inter-specific CoV on area-basis compared to mass-basis (14% compared to 22%). The CoV of leaf C content between species was low (only 9% between max and min values, Table S3), but this still indicate some species differences in the proportions of different types of organic compounds (carbohydrates, lignin, and proteins).

Potential nutrient limitations at the high elevation site

The ratio of elements to N used to indicate limiting levels of individual nutrients showed a strong P limitation among most species at the HE site (Figure 1b, Table S6). All species had a mean P/N ratio below 10% and most species (90% of ES and 75% of LS species) also below 8%, which were determined as the upper and lower levels for potential P limitations, respectively. For some species (#1-15) also K/N, S/N, Zn/N, Cu/N, B/N ratios displayed values below the levels that potentially can be limiting (Figure 1b; Table S6), while Ca/N, Mg/N, Fe/N and Mn/N ratios were well above the upper limit of the optimum ratio for all species. If using limiting leaf N:P ratios suggested by Koerselman & Meuleman (1996), most species will instead be co-limited by N and P, as they suggested N:P ratio > 16 and < 14, corresponding to P:N ratio < 6.3 and > 7.1, indicating P and N limitation, respectively, and co-limiting in between (Table S6).

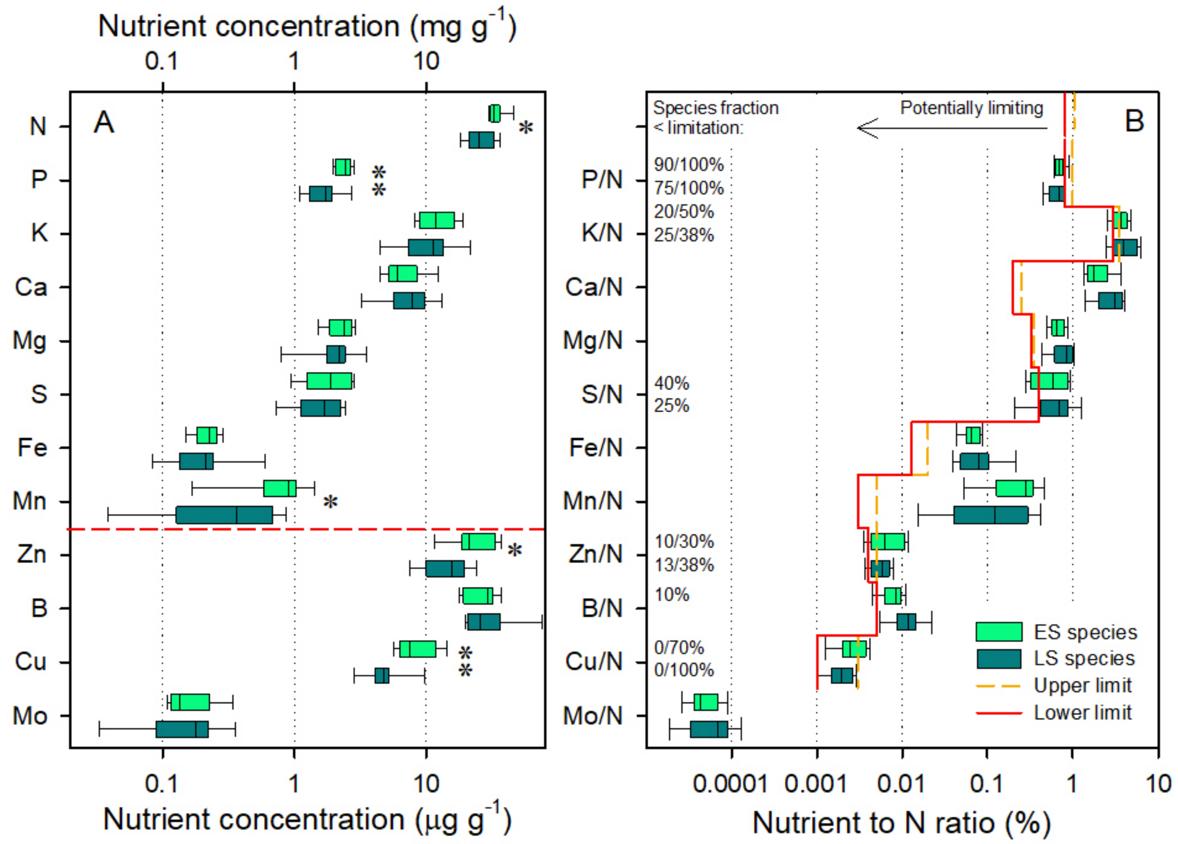
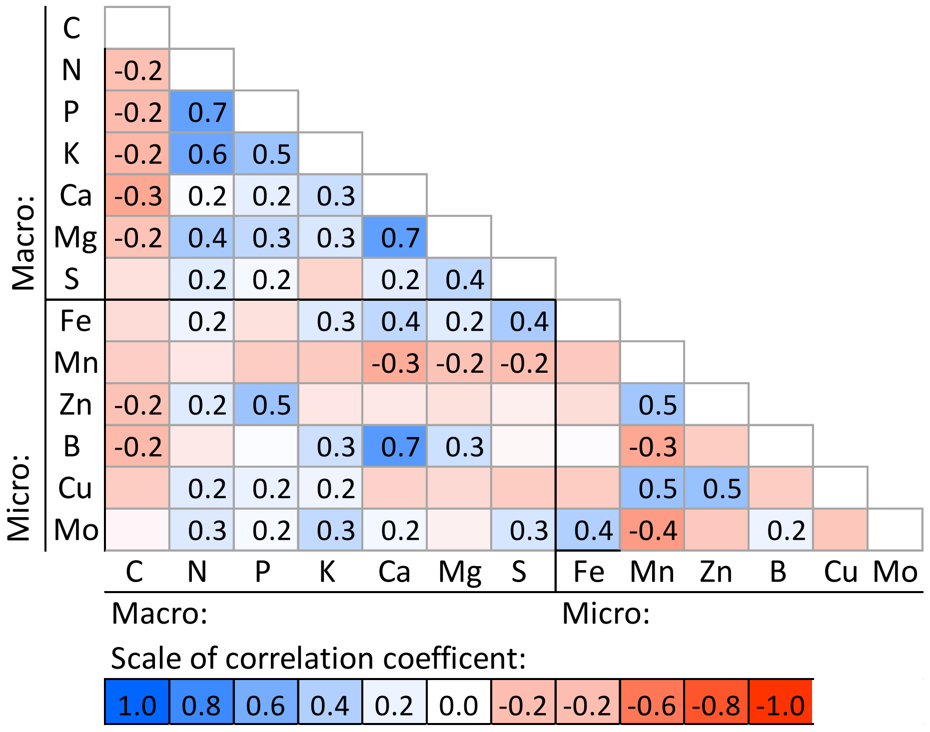


Figure 1: Leaf nutrient (mass based) element concentrations (A) and ratios to N (B) of 18 species at the HE-site (Sigira), based on mean values of each species and their classification to early (ES; n = 10) or late (LS; n = 8) successional strategies (Table 1). Concentrations (A) are given in mg g^{-1} and µg g^{-1} above and below the red dashed line, respectively, and ratios (B) are given as element to N ratio in percent. All x-axis scales are logarithmic. The boundary of the boxes indicates the 25th and 75th percentile, the error bars indicate the 90th and 10th percentiles, the line within the box marks the median. The red solid and yellow dashed lines in B indicate upper and lower literature values, respectively, of the optimal nutrient ratios defined as those when all nutrients co-limit growth simultaneously. The percent of species within each successional group below these lines is indicated to the left in B, with a slash between upper and lower limits. No optimal values were found for Mo. For values and references see Table S1. Significant differences between successional groups are indicated by: * $P < 0.05$; ** $P < 0.01$.

Relationships between different leaf macro- and micronutrients

Across all species and sites, both positive and negative relationships were observed within and among leaf macro- and micronutrients (Table 4). A strong positive and significant correlation (Pearson correlation coefficient mostly ≥ 0.5) was observed within the following three groups of elements: i) N, P and K; ii) Ca with Mg and B; iii) Mn, Zn and Cu. The elements of the second group also correlated relatively well to Fe and S (Pearson correlation 0.2-0.4). Most nutrients exhibited weak but significant positive correlations with N, P and K, except Mn as well as the pairs of P and Fe, K and S, and K and Mn. The strongest negative correlations (Pearson correlation ≤ -0.3) were observed for Mn versus Ca, B and Mo. Most elements had a weak negative correlation to C indicating a dilution effect by C on other elements.

Table 4: Pearson correlation coefficients between different leaf nutrients across species and sites. Bluish to blue colours represent increasing positive correlations while reddish to red colours represent decreasing negative correlations. The numbers in the scale state the upper value of each range. All cells with a value show regression that are significantly different from zero ($P < 0.05$).



		C	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Mo
Macro:	C													
	N	-0.2												
	P	-0.2	0.7											
	K	-0.2	0.6	0.5										
	Ca	-0.3	0.2	0.2	0.3									
	Mg	-0.2	0.4	0.3	0.3	0.7								
	S		0.2	0.2		0.2	0.4							
Micro:	Fe		0.2		0.3	0.4	0.2	0.4						
	Mn					-0.3	-0.2	-0.2						
	Zn	-0.2	0.2	0.5						0.5				
	B	-0.2			0.3	0.7	0.3			-0.3				
	Cu		0.2	0.2	0.2					0.5	0.5			
	Mo		0.3	0.2	0.3	0.2		0.3	0.4	-0.4		0.2		
		Macro:							Micro:					

Scale of correlation coefficent:

1.0	0.8	0.6	0.4	0.2	0.0	-0.2	-0.2	-0.6	-0.8	-1.0

Relationships between nutrient content in leaves and soil

The mass-based leaf content of N, P, Ca, Mg, Zn and Cu had a positive significant correlation to the total soil content of the corresponding element, while the correlation for K and Fe were negative (Table 5). No significant soil effect on the leaf content was observed for Mn and Mo, while the effect of soil S and B could not be tested as they were below the detection limits in our soil analysis (Table 3). The leaf N content was also positively correlated to the soil available N (NH_4^+ and NO_3^-) while leaf P was negatively correlated to soil available P. The soil pH significantly correlated to the leaf content of all macro and micronutrients except N and P (Table 5), but the effect of pH was negatively correlated to mass-based leaf content of K, Mn, Zn and Cu, indicating that increasing pH made them less available.

Table 5. Relationship between soil nutrients concentration or soil pH (KCl) and relative mass based leaf nutrient content across all species. Leaf nutrient concentration are standardised to % of maximum content within each species across sites. Leaf N is evaluated against both total and available soil N and P concentration. Available N is determined as sum of soil NO_3^- & NH_4^+.

	Relative leaf nut conc (mass) vs soil conc					Relative leaf nut conc (mass) vs soil pH				
Nutrient	Intercept	Slope	R^2	R	P-value	Intercept	Slope	R^2	R	P-value
N (tot)	81.2	2.9	0.04	0.20	**0.013**	92.0	-1.2	0.01	-0.10	0.20
N (available)	72.8	0.5	0.25	0.50	**<0.001**					
P (tot)	55.5	61.6	0.53	0.73	**<0.001**	78.1	-0.3	0.00	-0.01	0.89
P (available)	96.2	-1.7	0.09	-0.30	**<0.001**					
K	89.7	-11.5	0.06	-0.24	**0.002**	92.8	-2.6	0.03	-0.16	**0.041**
Ca	53.6	15.7	0.47	0.69	**<0.001**	-23.1	21.9	0.54	0.74	**<0.001**
Mg	48.9	45.9	0.26	0.51	**<0.001**	-3.2	17.4	0.44	0.67	**<0.001**
S	na	na	na	na	**na**	15.7	12.5	0.19	0.43	**<0.001**
Fe	83.9	-0.5	0.08	-0.29	**<0.001**	33.2	9.4	0.12	0.35	**<0.001**
Mn	46.5	0.0	0.003	0.05	0.51	177.4	-29.9	0.57	-0.75	**<0.001**
Zn	58.3	2.0	0.05	0.23	**0.003**	117.5	-10.2	0.27	-0.52	**<0.001**
B	na	na	na	na	na	-41.5	24.8	0.7	0.8	**<0.001**
Cu	63.3	0.4	0.07	0.27	**<0.001**	143.4	-17.3	0.40	-0.63	**<0.001**
Mo	50.2	2.2	0.01	0.08	0.31	5.3	11.6	0.11	0.34	**<0.001**

Elevation gradient site effect on leaf nutrient contents

Significant site, species, and site x species effects were observed for all leaf macro- and micronutrients contents when expressed on mass, area and N based ratio, except for mass and area-based C and K content which were not affected by site (Table 6). To reduce the effects of soil differences between sites, soil nutrient was used as a covariate in this analysis, when a significant correlation between leaf and soil contents was observed (Table 5) and when the effect of the covariate was significant (Table 6). This was valid for K, Mg and Zn independent of leaf nutrient basis, but also for mass-based N and Cu, area-based P and Mn, and ratios of N/C and Mn/N.

Mass-based contents of Ca, Mg, Fe, B and Mo increased with declining elevation, while Zn and Cu decreased significantly with decreased elevation (Table 6, Figure 2). Also, mass-based leaf content N and P declined downwards, however, the effect where more ambiguous as the ME sites had significantly lower contents compared to both HE and LE sites, but the LE site also had lower contents compared to the HE site (Figure 2). The site effect on leaf N and P content seem to be dominated by lower N and P availability at the ME site compared to the other two sites, possibly caused by the significantly lower soil NO_3^- and total P contents at the ME compared to the other sites (Table 3). The observed elevation trends of most area and N based leaf nutrients contents were similar to the trends of mass-based contents, except that no trend was observed for area-based Cu/N, while S/N increased with decreasing elevation (Table 6, Figure S3).

Table 6. Results from two-way ANOVA of the species (Sp) and site effects on mass- and area-based leaf nutrient contents and leaf element ratios across all species (n=18) and sites. The analysis includes the covariate of soil nutrient (nut.) of each element and soil available nutrient (Av. Nut. for N and P) but only when it is significant (P < 0.05). The main and interaction (inter.) effects are classified as significant at P<0.01 as all ANOVA not passed the variance homogeneity test. The results from Bonferroni post hoc test are given in Figure 2. The arrows indicate the direction of a possible warming effect at a significant site effect (P < 0.01), as explained in the table footnote.

	Mass based leaf content							Area based leaf content								Leaf element ratio						
		Main effect		Inter.	Covar				Main effect		Inter.	Covar					Main effect		Inter.	Covar		
Ele-ment	cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.		cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.		Ele-ment	cv	Site	Sp	Site x Sp	Soil nut.	Av. nut.	
C		0.4	**<0.001**	0.03					0.56	**<0.001**	**<0.001**											
N	x	**<0.001**	**<0.001**	**<0.001**	**0.04**	**0.01**	↘		**<0.001**	**<0.001**	**<0.001**	1	0.15	↘	N/C	x	**<0.001**	**<0.001**	**<0.001**	**0.05**	**0.02**	↘
P		**<0.001**	**<0.001**	**<0.001**	0.53	0.060	↘	x	**<0.001**	**<0.001**	**<0.001**	0.11	**0.01**	→	P/N		**<0.001**	**<0.001**	**<0.001**	0.22	0.1	↘
K	x	0.06	**<0.001**	**<0.001**	**0**			x	0.09	**<0.001**	**<0.001**	**0.03**			K/N	x	**<0.001**	**<0.001**	**<0.001**	**0.05**		→
Ca		**<0.001**	**<0.001**	**<0.001**	0.94		↗		**<0.001**	**<0.001**	**<0.001**	0.87		↗	Ca/N		**<0.001**	**<0.001**	**<0.001**	0.51		↗
Mg	x	**<0.001**	**<0.001**	**<0.001**	**0.03**		↗	x	**<0.001**	**<0.001**	**<0.001**	**0.02**		↗	Mg/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↗
S		**<0.001**	**<0.001**	**<0.001**			→		**<0.001**	**<0.001**	**<0.001**			→	S/N		**<0.001**	**<0.001**	**<0.001**			↗
Fe		**<0.001**	**<0.001**	**<0.001**	0.17		↗		**<0.001**	**<0.001**	**<0.001**	0.19		↗	Fe/N		**<0.001**	**<0.001**	**<0.001**	0.10		↗
Mn		**<0.001**	**<0.001**	**<0.001**	0.08		→	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↘	Mn/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		→
Zn	x	**<0.001**	**<0.001**	**<0.001**	**0.03**		↘	x	**<0.001**	**<0.001**	**<0.001**	**0**		↘	Zn/N	x	**<0.001**	**<0.001**	**<0.001**	**0.01**		↘
B		**<0.001**	**<0.001**	**<0.001**			↗		**<0.001**	**<0.001**	**<0.001**			↗	B/N		**<0.001**	**<0.001**	**<0.001**			↗
Cu	x	**<0.001**	**<0.001**	**<0.001**	**0.02**		↘		**<0.001**	**<0.001**	**<0.001**	0.27		↘	Cu/N		**<0.001**	**<0.001**	**<0.001**	0.16		→
Mo		**<0.001**	**<0.001**	**<0.001**	0.44		↗		**<0.001**	**<0.001**	**<0.001**	0.47		↗	Mo/N		**<0.001**	**<0.001**	**<0.001**	0.59		↗

↘ Decrease with warmer sites (down at both ME and LE or no/small effect at ME and down at LE compared to HE site).
↗ Increase with warmer sites (up at both ME and LE or no/small effect at ME and up at LE compared to HE site).
→ No warming effect (up at one site and down at one site or down/up at ME but no effect at LE compared to HE site).

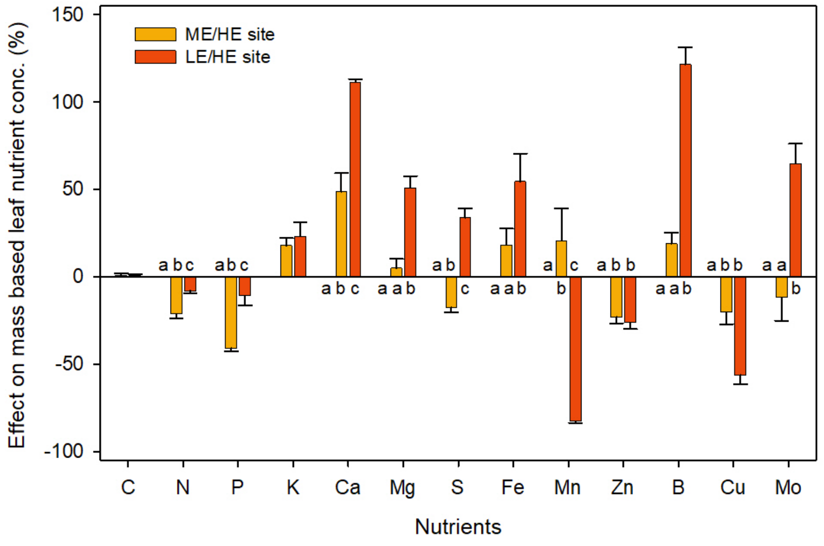


Figure 2. The effect on mass-based leaf nutrient concentration along the elevation gradient expressed as percent difference at ME and LE sites relative to the HE site, i.e. HE site is the zero line. Different letters (a, b, c, first letter for each nutrient represents the HE site) denote significant difference between sites. HE, high; ME, mid; LE, low elevation site. See Table 6 for results from statistical analyses.

As a consequence of the significant site by species interactions on mass-based leaf nutrient contents (Table 6), the site effects were analysed separately for each of the 18 species (Table 7, 8). Most species (8 to 16 out of 18) responded similarly to the elevation gradient, especially for leaf Ca, Mg and B which increased downwards and N, P, Zn, Mn and Cu which decreased downwards. Leaf S, Fe and Mo contents also increased downwards (Table 7, 8) but fewer species were significantly affected and the trends were partly conflicting. No specific group of species (successional or origin) stand out as being particularly affected by the elevation.

LMA effects on area-based leaf nutrient content

The decrease in leaf mass per unit area (LMA) at warmer sites in one third of the species, reported on the same trees in an earlier study (Manishimwe et al., 2022), contributed to significantly reducing the area-based leaf content of N, P, Zn, Mn and Cu of these species (#6) compared to species where LMA was not affected by elevation (#12, Figure 3). All these nutrients except Mn were also found to be potentially limiting.

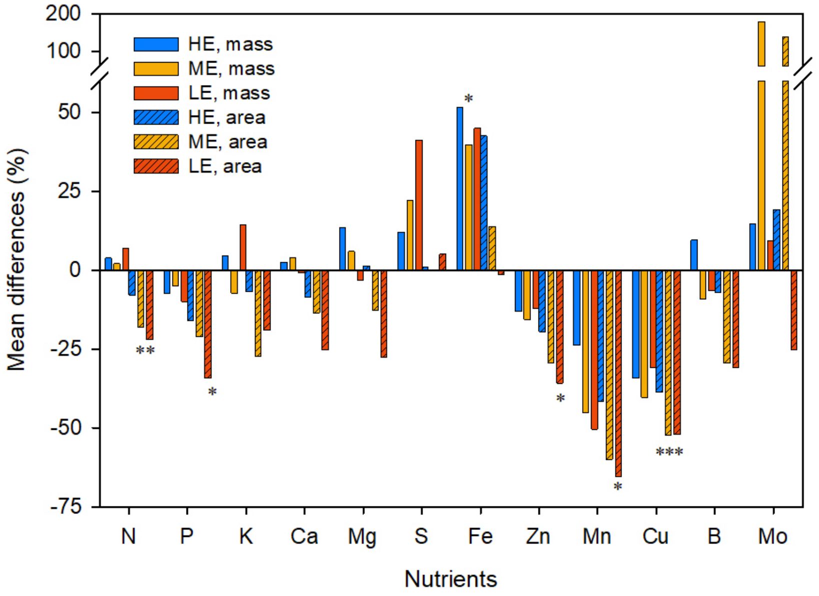


Figure 3. The mean difference in mass and area-based leaf nutrient contents between species with a significant warming decline in leaf mass per area unit (LMA, 6#) compared to species with unaffected LMA (#12) along the elevation gradient. * significant difference within site between the two groups of species (P < 0.05). HE, high; ME, mid; LE, low elevation site.

Table 7. Significance of site effects on leaf macronutrients in 18 tree species (Spec). SG, successional group (ES, early successional; LS, late successional); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest). Explanation of species codes is given in Table 1. H, M and L represent high (HE/Sigira), mid (ME/Rubona) and low (LE/Makera) elevation sites, respectively. Different letters (a, b, c) denote significant difference between sites. The arrows indicate the direction of warming effect of species with significant site effect (*P* < 0.05), see Table footnote. * and $^{+}$ indicates a significant covariate and non-significant post hoc test despite a significant site effect in the ANOVA, respectively.

Mass based:			C					N					P					K					Ca					Mg					S				
SG	FT	Spec	P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L	
ES	TMF	Bbr	0.57					0.687					**<0.001**	a	c	b	↘	0.087					**<0.001**	a	a	b	↗	**<0.001**	a	a	b	↗	**0.005**	a	a	b	↗
		Hmo	0.74					**0.001**	a	c	b	↘	**<0.001**	a	c	b	↘	0.91					0.061					**0.005**	a	a	b	↗	**<0.001**	a	b	c	→
		Mki	**0.001**	a	a	b	↗	**0.006**	a	ab	b	↘	**0.001**	a	b	a	→	**0.024**	a	b	a	→	**0.005**	a	a	b	↗	**0.046**	a	ab	b	↗	0.37				
		Mla	0.19					**0.001**	a	b	a	→	**<0.001**	a	b	a	→	$^{+}$**0.044**					**0.001**	a	a	b	↗	**0.035**	a	ab	b	↗	**0.025**	a	b	b	↘
		Pfu	0.18					**<0.001**	a	b	a	→	**<0.001**	a	b	a	→	*0.009	a	ab	b	↗	**<0.001**	a	a	b	↗	**0.007**	a	a	b	↗	**0.003**	a	b	a	→
	LVTF	Bmi	**0.024**	a	b	ab	→	**0.019**	a	b	b	↘	**0.008**	a	b	a	→	0.30					**0.002**	a	a	b	↗	**0.003**	a	a	b	↗	**<0.001**	a	b	c	↗
		Cme	0.47					**0.038**	ab	b	a	→	**0.009**	a	b	a	→	0.79					**<0.001**	a	b	c	↗	**0.001**	a	b	c	↗	**<0.001**	a	b	c	↗
		Dto	0.11					**0.007**	a	b	a	→	**<0.001**	a	b	a	→	0.21					**<0.001**	a	b	c	↗	**0.002**	a	a	b	↗	**<0.001**	a	b	c	↗
		Hma	0.63					**0.003**	a	b	b	↘	**0.008**	a	b	a	→	0.072					**0.036**	a	b	b	↗	**0.012**	a	ab	b	↗	0.12				
		Mlu	0.34					**0.017**	a	b	a	→	**0.001**	a	c	b	↘	**0.011**	a	ab	b	↘	**0.001**	a	b	c	↗	**0.017**	a	ab	b	↗	**0.029**	a	ab	b	↗
LS	TMF	Afa	0.13					***0.006**	a	a	b	↘	**0.009**	a	b	a	→	**0.026**	ab	a	b	↘	**0.002**	a	b	b	↗	**0.001**	a	b	c	↗	**0.009**	a	ab	b	↗
		Cgr	0.93					**0.001**	a	c	b	↘	**<0.001**	a	b	b	↘	0.77					**0.006**	a	a	b	↗	0.10					**<0.001**	a	b	c	↗
		Fsa	0.87					0.010	a	b	b	↗	<0.001	a	c	b	↘	***0.005**	a	b	c	↗	0.19					**0.000**	a	b	b	↗	**<0.001**	a	b	b	↘
		Paf	**0.001**	a	b	b	↘	***0.013**	a	b	a	→	0.060					**0.020**	ab	a	b	→	**<0.001**	a	b	c	↗	**0.003**	a	a	b	↗	0.11				
		Sgu	0.34					**0.018**	a	ab	b	↘	**<0.001**	a	c	b	↘	0.40					**0.001**	a	c	b	→	**0.006**	ab	a	b	↗	**0.001**	a	b	a	→
	LVTF	Cgo	**0.003**	a	b	a	→	**0.032**	a	ab	b	↗	0.20					0.080					**0.001**	a	a	b	↗	**0.012**	a	a	b	↗	**0.003**	a	a	b	↗
		Eex	**<0.001**	a	a	b	↘	**0.017**	a	b	ab	→	**0.035**	a	b	ab	→	0.29					**<0.001**	a	b	b	↗	**0.002**	a	b	b	↗	0.79				
		Fth	0.26					**0.022**	a	b	ab	→	**<0.001**	a	c	b	↘	$^{*,+}$**0.021**					**0.003**	a	b	b	↗	0.21					**0.017**	ab	b	a	→

↘ Decrease with warmer sites (down at both ME and LE or no/small effect at ME and down at LE compared to HE site).
↗ Increase with warmer sites (up at both ME and LE or no/small effect at ME and up at LE compared to HE site).
→ No warming effect (up at one site and down at one site or down/up at ME but no effect at LE compared to HE site).

Source of variance in leaf nutrients contents across species and sites

The partitioning of variation in mass-based leaf nutrient content across all species and sites revealed that species (within + between successional groups) was the main source of variation (70-85% for N, K and Zn; 50-70% for B and Cu; 40-50% for C, P, Ca, Mg, S, Fe and Mo; 35-40% for Mn; Figure 4a). Essentially the same partitioning of variance was found for the element content expressed on area and N-based ratios, except for area-based C and N for which the species source of variance shifted from 44% to 90% and 80% to 48 %, respectively (Figure 4 b-c). In general, only a minor portion of the species as source of variance was related to the successional group, but depending on element it varies between 0 to 37%, with major and significant contributions >25% by N, P, Mn, Zn and Cu (see also Figure 1A) Site contributed to between 0.4 to 37 % to the variance of mass-based content (>30% for P, Ca and Mn; 20-30% for Mg, S and B; 10-20% for Zn and Cu; <10% for C, N, K, Fe, Mo). The site contribution was mostly similar or slightly lower for both area and N based ratios. The maximum within-site (block) contribution to the variance was 1.7% (N based Mg), but normally much less regardless of element content basis and thus negligible. The remaining residual source of variance was most due to variation within or between sampled individuals and/or measurement errors.

Table 8. Significance of site effects on leaf micronutrients in 18 tree species. SG, successional group (ES, early successional; LS, late successional); FT, forest type (TMF, tropical montane forest; LVTF, Lake Victoria transitional forest). Explanation of species codes is given in Table 1. H, M and L represent high (HE/Sigira), mid (ME/Rubona) and low (LE/Makera) elevation sites, respectively. Different letters (a, b, c) denote significant difference between sites. The arrows indicate the direction of warming effect of species with significant site effect (*P* < 0.05), see Table footnote. * Indicate a significant covariate.

Mass based:			Fe					Zn					Mn					Cu					B					Mo				
SG	FT	Spec	P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L		P	H	M	L	
ES	TMF	Bbr	0.093					**0.007**	a	b	b	↘	**0.001**	a	a	b	↘	0.059					**<0.001**	a	a	b	↗	**0.001**	a	b	b	↘
		Hmo	0.52					**0.000**	a	b	c	↘	**0.001**	a	a	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	0.080				
		Mki	0.37					**0.002**	a	b	b	↘	**0.006**	a	a	b	↘	**<0.001**	a	b	b	↘	**<0.001**	a	a	b	↗	0.055				
		Mla	**0.009**	a	b	b	↗	0.23					**0.027**	ab	a	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	**0.012**	a	a	b	↗
		Pfu	0.80					0.089					**<0.001**	a	a	b	↘	**0.002**	a	b	b	↘	**<0.001**	a	b	c	→	0.60				
	LVTF	Bmi	0.84					0.41					**0.003**	a	a	b	↘	**0.003**	ab	b	a	→	**<0.001**	a	a	b	↗	0.50				
		Cme	0.17					* **0.004**	a	b	b	↘	**0.001**	a	b	a	→	**<0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	0.059				
		Dto	**0.002**	a	b	b	↗	0.11					**0.001**	a	a	b	↘	**0.001**	ab	b	a	→	**<0.001**	a	b	c	↗	**<0.001**	a	a	b	↗
		Hma	**0.015**	a	b	a	→	**0.034**	a	ab	b	↘	**<0.001**	a	b	c	↘	**<0.001**	a	b	c	→	**<0.001**	a	a	b	↗	**0.035**	ab	b	a	→
		Mlu	0.22					**0.005**	a	b	b	↘	**<0.001**	a	c	b	→	**<0.001**	a	b	c	→	**<0.001**	a	b	c	↗	**0.027**	ab	b	a	→
LS	TMF	Afa	0.082					* **0.002**	a	b	c	→	**<0.001**	a	a	b	↘	0.073					0.18					**0.047**	*			↗
		Cgr	**0.023**	a	a	b	↗	0.087					**<0.001**	a	b	b	↘	**0.002**	a	a	b	↘	**0.003**	a	a	b	↗	0.10				
		Fsa	**0.034**	a	b	b	↗	0.74					* **0.005**	a	ab	b	→	**0.004**	a	b	a	→	**0.002**	a	a	b	↗	**0.039**	*			↗
		Paf	**0.004**	a	ab	b	↗	0.30					**0.009**	ab	a	b	→	**0.011**	a	a	b	↘	**0.012**	a	ab	b	↗	0.16				
		Sgu	0.36					**0.001**	a	b	a	→	**<0.001**	a	a	b	↘	**0.002**	a	a	b	↘	**<0.001**	a	a	b	↗	0.86				
	LVTF	Cgo	**<0.001**	a	c	b	→	**0.003**	a	b	b	↘	**<0.001**	a	b	c	↘	0.62					0.30					**0.007**	a	b	ab	→
		Eex	**0.010**	a	b	b	↗	**0.032**	a	a	b	↘	* **0.024**	ab	b	a	→	**0.003**	a	a	b	↘	**0.002**	a	a	b	↗	**0.028**	a	ab	b	→
		Fth	**0.004**	a	b	b	↗	**0.006**	a	b	b	↘	**0.001**	a	a	b	↘	**<0.001**	a	a	b	↘	**<0.001**	a	a	b	↗	**0.002**	a	a	b	↗

↘ Decrease with warmer sites (down at both ME and LE or no/small effect at ME and down at LE compared to HE site).
↗ Increase with warmer sites (up at both ME and LE or no/small effect at ME and up at LE compared to HE site).
→ No warming effect (up at one site and down at one site or down/up at ME but no effect at LE compared to HE site).

4. Discussion

In this experiment we demonstrated that leaf nutrient contents varied greatly among 18 common African tropical tree species (factor 2.5-40) grown in mixed multispecies stands at a high elevation site (Figure 1A). Nutrients other than N and P varied the most. These results are in line with suggestions of a high degree of species-specificity in the mechanisms controlling nutrient uptake and accumulation in leaves, which in turn suggest large interspecific variation in nutrient requirements and hyperaccumulation ability (Chapin et al., 1986; Leitenmaier & Küpper, 2013; Rascio & Navari-Izzo, 2011; Uchida, 2000). Based on our literature review of optimal N based nutrient ratios (Table S2), leaf P was considered potentially growth limiting for most species at the high elevation site, with K, S, Zn, Cu co-limiting for some of the species (Figure 1B, table S6). By growing the same plant material at three sites spanning an elevation of 1100 m and 5.4 °C in MAT, with irrigation to compensate for site differences in precipitation, we determined elevational trends of 13 essential nutrients and interpret these in relation to growth temperature and soil properties at different sites.

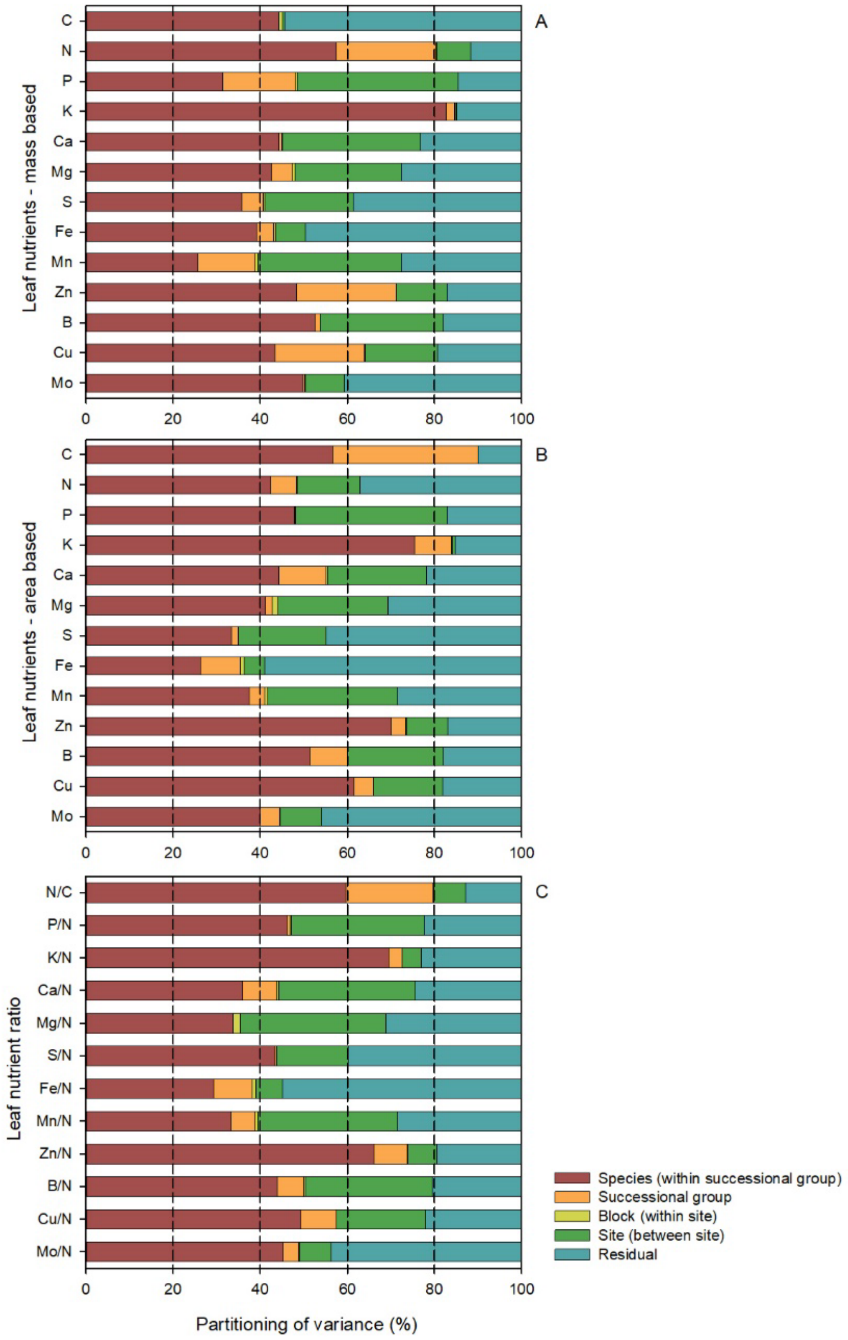


Figure 4 A-C: Partitioning of variance (%) in mass (A), area (B) and element ration of leaf nutrient contents attributed to species (within successional group), successional group, blocks within sites (3), and sites. Residual account for variation among trees.

The relationship between nutrient contents in leaf and soil

With a few exceptions, leaf nutrient contents were influenced by soil nutrient content and soil pH, supporting our first hypothesis (H#1; Table 4 and 5). Several of the mass-based leaf nutrients were positively related to the soil concentration of the respective element, but relationships were rather weak except for Ca, Mg, N (*vs* available soil N) and P (*vs* total soil P) which had $R^2 > 0.25$ (Table 5). Surprisingly, mass-based leaf P content was negatively related to soil available P concentration while it was strongly positively related to total soil P concentration ($R^2 = 0.53$). Soil P occurs in several fractions with varying lability but many of these can be transformed into phyto-available forms by microorganism (Yang et al., 2021). The pool of soil P available to plants may therefore have been underestimated, as also supported by a comparison of different available soil P methods by Johnson et al. (2003) showing that the results of the Bray 1 method, used in this study, do not fully represent the labile fractions of soil P in at least some soils. Also leaf K and Fe content were negatively related to their concentrations in the soil, but weakly so. Overall, the results on relationships between leaf and soil nutrients are in line with previous studies, reporting mostly positive, but also some negative relationships (e.g., K, S, Fe) (Bai et al., 2019; Heineman et al., 2016; Jones et al., 2019; Ordoñez et al., 2009).

Mass-based leaf contents of Ca, Mg, S, Fe, B, Mo were positively related to soil pH, while relationships were negative for Mn, Zn and Cu. These results mostly follow how pH affect the soil availability of these elements (Barman et al., 2014; Barrow & Hartemink, 2023; De Mello Prado, 2021; Neugebauer et al., 2018), except for Fe which in its ionic form has declining availability with increasing pH (Colombo et al., 2014). However, Fe is commonly taken up by plants in its chelated form, which is less pH sensitive (De Mello Prado, 2021). Leaf N, P and K were not, or weakly, affected by soil pH, although the phyto-availability of P and K normally increases up to a pH of around 6. The role of pH for N availability is more complex as it occurs in the form of both nitrate and ammonium, but also because the N mineralisation is regulated by several factors other than pH (De Mello Prado, 2021; Barrow & Hartemink, 2023).

Elevation gradient of leaf nutrients

Down the elevation gradient, mass-based leaf contents of Ca, Mg, Fe, B and Mo increased while N, P, Zn, and Cu decreased, while shifts in K, S and Mn were smaller or varied more among species (Table 6-8, Figure 2). The decrease in leaf N and P compared to the HE site was larger at the ME site than at the LE site, but the declines were significant at both places. Although the significantly higher soil bulk density at ME compared to the other sites partly compensated for the low soil concentration of nitrate and total P, the lower leaf N and P contents at the ME site likely also reflect lower soil fertility. The results on leaf N content resemble observations in a study with seedlings of two species grown in pots with the same soil (from HE site) at the three Rwanda TREE sites. In that study both mass- and area-based leaf N content of *Harungana montana* significantly dropped at the ME site compared to both HE and LE sites, while the leaf N contents of *Syzygium guineense* dropped significantly at both ME and LE sites compared to the HE site (Dusenge et al., 2021). The results of that study and those presented here thus together support that warming contributed to decreased leaf N contents down the elevation gradient and that these are not only caused by site differences in soil fertility. Moreover, our results with a decline in mass-based leaf N and P content with decreasing elevation and increasing temperature agrees with observations in an elevation gradient study of mature forest in Uganda, also located in the Albertine ecoregion (Okello et al., 2022), as well as in global and regional studies over the Americas and Amazonia (Reich & Oleksyn, 2004; Šímová et al., 2019; Fyllas et al., 2009). However, there are also observations where leaf N and/or P increased with decreasing elevation in two studies in the Andes (Soethe et al., 2008; van de Weg et al., 2009), in Hawaii (Vitousek et al., 1988) and in one warming experiment in southern China (Wu et al., 2019), as well as observations of no trends with elevation in a study on mature trees of four species in Rwanda (Mujawamariya et al., 2018). Clearly, more field data is needed to understand the patterns in leaf N and P along tropical elevation gradients.

Leaf P, Zn and Cu were all potentially limiting at the high elevation site and declined down the elevation gradient (Figure 1B). Leaf N also declined downwards and may contribute to the potential limitation of plant processes as the index developed by Koerselman & Meuleman (1996) suggest that P become limiting instead of N at slightly lower P:N ratios (7.1 instead of 8-10 and that the P limitation gradually increase down to 6.3). Thus, this index rather indicated that most species in our study are co-limited by N and P and not only by P (table S6). However, especially, leaf P and Zn likely become more limiting at lower and warmer sites as they decline more than leaf N content, as also indicted by downward decreases in N based ratios of these two elements (Table 6, Figure S2).

In one third of the investigated species, leaf mass per area unit decreased at warmer sites (Manishimwe et al., 2021). The observed downward declines in leaf N, P, Zn and Cu of these species were therefore reinforced when leaf content was expressed on area instead of mass (Figure 3). In spite of potential amplification of P and Zn limitation with warming, most species grew faster at warmer sites, although some did not respond or grew slower (Ntirugulirwa et al., 2023). This reflects a complex interaction between nutrient contents, leaf morphology and temperature on plant growth that remains to be elucidated. Overall, the elevation gradient responses of leaf nutrient contents mostly support our second hypothesis (H#2) that different leaf nutrients responded differently, but most importantly that leaf N and P declined down the elevation gradient. However, we did not observe a general downward increase in N based ratios (last part of H#2) which would likely occur if N became less limiting at warmer sites, as suggested in several studies

(Austin & Vitousek, 1998; Fisher et al., 2013; Tanner et al., 1990; Tanner et al., 1992; Vitousek, 1984; Wright et al., 2011).

Leaf nutrients in early and late successional species

The significantly lower leaf N, P, Mn, Zn and Cu contents in LS compared to ES species (Figure 1) are in line with the different growth strategies of these successional groups (Poorter et al., 2004; Schönbeck et al., 2015) and the general difference in nutrient availability at late compared to early successional stages (Hernández-Vargas et al., 2019; Lohbeck et al., 2015; Prado-Junior et al., 2016; Sterck et al., 2011). However, all significant mass-based differences between successional groups disappeared when expressed on area-basis (Table S5). Thus, our third hypothesis (H#3) regarding successional group differences in mass-based nutrient contents but not in area-based nutrient contents was mostly confirmed.

Interestingly, three (P, Cu and Zn) of the five nutrients which significantly differed between the successional groups (on mass basis) were assessed as strongly or co-limiting (c.f. Figure 1A & B). The acquisition of these nutrients therefore likely plays a role in the competition between the successional groups, affecting the rate of succession and tree community composition. The result is also in line with the observation that soil nutrient distributions substantially influence local species distributions (John et al., 2007).

Source of variance in leaf nutrients across species and sites

Our finding that species was a much larger source of variation in leaf nutrient contents than site supports our fourth hypothesis (H#4; Figure 4). It is also in line with neotropical observations that the variation in leaf chemical and morphological traits is much more strongly explained by phylogeny (species, genus, family) than by environment (i.e., site) (Asner et al., 2014; Messier et al., 2017). The strongest site influences (> 20% of the total variance) were observed for leaf P, Ca, Mg, S, Mn and B (Figure 4A). However, among these only leaf P was both potentially limiting and declining down the elevation gradient, as discussed above. The finding that species identity (including species successional strategy) explained a considerably larger fraction of the total variation than variation caused by site differences in soil and climate conditions is important in a biogeochemical context. It implies that the nutrient cycling in Afromontane forests in a changing climate likely will depend more strongly on shifts in tree community composition than on intraspecific warming-induced changes in leaf nutrient content.

5. Conclusion

We showed that different leaf nutrients responded differently along the elevation gradient, likely as a combined effect of warming, soil nutrient content and nutrient availability mediated by pH. Leaf content of the potentially most growth-limiting nutrients N and P, but also Zn and Cu declined down the elevation gradient, so their potential limitation increased with warming. The downward reduction in the leaf nutrients were reinforced when expressed on area instead of mass basis for species in which leaf mass per area unit (LMA) declined with warming. Early successional species had higher mass-based leaf contents of N, P, Mn, Zn and Cu compared to late successional species, while the differences disappeared when expressed on area basis. The strongly contrasting leaf nutrient contents of different species explained a higher fraction of the total variation in leaf nutrients than site, despite large variation in soil conditions and climate. This means that nutrient cycling in Afromontane forests in a changing climate may depend more strongly on shifts in tree community composition than on intraspecific warming-induced shifts in leaf nutrient content.

Supplement. Additional supporting information is found online in the Supplement.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions. AM, GW, JU, and DN planned and designed the experiment; AM, BN, and EZ conducted field measurements; AM and GW complied and analysed the data; EB helped with characterizing the species. AM and GW wrote the manuscript with important contribution by JU.

Conflicts of Interest. The authors declare no conflict of interest.

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Supplementary Material

Table S1. Taxonomy[1] for species and their main forest type[2] of origin, classification into successional group, their leaf type and shape[3], tree stature and habitat.

Species code	Scientific name and author	Family name	Leaf Type & Shape	Description
Early successional species from Tropical montane rainforest:				
Bbr	*Bridelia brideliifolia,* (Pax) Fedde	Euphorbiaceae/ Phyllanthaceae	Simple alternate, Elliptic	Shrub-tree (up to 30 m height) in evegreen and rainforests, and associated bushland and thickets at 1700-2500 m a.s.l.
Hmo	*Harungana montana,* Spirlet	Hypericaceae	Simple opposite, Ovate	Tree (15-20 m height) in montane rain forest at 2000-2300 m a.s.l
Mki	*Macaranga kilimandscharica,* Pax	Euphorbiaceae	Simple alternate, Ovate truncate	Tree (25 m height) in montane rainforests at 1700-2500 m a.s.l
Mla	*Maesa lanceolate,* (Henriq.) F. White	Myrsinaceae/ Primulaceae	Simple alternate, Ovate	Shrub-tree (up to 24 m height) in dry, humid, montane, subhumid, anthropic forests, woodlands and wooded grasslands at 2000-3000 m a.s.l
Pfu	*Polyscias fulva,* (Hiern) Harms	Araliaceae	Pinnately Compound, Oblong	Tree (up to 30 m height) in montane rainforest at 1700-2900 m a.s.l.
Late successional species from Tropical montane rainforest:				
Cgr	*Carapa grandiflora,* Sprague	Meliaceae	Pinnately Compound, Oblong	Tree (15-25 m height) in mountain rainforest at 1200-2500 m a.s.l
Paf	*Prunus Africana,* (Hook.f.) Kalkman	Rosaceae	Simple alternate, Oblong	Tree (6-40 m height) in upland rain forest, montane and riverine forests, moist evergreen forest, Brachystegia woodland, edge of gallery forest
Fsa	*Faurea saligna, Harv*	Proteaceae	Simple alternate, Oblong acute	Tree (17-20 m height) in Brachystegia woodland or grassland at 700-1800 m a.s.l
Afa	*Afrocarpus falcatus+,* (Thunb.) C.N.Page	Podocarpaceae	Simple alternate, Oblong linear	Conifer tree (25-40 m) in montane rainforest 1800-3000 m a.s.l.)
Sgu	*Syzygium guineense,* (Willd.) DC.	Myrtaceae	Simple opposite, Elliptic	Rhizotomous subshrub-tree (up to 30 m height) at 1-2600 m a.s.l, widespread distribution range
Early successional species from Lake Victoria transitional rainforest:				
Bmi	*Bridelia micrantha,* (Hochst.) Baill	Euphorbiaceae/ Phyllantaceae	Simple alternate, Elliptic	Shrub-tree (up to 27 m height) in evergreen forests, associted with bushland and thicket, riverine and gully forests, woodlands; seasonally flooded grassland, mangrove swamp margins.
Cme	*Croton megalocarpus,* Hutch.	Euphorbiaceae	Simple opposite, Ovate	Tree (up to 35 m height) in evergreen; riverine and gully forests; Brachystegia woodland;on old lava flows; in mixed rain-forest at (35-)1500-2400 m a.s.l.
Dto	*Dombeya torrida,* (J.F.Gmel.) Bamps	Sterculiaceae/ Malvaceae	Simple alternate, Elliptic lobate	Tree (up to 24 m height) in Montane forest and scrub, bushland and grassland, cultivated areas at 1100-3100 m a.s.l.
Hma	*Harungana madagascariensis,* Lam. ex Poir	Hypericaceae	Simple opposite, Ovate	Tree (up to 27 m height) in Rain-forest, remnants, fringing forest in savanna; secondary regrowth forets and widespread common; at 1 - 450 m a.s.l (coastal) and 800 - 1900 m a.s.l (inland)
Mlu	*Markhamia lutea,* (Benth.) K.Schum	Bignoniaceae	Pinnately Compound,	Tree (up to 15 m) in african upland forest 900-2000 m a.s.l.
Late successional species from Lake Victoria transitional rainforest:				
Cgo	*Chrysophyllum gorungosanum,* Engl	Sapotaceae	Simple alternate, Obovate	Tree (up to 40 m height) in upland forest 1600-2450 m a.s.l.
Eex	*Entandrophragma exselsum,* (Dawe & Sprague) Sprague	Meliaceae	Pinnately Compound, Elliptic	Tree (30-60 m height) in montane rainforest; riverine forest; rarely occurs almost pure; at 925-2220 m a.s.l
Fth	*Ficus thonninghii,* Blume	Moraceae	Simple alternate, Elliptic	Shrub-tree (8-50 m height)in gallery with other species; savanna; lake shores; miombo woodland.

[1] Taxonomy information from The plant list (http://www.theplantlist.org, 9 December 2021) and for family names, both classic and Angiosperm Phylogeny Group (APG III) system are given when applicable (*Angiosperm Phylogeny Group (2009), An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III", Botanical Journal of the Linnean Society,* ***161*** *(2): 105–121,* doi:10.1111/j.1095-8339.2009.00996.x; [2]Forest type follows the Potential Natural Vegetation's by Kindt, R., van Breugel, P., Lillesø, J-P.B., Minani, V., Ruffo, C.K., Gapusi, J., Jamnadass, R. and Graudal, L. (2014), Potential natural vegetation of eastern Africa, Volume 9. Atlas and tree species composition for Rwanda. Copenhagen, Denmark: Department of Geosciences and Natural Resource Management, University of Copenhagen, 93 pp.; [3]Leaf type and shape classifications follow Ellis, B., Daly, D.C., Hickery, L.J., Johnson, K.R., Mitchell, J.D., Wilf, P., Wing, S.L. (2009) Manual of Leaf Architecture; Cornell University, Press: Ithaca, NY, USA, p. 190.

Table S2. The optimal nutrient ratios defined as when all nutrients limit growth simultaneously. The values are given for nutrient to N ratios (%) in leaves and are derived from: Ericsson and Kähr (1993, 1995), Göransson (1993), Göransson (1994), Göransson (1998), Göransson (1999), Knecht and Göransson (2004) and Linder (1995).

Nutrient	Optimal ratios	
ratios	Lower	Upper
P/N	8	10
K/N	30	35
Ca/N	2	2.5
Mg/N	3.3	3.5
S/N	4	4
Fe/N	0.13	0.2
Mn/N	0.03	0.05
Zn/N	0.04	0.05
B/N	0.05	0.05
Cu/N	0.01	0.03
Mo/N	no data	no data

Table S3. Leaf nutrient element concentrations (means ± SD and range) of 18 species at the high elevation site (HE/Sigira) based on mean values of each species and their classification to early (ES; n = 10) or late (LS; n = 8) successional strategies (Table 1). Times diff, times higher concentration of the highest compared to lowest range; Diff (%), the concentration in LS in relation to ES species; P-values are the results of a t-test of the difference between ES and LS species; bold value, P < 0.05.

		All species (18)				Species of different successional groups					
					Diff	ES		LS		Diff	
Element	Unit	Mean	SD	Range	range (%)	Mean	SD	Mean	SD	LS/ES (%)	P-value
C	mg g^{-1}	467 ±	13	448 - 490	9	465 ±	11	469 ±	15	1.0	0.48
N	mg g^{-1}	30.8 ±	6.9	18.1 - 47.1	161	34.3 ±	5.4	26.4 ±	6.3	-23.0	**0.011**
P	mg g^{-1}	2.1 ±	0.5	1.1 - 2.8	157	2.4 ±	0.3	1.7 ±	0.5	-27.7	**0.004**
K	mg g^{-1}	12.1 ±	4.6	4.4 - 21.8	391	12.7 ±	4.1	11.3 ±	5.3	-10.5	0.56
Ca	mg g^{-1}	7.4 ±	2.7	3.2 - 13.2	313	7.0 ±	2.6	7.8 ±	3.0	12.2	0.53
Mg	mg g^{-1}	2.2 ±	0.6	0.8 - 3.6	350	2.3 ±	0.5	2.2 ±	0.8	-5.5	0.68
S	mg g^{-1}	1.8 ±	0.7	0.7 - 2.9	291	2.0 ±	0.8	1.7 ±	0.6	-15.4	0.38
Fe	mg g^{-1}	0.2 ±	0.1	0.08 - 0.60	624	0.22 ±	0.05	0.23 ±	0.16	4.3	0.86
Mn	µg g^{-1}	631 ±	415	38 - 1464	3720	825 ±	402	388 ±	300	-53.0	**0.021**
Zn	µg g^{-1}	20.5 ±	8.8	7.5 - 36.9	392	24.7 ±	8.8	15.4 ±	5.5	-37.8	**0.020**
B	µg g^{-1}	29.7 ±	13.3	17.7 - 76.3	332	27.4 ±	7.0	32.5 ±	18.8	18.9	0.43
Cu	µg g^{-1}	7.3 ±	3.3	2.9 - 14.5	405	9.0 ±	3.2	5.1 ±	2.0	-43.4	**0.009**
Mo	µg g^{-1}	0.17 ±	0.09	0.03 - 0.36	980	0.17 ±	0.09	0.18 ±	0.10	0.6	0.98

Table S4 Mass based leaf nutrient content in 18 species at the high elevation site (HE/Sigira). Values in green > 10% above mean; red < 10% below mean, black, between + and – 10%. ES and LS, early and late successional species; CV, coefficient of variation. Explanation of species codes is given in Table 1.

Species	SG	FT	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)	S (mg/g)	Fe (mg/g)	Zn (µg/g)	Mn (µg/g)	Cu (µg/g)	B (mg/g)	Mo (µg/g)
Bbr	ES	TMF	30.7 ± 0.9	2.4 ± 0.0	9.5 ± 0.3	7.0 ± 0.6	2.1 ± 0.3	2.3 ± 0.2	0.18 ± 0.01	36.9 ± 3.5	1464 ± 265	7.1 ± 1.6	25.7 ± 3.8	0.14 ± 0.02
Hmo	ES	TMF	33.9 ± 1.8	2.5 ± 0.1	8.4 ± 0.9	12.4 ± 1.6	2.8 ± 0.1	2.7 ± 0.1	0.25 ± 0.08	35.9 ± 2.3	1156 ± 350	14.5 ± 1.9	28.7 ± 2.5	0.13 ± 0.03
Mki	ES	TMF	31.1 ± 0.5	2.0 ± 0.2	10.2 ± 1.0	6.2 ± 0.9	2.4 ± 0.3	2.7 ± 0.3	0.21 ± 0.02	19.4 ± 1.7	843 ± 301	8.0 ± 0.7	29.7 ± 5.7	0.12 ± 0.02
Mla	ES	TMF	32.2 ± 2.1	2.3 ± 0.1	15.6 ± 2.0	5.8 ± 0.6	1.9 ± 0.1	2.9 ± 0.6	0.15 ± 0.02	11.0 ± 0.9	168 ± 12	6.9 ± 0.9	30.3 ± 3.2	0.20 ± 0.06
Pfu	ES	TMF	33.2 ± 2.2	2.1 ± 0.1	13.3 ± 0.3	5.6 ± 0.8	2.4 ± 0.2	1.3 ± 0.1	0.27 ± 0.07	20.7 ± 2.4	967 ± 123	6.4 ± 0.6	37.3 ± 3.8	0.11 ± 0.02
Bmi	ES	LVTF	29.7 ± 1.7	2.7 ± 0.4	9.0 ± 1.1	4.4 ± 1.8	1.5 ± 0.6	0.9 ± 0.1	0.26 ± 0.07	31.1 ± 7.1	1000 ± 219	6.1 ± 0.2	19.3 ± 3.5	0.11 ± 0.03
Cme	ES	LVTF	39.7 ± 0.3	2.6 ± 0.3	16.3 ± 4.7	5.4 ± 0.5	2.4 ± 0.2	1.1 ± 0.2	0.25 ± 0.06	16.6 ± 1.5	760 ± 153	11.4 ± 1.5	17.7 ± 4.2	0.34 ± 0.18
Dto	ES	LVTF	47.1 ± 0.5	2.8 ± 0.2	19.3 ± 3.3	7.8 ± 1.1	2.9 ± 0.4	1.5 ± 0.1	0.20 ± 0.01	20.7 ± 1.2	720 ± 56	5.6 ± 0.7	36.3 ± 1.5	0.12 ± 0.02
Hma	ES	LVTF	30.3 ± 1.7	2.0 ± 0.0	8.3 ± 0.8	10.5 ± 0.5	2.7 ± 0.1	2.8 ± 0.2	0.18 ± 0.03	32.9 ± 3.6	986 ± 74	11.4 ± 0.6	30.0 ± 4.6	0.15 ± 0.05
Mlu	ES	LVTF	35.0 ± 2.0	2.7 ± 0.2	16.7 ± 1.3	4.6 ± 0.8	1.7 ± 0.3	1.5 ± 0.2	0.29 ± 0.08	21.5 ± 1.4	185 ± 16	12.9 ± 1.1	18.7 ± 0.6	0.31 ± 0.09
Afa	LS	TMF	20.4 ± 1.6	1.7 ± 0.1	12.0 ± 0.9	7.7 ± 0.5	1.7 ± 0.1	1.1 ± 0.1	0.21 ± 0.01	13.9 ± 0.5	859 ± 146	4.3 ± 0.2	23.7 ± 0.6	0.14 ± 0.03
Cgr	LS	TMF	25.0 ± 1.1	1.9 ± 0.1	7.8 ± 0.5	10.2 ± 0.8	2.2 ± 0.4	2.1 ± 0.1	0.24 ± 0.06	13.8 ± 1.3	174 ± 13	4.5 ± 0.6	37.0 ± 5.0	0.22 ± 0.06
Fsa	LS	TMF	18.1 ± 0.7	1.1 ± 0.1	4.4 ± 0.3	3.2 ± 0.3	0.8 ± 0.1	2.3 ± 0.1	0.08 ± 0.01	7.5 ± 1.2	367 ± 47	5.1 ± 0.2	21.7 ± 4.0	0.03 ± 0.01
Paf	LS	TMF	36.2 ± 2.8	1.8 ± 0.3	13.6 ± 2.5	5.1 ± 1.4	2.0 ± 0.2	0.7 ± 0.1	0.14 ± 0.02	17.4 ± 3.4	112 ± 31	5.2 ± 0.4	19.7 ± 2.9	0.13 ± 0.07
Sgu	LS	TMF	24.0 ± 0.6	1.4 ± 0.0	7.2 ± 0.9	8.2 ± 0.9	2.4 ± 0.3	1.6 ± 0.1	0.13 ± 0.02	8.8 ± 0.2	792 ± 166	4.0 ± 0.2	33.3 ± 4.0	0.08 ± 0.01
Cgo	LS	LVTF	27.8 ± 0.4	1.3 ± 0.1	13.4 ± 1.6	7.8 ± 0.4	2.1 ± 0.3	2.5 ± 0.5	0.60 ± 0.09	17.4 ± 2.4	387 ± 14	2.9 ± 0.4	28.3 ± 4.2	0.36 ± 0.03
Eex	LS	LVTF	25.1 ± 1.2	1.9 ± 0.1	10.5 ± 1.1	7.3 ± 1.0	2.5 ± 0.2	1.8 ± 0.0	0.24 ± 0.04	20.0 ± 3.2	38 ± 4	5.1 ± 0.7	20.3 ± 2.1	0.21 ± 0.05
Fth	LS	LVTF	34.4 ± 3.1	2.8 ± 0.1	21.8 ± 0.3	13.2 ± 3.9	3.6 ± 0.9	1.3 ± 0.1	0.22 ± 0.04	24.1 ± 2.6	371 ± 85	9.8 ± 0.4	76.3 ± 15.3	0.22 ± 0.08
Mean/SD			30.8 ± 6.9	2.1 ± 0.5	12.1 ± 4.6	7.4 ± 2.7	2.2 ± 0.6	1.8 ± 0.7	0.23 ± 0.1	20.5 ± 8.8	631 ± 415	7.3 ± 3.3	29.7 ± 13.3	0.17 ± 0.1
CV			22	25	38	37	27	38	47	43	66	46	45	52
Max to min ratio (%)			161	157	391	313	350	291	624	392	3720	405	332	980
Mean/SD ES			34.3 ± 5.4	2.4 ± 0.3	12.7 ± 4.1	7.0 ± 2.6	2.3 ± 0.5	2.0 ± 0.8	0.22 ± 0.05	24.7 ± 8.8	825 ± 402	9.0 ± 3.2	27.4 ± 7.0	0.2 ± 0.1
Mean/SD LS			26.4 ± 6.3	1.7 ± 0.5	11.3 ± 5.3	7.8 ± 3.0	2.2 ± 0.8	1.7 ± 0.6	0.23 ± 0.16	15.4 ± 5.5	388 ± 300	5.1 ± 2.0	32.5 ± 18.8	0.2 ± 0.1
P-value, ES vs LS			**0.013**	**0.008**	0.57	0.54	0.69	0.37	0.87	**0.015**	**0.018**	**0.007**	0.48	0.98

Table S5. Area based leaf nutrient content in 18 species at the high elevation site (HE/Sigira). Values in green > 10% above mean; red < 10% below mean, black, between + and – 10%. ES and LS, early and late successional species; CV, coefficient of variation. Explanation of species codes is given in Table 1.

Species	SG	FT	N (g/m2)	P (g/m2)	K (g/m2)	Ca (g/m2)	Mg (g/m2)	S (g/m2)	Fe (mg/m2)	Zn (mg/m2)	Mn (mg/m2)	Cu (mg/m2)	B (g/m2)2	Mo (µg/m2)
Bbr	ES	TMF	2.6 ± 0.2	0.21 ± 0.01	0.81 ± 0.01	0.59 ± 0.05	0.18 ± 0.03	0.19 ± 0.02	15.5 ± 0.7	3.1 ± 0.4	124.7 ± 26	0.6 ± 0.14	2.2 ± 0.4	12.1 ± 0.9
Hmo	ES	TMF	3.2 ± 0.2	0.24 ± 0.01	0.80 ± 0.11	1.18 ± 0.11	0.26 ± 0.01	0.26 ± 0.02	23.4 ± 7.5	3.4 ± 0.1	109.7 ± 32	1.4 ± 0.17	2.7 ± 0.3	12.3 ± 2.7
Mki	ES	TMF	2.3 ± 0.0	0.15 ± 0.02	0.76 ± 0.06	0.46 ± 0.06	0.18 ± 0.02	0.20 ± 0.02	15.7 ± 1.4	1.5 ± 0.1	62.8 ± 21	0.6 ± 0.04	2.2 ± 0.4	8.7 ± 1.3
Mla	ES	TMF	2.4 ± 0.1	0.17 ± 0.00	1.18 ± 0.18	0.44 ± 0.05	0.14 ± 0.01	0.22 ± 0.05	11.1 ± 0.8	0.8 ± 0.1	12.7 ± 1	0.5 ± 0.06	2.3 ± 0.2	15.2 ± 4.3
Pfu	ES	TMF	3.0 ± 0.4	0.19 ± 0.02	1.20 ± 0.06	0.51 ± 0.09	0.22 ± 0.03	0.12 ± 0.01	24.8 ± 6.6	1.9 ± 0.2	88.2 ± 17	0.6 ± 0.09	3.4 ± 0.4	10.2 ± 0.7
Bmi	ES	LVTF	3.1 ± 0.3	0.27 ± 0.04	0.92 ± 0.08	0.45 ± 0.16	0.15 ± 0.05	0.10 ± 0.01	26.2 ± 5.7	3.2 ± 0.6	102.0 ± 17	0.6 ± 0.04	2.0 ± 0.3	11.2 ± 2.1
Cme	ES	LVTF	3.0 ± 0.2	0.20 ± 0.01	1.22 ± 0.28	0.41 ± 0.02	0.18 ± 0.02	0.08 ± 0.01	18.9 ± 6.4	1.3 ± 0.0	58.1 ± 16	0.9 ± 0.05	1.3 ± 0.2	25.3 ± 12.7
Dto	ES	LVTF	2.5 ± 0.1	0.15 ± 0.02	1.05 ± 0.23	0.42 ± 0.08	0.16 ± 0.03	0.08 ± 0.00	10.9 ± 0.5	1.1 ± 0.1	38.8 ± 4	0.3 ± 0.06	2.0 ± 0.2	6.3 ± 0.9
Hma	ES	LVTF	3.0 ± 0.1	0.20 ± 0.01	0.82 ± 0.09	1.05 ± 0.12	0.27 ± 0.03	0.28 ± 0.02	18.4 ± 4.0	3.3 ± 0.6	98.3 ± 10	1.1 ± 0.04	3.0 ± 0.6	15.5 ± 5.6
Mlu	ES	LVTF	2.3 ± 0.2	0.17 ± 0.01	1.08 ± 0.14	0.30 ± 0.06	0.11 ± 0.02	0.10 ± 0.01	18.6 ± 5.6	1.4 ± 0.2	12.0 ± 1	0.8 ± 0.05	1.2 ± 0.1	20.1 ± 5.0
Afa	LS	TMF	2.8 ± 0.1	0.23 ± 0.02	1.68 ± 0.22	1.08 ± 0.13	0.24 ± 0.03	0.15 ± 0.02	29.3 ± 1.7	1.9 ± 0.1	120.5 ± 27	0.6 ± 0.01	3.3 ± 0.2	20.1 ± 5.1
Cgr	LS	TMF	2.7 ± 0.2	0.21 ± 0.02	0.85 ± 0.04	1.11 ± 0.08	0.24 ± 0.04	0.23 ± 0.02	26.7 ± 7.5	1.5 ± 0.1	19.0 ± 1	0.5 ± 0.08	4.1 ± 0.7	24.2 ± 5.9
Fsa	LS	TMF	2.6 ± 0.1	0.16 ± 0.01	0.64 ± 0.00	0.47 ± 0.04	0.11 ± 0.00	0.33 ± 0.01	12.1 ± 1.1	1.1 ± 0.1	53.3 ± 7	0.7 ± 0.01	3.1 ± 0.4	4.9 ± 1.1
Paf	LS	TMF	3.0 ± 0.1	0.15 ± 0.02	1.13 ± 0.18	0.42 ± 0.12	0.17 ± 0.02	0.06 ± 0.01	11.7 ± 1.9	1.4 ± 0.3	9.3 ± 2	0.4 ± 0.05	1.6 ± 0.3	11.0 ± 6.5
Sgu	LS	TMF	3.3 ± 0.2	0.20 ± 0.01	1.00 ± 0.12	1.14 ± 0.10	0.33 ± 0.03	0.22 ± 0.02	18.6 ± 2.9	1.2 ± 0.0	110.0 ± 21	0.6 ± 0.03	4.6 ± 0.7	10.7 ± 1.0
Cgo	LS	LVTF	2.5 ± 0.1	0.12 ± 0.01	1.22 ± 0.14	0.72 ± 0.04	0.20 ± 0.02	0.23 ± 0.04	55.2 ± 8.1	1.6 ± 0.2	35.4 ± 2	0.3 ± 0.04	2.6 ± 0.4	32.9 ± 3.5
Eex	LS	LVTF	3.1 ± 0.2	0.24 ± 0.02	1.29 ± 0.10	0.89 ± 0.11	0.30 ± 0.02	0.22 ± 0.01	29.0 ± 4.2	2.4 ± 0.4	4.7 ± 0	0.6 ± 0.09	2.5 ± 0.3	26.2 ± 5.4
Fth	LS	LVTF	3.8 ± 0.6	0.30 ± 0.03	2.39 ± 0.15	1.47 ± 0.54	0.40 ± 0.12	0.14 ± 0.02	24.0 ± 5.6	2.6 ± 0.1	41.1 ± 12	1.1 ± 0.12	8.5 ± 2.3	23.7 ± 7.0
Mean/SD			2.9 ± 0.4	0.20 ± 0.05	1.11 ± 0.40	0.7 ± 0.36	0.21 ± 0.08	0.18 ± 0.08	21.7 ± 10.4	1.9 ± 0.9	61.1 ± 42	0.7 ± 0.3	2.9 ± 1.6	16.2 ± 7.9
CV			14	24	36	49	36	43	48	44	69	42	56	49
Max to min ratio (%)			68	163	271	389	254	436	404	307	2554	424	601	576
Mean/SD ES			2.7 ± 0.4	0.19 ± 0.04	0.98 ± 0.2	0.58 ± 0.29	0.19 ± 0.1	0.16 ± 0.08	18.36 ± 5.3	2.1 ± 1.0	71 ± 40	0.7 ± 0.3	2.2 ± 0.7	13.7 ± 5.6
Mean/SD LS			3.0 ± 0.4	0.20 ± 0.06	1.28 ± 0.5	0.91 ± 0.36	0.25 ± 0.1	0.20 ± 0.08	25.83 ± 13.8	1.7 ± 0.6	49 ± 44	0.6 ± 0.2	3.8 ± 2.1	19.2 ± 9.5
P-value, ES vs LS			0.20	0.78	0.19	0.054	0.11	0.36	0.18	0.363	0.301	0.288	0.077	0.17

Table S6. Leaf element ratios at the high elevation site (HE/Sigira) of N to C and other nutrients to N and values potentially below the limitation*. ES and LS, early and late successional species; CV, coefficient of variation. Explanation of species codes is given in Table 1. Values in green > 10% above mean; red < 10% below mean, black, between + and – 10%. Values marked with grey are below limiting ratio shown at the last two lines. If two levels of limiting ratios are identified, values below the upper but above the lower limiting values are marked in light grey, while values below the lower are marked in the dark grey.

Species	SG	FT	N/C (%)	P/N (%)	K/N (%)	Ca/N (%)	Mg/N (%)	S/N (%)	Fe/N (%)	Zn/N (%)	Mn/N (%)	Cu/N (ppm)	B/N (ppm)	Mo/N (ppm)
Bbr	ES	TMF	6.9 ± 0.2	7.96 ± 0.3	31.1 ± 1.7	22.8 ± 2.1	6.7 ± 0.9	7.4 ± 0.5	0.60 ± 0.05	0.120 ± 0.008	4.8 ± 0.78	231 ± 49	834 ± 107	4.7 ± 0.6
Hmo	ES	TMF	7.1 ± 0.2	7.32 ± 0.3	24.8 ± 3.6	36.7 ± 5.6	8.2 ± 0.5	8.1 ± 0.7	0.72 ± 0.21	0.106 ± 0.009	3.4 ± 0.89	427 ± 41	845 ± 47	3.9 ± 1.1
Mki	ES	TMF	6.6 ± 0.1	6.38 ± 0.6	32.8 ± 2.6	19.9 ± 2.6	7.8 ± 0.7	8.7 ± 0.7	0.68 ± 0.06	0.063 ± 0.004	2.7 ± 0.93	256 ± 18	953 ± 168	3.8 ± 0.5
Mla	ES	TMF	6.8 ± 0.1	7.09 ± 0.3	48.7 ± 7.4	18.2 ± 2.9	6.0 ± 0.4	9.0 ± 2.3	0.46 ± 0.04	0.034 ± 0.003	0.5 ± 0.07	214 ± 37	947 ± 130	6.2 ± 1.4
Pfu	ES	TMF	6.9 ± 0.6	6.20 ± 0.0	40.3 ± 3.2	16.9 ± 1.5	7.1 ± 0.3	3.9 ± 0.5	0.82 ± 0.15	0.062 ± 0.006	2.9 ± 0.28	194 ± 8	1134 ± 177	3.4 ± 0.6
Bmi	ES	LVTF	6.5 ± 0.1	9.10 ± 2.0	30.5 ± 5.3	15.2 ± 6.9	5.1 ± 2.2	3.1 ± 0.2	0.87 ± 0.28	0.106 ± 0.030	3.4 ± 0.95	205 ± 16	655 ± 154	3.7 ± 1.1
Cme	ES	LVTF	8.8 ± 0.2	6.54 ± 0.7	41.2 ± 11.9	13.7 ± 1.2	6.1 ± 0.4	2.8 ± 0.5	0.62 ± 0.16	0.042 ± 0.004	1.9 ± 0.38	288 ± 37	446 ± 108	8.7 ± 4.7
Dto	ES	LVTF	10.2 ± 0.2	6.03 ± 0.5	41.1 ± 7.1	16.6 ± 2.4	6.2 ± 0.8	3.2 ± 0.2	0.43 ± 0.01	0.044 ± 0.003	1.5 ± 0.13	119 ± 17	772 ± 39	2.5 ± 0.4
Hma	ES	LVTF	6.6 ± 0.4	6.54 ± 0.4	27.4 ± 3.6	34.8 ± 3.0	9.0 ± 0.9	9.3 ± 0.8	0.61 ± 0.12	0.109 ± 0.015	3.3 ± 0.39	377 ± 2	995 ± 173	5.1 ± 1.7
Mlu	ES	LVTF	7.5 ± 0.4	7.60 ± 0.5	47.8 ± 3.5	13.3 ± 2.1	4.9 ± 0.7	4.3 ± 0.8	0.83 ± 0.28	0.062 ± 0.005	0.5 ± 0.07	370 ± 49	535 ± 44	9.0 ± 2.7
Afa	LS	TMF	4.5 ± 0.2	8.27 ± 1.0	59.2 ± 9.0	38.2 ± 5.6	8.3 ± 1.1	5.3 ± 0.6	1.03 ± 0.06	0.068 ± 0.003	4.3 ± 1.06	212 ± 6	1164 ± 101	7.1 ± 1.8
Cgr	LS	TMF	5.5 ± 0.3	7.71 ± 0.5	31.3 ± 1.9	40.8 ± 5.1	8.9 ± 2.0	8.4 ± 0.5	0.97 ± 0.20	0.055 ± 0.008	0.7 ± 0.08	181 ± 15	1475 ± 139	9.0 ± 2.8
Fsa	LS	TMF	3.7 ± 0.2	6.11 ± 0.1	24.5 ± 0.9	17.7 ± 1.0	4.4 ± 0.3	12.6 ± 0.2	0.46 ± 0.03	0.042 ± 0.007	2.0 ± 0.19	282 ± 5	1197 ± 192	1.9 ± 0.4
Paf	LS	TMF	7.6 ± 0.6	5.01 ± 0.5	37.4 ± 4.3	14.1 ± 3.9	5.6 ± 1.0	2.0 ± 0.4	0.39 ± 0.06	0.048 ± 0.008	0.3 ± 0.06	145 ± 18	551 ± 128	3.7 ± 2.4
Sgu	LS	TMF	5.2 ± 0.2	5.85 ± 0.3	29.8 ± 3.1	34.2 ± 4.6	10.0 ± 1.4	6.7 ± 0.2	0.55 ± 0.05	0.036 ± 0.002	3.3 ± 0.78	166 ± 6	1384 ± 134	3.2 ± 0.2
Cgo	LS	LVTF	5.9 ± 0.1	4.53 ± 0.3	48.2 ± 6.3	28.2 ± 1.1	7.7 ± 1.0	8.9 ± 1.7	2.17 ± 0.29	0.062 ± 0.008	1.4 ± 0.04	103 ± 16	1019 ± 151	13.0 ± 1.3
Eex	LS	LVTF	5.2 ± 0.3	7.65 ± 0.1	41.9 ± 5.3	29.0 ± 2.6	9.8 ± 0.5	7.2 ± 0.3	0.95 ± 0.20	0.079 ± 0.009	0.2 ± 0.02	201 ± 19	812 ± 109	8.4 ± 1.4
Fth	LS	LVTF	7.7 ± 1.1	8.03 ± 0.4	63.7 ± 6.8	38.1 ± 9.2	10.3 ± 1.8	3.8 ± 0.1	0.63 ± 0.06	0.071 ± 0.013	1.1 ± 0.16	286 ± 15	2220 ± 387	6.6 ± 2.9
Mean/SD			6.6 ± 1.5	6.9 ± 1.18	39.0 ± 11.3	24.9 ± 9.95	7.3 ± 1.82	6.4 ± 2.91	0.77 ± 0	0.07 ± 0.027	2.1 ± 1.43	236 ± 89	997 ± 415	5.8 ± 2.9
CV			23	17	29	40	25	46	52	40	67	38	42	51
Max to min ratio (%)			177	101	159	207	135	513	457	249	3003	313	398	598
Mean/SD ES			7.4 ± 1.2	7.1 ± 0.9	36.6 ± 8.4	20.8 ± 8.4	6.7 ± 1.3	6.0 ± 2.7	0.66 ± 0.15	0.07 ± 0.03	2.5 ± 1.4	268 ± 97	812 ± 214	5.1 ± 2.2
Mean/SD LS			5.6 ± 1.4	6.6 ± 1.5	42.0 ± 14.1	30.0 ± 9.8	8.1 ± 2.1	6.8 ± 3.3	0.89 ± 0.57	0.06 ± 0.02	1.7 ± 1.5	197 ± 63	1228 ± 500	6.6 ± 3.6
P-value, ES vs LS			**0.014**	0.49	0.36	0.053	0.13	0.56	0.30	0.16	0.23	0.079	0.055	0.32
Limitation, lower				8	30	2.0	3.3	4.0	0.1	0.04	0.03	100	500	NA
Limitation, upper				10	35	2.5	3.5		0.2	0.05	0.05	300		NA

*The limiting values are derived from: Ericsson and Kähr (1993, 1995), Göransson (1993), Göransson (1994), Göransson (1998), Göransson (1999), Knecht and Göransson (2004) and Linder (1995). See Table S1 for limiting values.

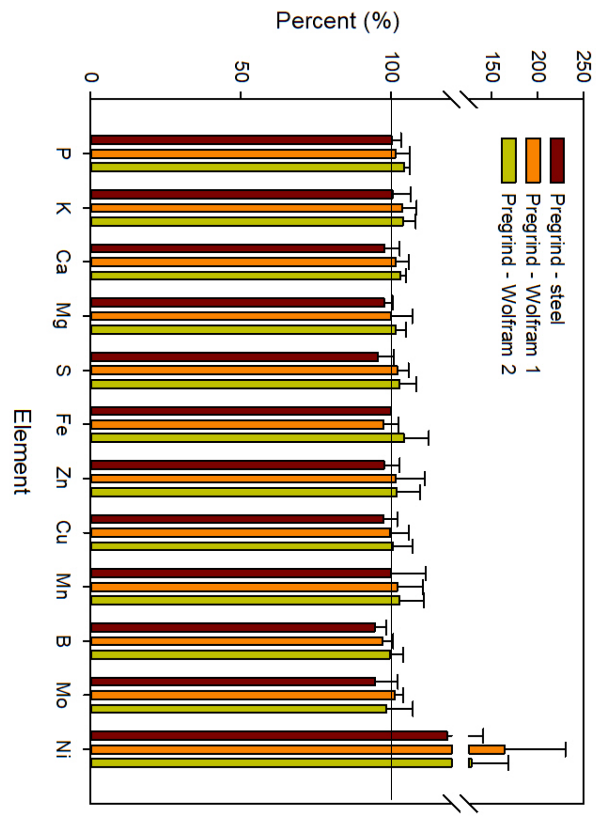

Figure S1. Effects of griding method and materials using a ball mill on leaf element composition. Previous to the ball mill grinding, the leaves were pregrind in a coffee grinder or disintegrated by hand wearing lab glows. Three types of grinding jars (steel and two types of Wolfram carbide jars, old (1) and new (2)) were tested. The results are given as element concentration in percent of disintegrating the leaves by hand and using the new Wolfram carbide jar (2). Leaves from *Rhododendron sp*. and *Ilex aquifolium* sampled in the Botanical garden of Gothenburg were used. They were dried in 70 °C before gridning. For each sample and species, 2 replicates were used. No statistically significant difference between methods was obtained.

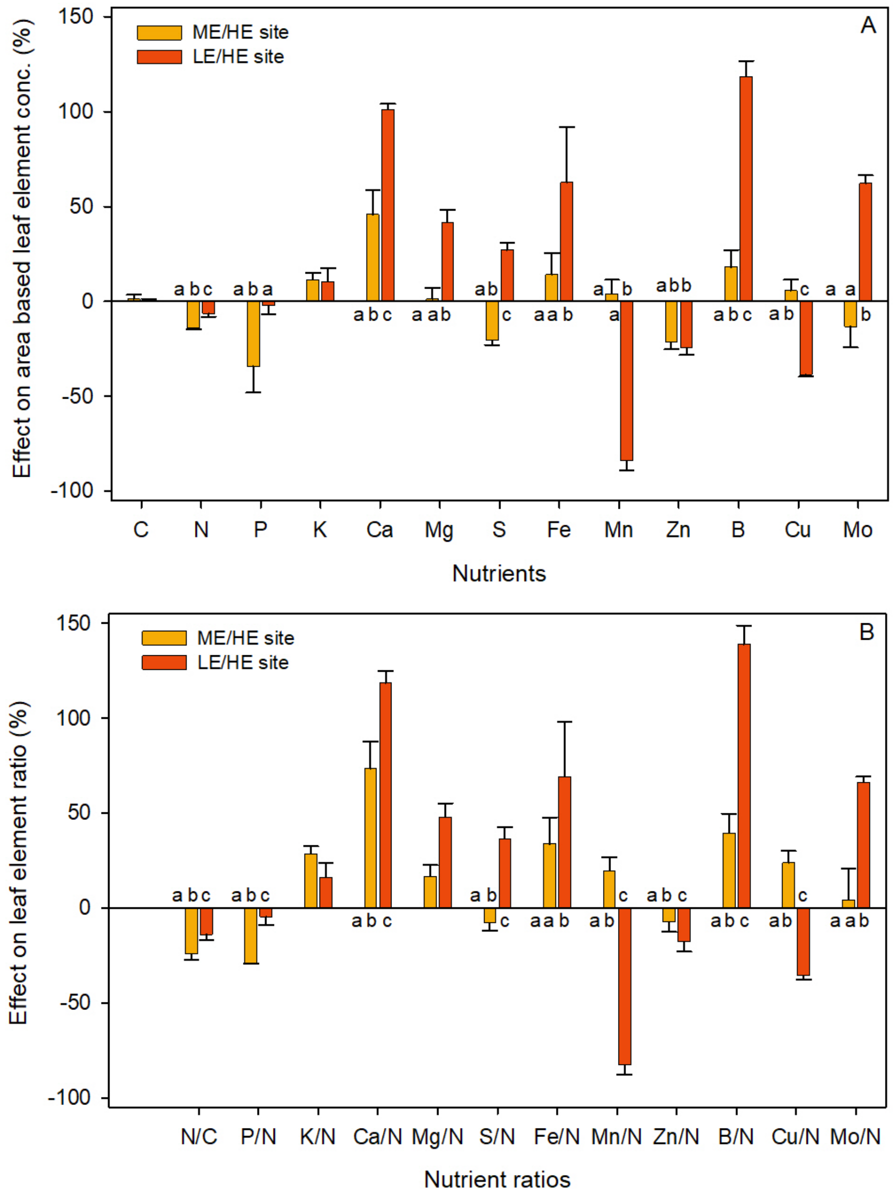

Figure S2 A, B. The effect on area (A) and element ratio to N (B) of leaf nutrients along the elevation gradient expressed as percent change at ME and LE sites relative to the HE site, i.e. HE site is the zero line. Different letters (a, b, c, first letter for each nutrient represents the HE site) denote significant difference between sites. HE, high; ME, mid; LE, low elevation site. See Table 6 for results from statistical analysis.

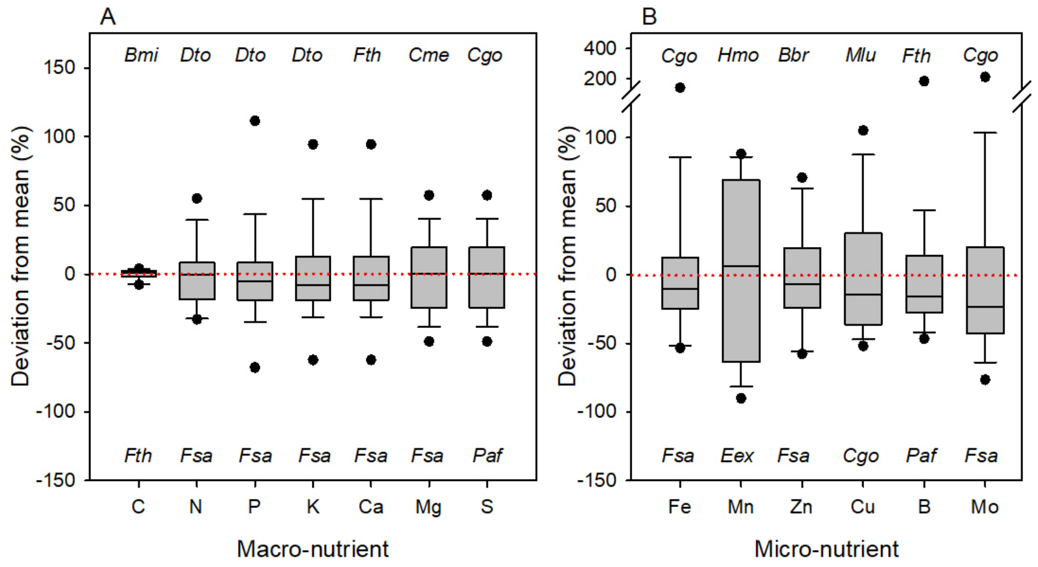

Figure S3. A-B. Deviation of leaf concentration of macro (A) and micronutrients (B) in percent of mean across all species (n=18). The boundary of the box indicates the 25th and 75th percentile, the error bars indicate the 90th and 10th percentiles, the line within the box marks the median and the red dotted line indicates the mean. The black dots indicate the lowest and highest values for the species indicated by the abbreviations at the lower and upper part of the figures, respectively. Note that some of the upper black dots in b are above the break of the y-axis.

Paper III

forests

MDPI

Article

Climate Sensitivity of Tropical Trees Along an Elevation Gradient in Rwanda

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Abstract: Elevation gradients offer excellent opportunities to explore the climate sensitivity of vegetation. Here, we investigated elevation patterns of structural, chemical, and physiological traits in tropical tree species along a 1700–2700 m elevation gradient in Rwanda, central Africa. Two early-successional (*Polyscias fulva*, *Macaranga kilimandscharica*) and two late-successional (*Syzygium guineense*, *Carapa grandiflora*) species that are abundant in the area and present along the entire gradient were investigated. We found that elevation patterns in leaf stomatal conductance (g_s), transpiration (E), net photosynthesis (A_n), and water-use efficiency were highly season-dependent. In the wet season, there was no clear variation in g_s or A_n with elevation, while E was lower at cooler high-elevation sites. In the dry season, g_s, A_n, and E were all lower at drier low elevation sites. The leaf-to-air temperature difference was smallest in *P. fulva*, which also had the highest g_s and E. Water-use efficiency (A_n/E) increased with elevation in the wet season, but not in the dry season. Leaf nutrient ratios indicated that trees at all sites are mostly P limited and the N:P ratio did not decrease with increasing elevation. Our finding of strongly decreased gas exchange at lower sites in the dry season suggests that both transpiration and primary production would decline in a climate with more pronounced dry periods. Furthermore, we showed that N limitation does not increase with elevation in the forests studied, as otherwise most commonly reported for tropical montane forests.

Keywords: elevation gradient; leaf traits; photosynthesis; Rwanda; stomatal conductance; transpiration; tropical trees

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1. Introduction

Tropical forests play a significant role as a carbon sink, currently dampening the ongoing rise in atmospheric CO_2 [1]. Through their high rates of evapotranspiration, they also strongly control land hydrology and energy partitioning, thus affecting both local and regional climate [2]. In spite of the importance of tropical forests for global, regional, and local climate and climate change, their climate sensitivity is poorly known compared to that of temperate and boreal forests [3–5]. Knowledge gaps are particularly severe for tropical montane forests [6–8]. The montane tropics are likely to undergo considerable change under global warming, with plants facing dual challenges of climatic change and invading lowland species [8,9]. A better understanding of the climate sensitivity of tropical tree species is fundamental to forecasting these changes.

Tropical species in general are considered to have a narrow thermal niche as a consequence of the small temporal variation in temperature [10,11]. They are also thought to operate at, or near, their physiological thermo-optimum, such that warming might be negative for physiological performance and growth [12–14]. The threat of climate change to tropical tree species is likely to be particularly severe for tree species with a limited ability to migrate, such as large-seeded species and montane species that are already at the top [9,15]. The speed of climate warming is generally higher than the potential speed of montane tree species migration to a higher altitude to maintain their thermal niches [15]. This will compromise the growth and survival of tropical trees unless they can thermally acclimate to warmer conditions. There is evidence of recent changes in tree community composition in montane rainforests, driven primarily by increased tree mortality within the warmer part of a given species' distribution range [16].

Not only warming, but also more pronounced rainfall seasonality and increased frequency and severity of droughts, have been projected for many tropical forests in the future [17]. Lower soil water availability strongly limits CO_2 and H_2O fluxes between tropical ecosystems and the atmosphere during drier periods [18,19]. Low precipitation and heat may interact such that heat increases the severity of drought stress and vice versa, as observed in controlled experiments [20]. Warming leads to more pronounced droughts as a consequence of increased atmospheric vapor pressure deficit (VPD) and evapotranspiration. Drought stress may cause increased heat stress due to the limited capacity for transpirational leaf cooling under dry conditions. Increased knowledge about these interactions is crucial to forecast forest–atmosphere interactions in a changing climate.

There are indications that late-successional (LS) species are more sensitive to warming than early-successional (ES) species. In a controlled experiment with seedlings, warming negatively affected the growth and photosynthesis of LS species, but not ES species [21]. In the same experiment, LS species had lower photosynthetic thermal optimum temperatures than ES species [22]. In a common garden experiment with seedlings of tropical tree species in Rwanda, photosynthetic heat stress was considerably higher in montane LS species compared to ES or exotic plantation species [23]. This was the combined result of low stomatal conductance (g_s) and a large leaf size, leading to higher leaf temperatures (T_{leaf}) in LS species. The thermal optima of photosynthetic biochemistry did not differ among species in that study, suggesting that interspecific variation in warming responses might be more strongly linked to leaf traits controlling the leaf energy balance than to differences in biochemical temperature sensitivities. It should be noted that all the studies mentioned above were conducted on seedlings and their relevance for mature trees in the field is thus uncertain.

Elevation gradients offer the potential to study climate change responses of montane plants and ecosystems. Of course, factors other than temperature co-vary with elevation, such as cloudiness and radiation, precipitation, soil conditions, and atmospheric absolute O_2 and CO_2 concentrations. However, with careful interpretation, elevation gradients can serve as laboratories for global change research to explore the temperature and precipitation controls on plant resource acquisition and ecosystem functioning under ecologically realistic conditions [7,24]. Previous tropical elevation gradient studies have shown that leaf transpiration (E) typically decreases with increasing elevation, likely as a result of decreasing temperature and VPD at a higher elevation [25–27]. For photosynthesis, however, different tropical studies have reported different altitudinal trends. Light-saturated net photosynthesis (A_{sat}) did not vary with elevation in a dominant tree species in Hawaiian forest ecosystems along a 100–2500 m gradient [28]. Similarly, there was no change in A_{sat} with elevation in 40 tropical tree species along a 1000–3000 m elevation gradient in the Ecuadorian Andes [29]. However, there have also been reports of both decreases [30,31] and increases in A_{sat} with elevation in other studies [32,33]. A Rwandan study specifically investigating biochemical photosynthetic capacity (at a given temperature) at two elevations demonstrated higher values at a high elevation [34]. A few studies have also investigated the $^{13}C/^{12}C$ carbon isotope composition of leaf tissues to explore possible patterns in water-use efficiency with elevation. They all reported higher $\delta^{13}C$ ratios, and thus lower ^{13}C discrimination, with increasing elevation [35–37]. This implies that the so-called intrinsic water-use efficiency (iWUE), which is the ratio of net photosynthesis (A_n) to g_s, increased with elevation in these studies.

A limitation of these previous studies with different tree species is that sites at different elevations also had different tree species, making it hard to distinguish between the roles played by genetic adaptation and acclimation in explaining observed differences in tree and forest traits. Such separation requires the study of the same species (within a narrow genotype range) along the entire gradient. This is the approach taken in the present study.

The common view is that nutrient limitations change with elevation in tropical regions, such that lowland forests are typically phosphorous (P) limited, while cooler montane forests are rather nitrogen (N) limited due to slower microbial N mineralization rates [38–40]. Indeed, decreasing leaf N content or N:P ratio with increasing altitude has been observed in several Neotropical studies [35,41–43], as well as in Hawaiian montane forests [36]. However, a study in Borneo reported that leaf N and P contents expressed per unit leaf area increased with altitude [44]. In addition, a recent study showed that tropical montane trees in Rwanda exhibited high leaf N concentrations and intermediate N:P ratios compared to what has been reported for other tropical montane forests, indicating P limitation or N-P co-limitation [45]. This study did not explore if relative nutrient limitations (e.g., N:P ratio) varied with elevation, since it was conducted in plots at rather similar elevations. Together, the above-mentioned studies show that there is no simple pattern in terms of how N limitations vary along elevation gradients in tropical forests, and that more research is needed to explore how these depend on regional and local conditions.

The overall aim of this study is to explore how leaf and tree traits and resource use vary with climate along a tropical elevation gradient in Rwanda, located on the Albertine Rift in central Africa. We measured structural, chemical, and physiological traits in a set of species with contrasting successional strategies to address the following hypotheses: (i) Leaf transpiration decreases while photosynthesis is relatively stable and water use efficiency increases with elevation; (ii) leaf temperatures greatly exceed air temperatures in late-successional species, but less so in early-successional species with higher transpiration and smaller leaves; and (iii) N limitation does not increase with elevation, as otherwise most commonly reported.

2. Materials and Methods

2.1. Sites, Climate, Species, and Measurement Campaigns

This study was conducted in Ruhande Arboretum ("Arboretum" hereafter) plus four sites in Nyungwe National Park ("Nyungwe" hereafter). The Arboretum is a forest plantation located in the transitional tropical rainforest zone in southern Rwanda (2°36′55.2″ S, 29°44′53.8″ E, 1638–1737 m elevation; [46]). The Arboretum was established in 1934 and since then, 227 tree species (50 native to Rwanda) have been planted, in most part, as replicated ($n = 3$) monospecific 50 × 50 m plots within its 200 ha plantation area. Nyungwe is a tropical montane rainforest located in southwestern Rwanda (2°17′–2°50′ S, 29°07′–29°26′ E, 1600–2950 m elevation; [47]). Nyungwe National Park is one of the top priority sites for conservation in the Albertine Rift based upon the numbers of endemic and globally threatened species [48]. Nyungwe covers 1013 km^2 and forms, together with the contiguous Kibira national park in Burundi, the largest block of tropical montane forest remaining in Africa [49].

Dominant natural vegetation zones in Rwanda are: Afromontane rainforest, Lake Victoria transitional rainforest, and evergreen and semi-evergreen bushland and thicket [50]. This study was conducted at sites in montane (Nyungwe) and transitional (Arboretum) rainforest areas. Overall, temperature decreases and precipitation increases with increasing elevation, but there is also an east-west gradient such that precipitation at a given elevation is lower further east (Table 1). The dry season in Nyungwe is in July and August, while it may last from May to September and is more pronounced in the Arboretum [46,51].

Data were collected at five sites along an elevational gradient: The lowest and warmest Arboretum site (A) at ~1700 m elevation and four sites in Nyungwe: Cyamudongo (C; 1800–1900 m), Nyungwe West (N1) (~1950 m), Nyungwe East (N2; ~2500 m), and Nyungwe Bigugu Mountain (N3; ~2700 m). While N1, N2, and N3 are all within the main forest block of Nyungwe, C is a smaller (4.3 km^2) relict forest area, located at 10 km west of the Nyungwe forest and surrounded by agricultural land. The elevation gradient ranging from ~1700 m to ~2700 m spanned a range of mean annual temperatures (MAT) at 1.7 m of approximately 13–19 °C (Table 1). Additionally, mean daytime temperatures and minimum (1%ile) and maximum (99%ile) temperatures declined systematically with increasing elevation. VPD was markedly lower and radiation somewhat lower at a high elevation. Annual precipitation spanned from ~1000 mm in A to ~3000 mm in N1. The three-month period preceding and covering the measurement campaigns during wet and dry seasons was markedly warmer, drier, and exhibited higher VPD at the low A site compared with N1 and N2. Both temperature and precipitation were measured over several years with weather stations at three of the sites (A, N1, N2; [46,51]). At C and N3, only temperature and air humidity at 1.7 m above the ground were measured using small temperature mini-loggers (Model TinyTag Plus 2, Gemini data loggers Ltd., United Kingdom) during a period of six to seven weeks (September to November 2017) to allow for comparisons with the other sites.

Table 1. Elevation, annual weather, and weather before and during experimental periods at the sites along the elevation gradient. MAT, mean annual air temperature; T day, mean annual daytime temperature; T 1%ile, T 99%ile, the warmest and coldest temperature percentile, respectively; VPD day, mean annual daytime vapor pressure deficit; MAP, mean annual precipitation; PPFD day, annual mean daytime photosynthetic photon flux density; T 10–16 and VPD 10–16, the average T and VPD between 10–16 h during the experimental periods, respectively; P 3 month, the total three-month precipitation before and during the experimental periods; Dry experiment period, 2–30 September 2017; wet periods, 15 February to 21 March 2017 and 24 January to 4 March 2018. Daytime is when PPFD > 2 µmol m^{-2} s^{-1}. Variation is given as standard deviation between years.

Parameter	Arboretum (A)	Cyamudongo (C)	Nyungwe West (N1)	Nyungwe East (N2)	Bigugu Mountain (N3)
Elevation (m a.s.l.)	1700	1850	1950	2500	2700
MAT (°C)	19.5 ± 0.2	17.9	16.2 ± 0.4	14.3 ± 0.2	13.3
T day (°C)	21.1 ± 0.1	19.2	18.2 ± 0.5	15.6 ± 0.2	14.7
T 1%ile (°C)	16.0 ± 0.5		13.3 ± 0.4	11.7 ± 0.3	
T 99%ile (°C)	24.1 ± 0.2		20.4 ± 0.3	18.1 ± 0.2	
MAP (mm)	979 ± 206		3016 ± 63	1657 ± 163	
VPD-day (kPa)	1.02 ± 0.04		0.44 ± 0.06	0.39 ± 0.09	
PPFD day (µmol m^{-2} s^{-1})	733 ± 25		572 ± 17	633 ± 32	
Experiment-dry					
T 10–16 (°C)	23.3		18.6	17.0	
VPD 10–16 (kPa)	1.48		0.27	0.40	
P 3 month (mm)	135			309	
Experiment-wet					
T 10–16 (°C)	22.6 ± 0.8		17.8 ± 0.4	16.9 ± 0.3	
VPD 10–16 (kPa)	1.27 ± 0.09		0.11 ± 0.01	0.27 ± 0.01	
P 3 month (mm)	360 ± 185			499 ± 125	

MAT and MAP at A, N1 and N2 are based on 4 years measurement (June 2013–May 2017), except MAP at N2 which was based on 2 years measurement (June 2013–May 2015). MAT at C and N3 are scaled from 6 weeks measurements based on long term studies at neighboring sites.

Data were collected from two early-successional (ES) and two late- successional (LS) species which are abundant in Nyungwe and could mostly be found along the entire elevation gradient: *Syzygium guineense* (LS), *Carapa grandiflora* (LS), *Macaranga kilimandscharica* (ES), and *Polyscias fulva* (ES). The first three species are the three most abundant tree species in Nyungwe, together accounting for 42% of the large trees (DBH > 30 cm) in the forest [49]. There were two cases where a species could not be found at one of the sites: *S. guineense* was not found at Cyamudongo (1800–1900 m elevation) and *C. grandiflora* was not present at 2700 m altitude as this species is distributed between 1600–2500 m in Rwanda [52]. Further extension of the elevation gradient was not possible due to species distribution ranges; Nyungwe lacks large trees much beyond 2700 m in Nyungwe (highest peak at 2950 m). Six trees per species were sampled at each site.

Data collection was conducted during three campaigns: two wet season campaigns between 15 February and 21 March in 2017 and between 24 January and 4 March in 2018; and one dry season campaign during 2–30 September in 2017. Six trees per species and site were measured in each campaign, aiming at selecting trees with diameters at breast height (DBH) of 15–40 cm. Such trees were found, except for *Polyscias fulva* at N3, where smaller trees had to be sampled (Table S1). The same trees were measured during each campaign.

2.2. Leaf Gas Exchange

Leaf gas exchange was measured for one site per day with the order of species on each day being randomized. Measurements were conducted during 10:00–16:00 h. Three leaves per tree were measured, resulting in a total of 72 measurements (4 species × 6 trees × 3 leaves) per site and measurement campaign. A 20 m long carbon fiber telescopic pole was used to access branches in the upper canopy and the measurements were taken as soon as possible after the branch was cut (usually within 15 min) to minimize possible post-cut decreases in g_s. Gas exchange measurements were made with a LI6400 (Li-COR, Inc. Lincoln: Nebraska, NE, USA) during the latter two campaigns (one wet and one dry) and with an SC-1 porometer (Decagon devices Inc. Pullman: Washington, DC, USA) during the first campaign (wet). In a separate data collection, both instruments were used to measure g_s on the same leaves (and leaf parts) of different species exhibiting a broad range of g_s. This instrument comparison showed that the SC-1 measured considerably higher g_s than the LI6400 (Figure S1). The relationship between the SC-1 and LI6400 data (equation in Figure S1) was used to adjust g_s data collected by the SC-1 since the LI6400 was considered more reliable.

Measurements with the LI6400 were made using the standard 2 × 3 cm leaf chamber and light source (6400-02B LED Light Source, LI-COR Biosciences: Lincoln, NE, USA) using a flow rate of 300–400 µmol m^{-2} s^{-1}. The photosynthetic photon flux density (PPFD) inside the leaf chamber was set at 1800 µmol m^{-2} s^{-1}. The CO$_2$ of air entering the leaf chamber was set at 415 µmol mol^{-1}, leading to a CO$_2$ concentration near 400 µmol m^{-2} s^{-1} inside the leaf chamber. The chamber air temperature was set to approximate ambient and was changed throughout the measurement day. The air desiccant of the instrument was not used.

2.3. Leaf Temperature

The same leaves measured for gas exchange were also measured for leaf temperature (T_{leaf}). This was done just after the gas exchange measurement, using an infrared thermometer (Trotec BP10 IR, Trotec Laser GmbH: Ismaning, Germany). When measuring T_{leaf}, the leaf was held in a horizontal position, with the thermometer at a 45° angle to the leaf and a distance of 5 cm from the leaf surface to avoid leaf shading. Wind speed (u) varied considerably among measurement days. To account for the influence of varying wind speed on T_{leaf}, all observations of the leaf-to-air temperature difference ($T_{\mathrm{leaf}} - T_{\mathrm{air}}$) were standardized to a wind speed of 1 m s^{-1} as follows:

The leaf boundary layer conductance for heat (g_{heat}) was calculated as

$$g_{\mathrm{heat}} = 0.135 * \left(\frac{u}{d}\right)^{0.5} \tag{1}$$

Values of g_{heat} were calculated both at measured wind speed (g_{heatobs}) and at a wind speed of 1 m s^{-1} (g_{heatstd}). The standardized $T_{\mathrm{leaf}} - T_{\mathrm{air}}$ at a 1 m s^{-1} wind speed (ΔT_{std}) was then calculated from the ratio of these conductances and the observed T_{leaf}–T_{air} (ΔT_{obs}):

$$\Delta T_{\mathrm{std}} = \Delta T_{\mathrm{obs}} * \frac{g_{\mathrm{heatobs}}}{g_{\mathrm{heatstd}}} \tag{2}$$

Air temperature and wind speed were measured adjacent to the leaf at the time of T_{leaf} measurements, using a portable weather station (WMR86N, Oregon Scientific, Tualatin, OR, USA). PPFD was measured adjacent to the leaf at a horizontal angle, using a portable PPFD sensor (MQ-500, Apogee Instruments, Inc.: Logan, UT, USA). The portable weather station was placed in the shade, while the PPFD sensor was exposed to the sky.

2.4. Tree and Leaf Structural Traits

Tree stem DBH was determined using diameter tape, while tree height was measured using a clinometer (Vertex IV, Haglöfs Sweden AB: Långsele, Sweden). Wood density was measured by taking wood cores at the breast height with an increment borer (Haglöf Sweden AB: Långsele, Sweden). Thereafter, the diameter (5.15 mm) and length (below bark to centre of the stem) of the fresh cores and the mass of the oven dried (70 °C) cores were used to calculate the wood density (g cm^{-3}).

Leaf structural traits were measured on the same leaves measured for gas exchange. Leaf length and width were measured to the nearest mm by a ruler. Leaf thickness was measured at three locations of each leaf, to the nearest 0.01 mm using an electronic caliper, avoiding major veins. On each measured leaf, three to five leaf discs of a known area were sampled and were oven-dried at 70 °C for at least 48 h and then weighed for leaf mass per unit leaf area (LMA) determination.

2.5. Chemical Traits

A proxy of area-based leaf chlorophyll content was measured using an SPAD-502 meter (Konica Minolta Sensing, Inc., Ltd.: Osaka, Japan). For each leaf, the average SPAD value was calculated from 10 measurements evenly distributed across the leaf surface. Analyses of leaf nutrient content and stable carbon isotope composition were done on dried leaf material from the first measurement campaign. Dry leaf samples were ground into a fine powder using a ball mill (model MM 301, Retsch: Haan, Germany). Samples were analyzed for C and N and their stable isotopes (^{12}C, ^{13}C, ^{14}N, ^{15}N) using a continuous flow isotope ratio mass spectrometer (UC Davis Stable Isotope Facility: Davis, CA, USA). A set of 37 non-N elements were determined using elements inductively coupled plasma mass spectrometry (VG101 analysis, ACME Analytical Laboratories: Vancover, BC, Canada), including the plant macronutrients P, K, S, Ca, and Mg and the micronutrients Cu, Zn, Mn, Fe, and B.

Values of ^{13}C abundance in air (δ_a) and leaf tissue (δ_l) expressed in per mil (‰) relative to the standard Pee Dee Belemnite were used to calculate leaf ^{13}C discrimination (Δ_{leaf}; [53]) as:

$$\Delta_{\text{leaf}} = \frac{\delta_a - \delta_l}{1 + \delta_l} \tag{3}$$

Values of δ_{leaf} were calculated from leaf samples analyzed for stable C isotopic composition as described above and δ_a was taken as $-8.4‰$.

The ratio of the CO_2 concentration inside the leaf (c_i) to that of the surrounding air (c_a) was calculated from Δ_{leaf} as:

$$\frac{c_i}{c_a} = (\Delta_{\text{leaf}} - a)/(b - a) \tag{4}$$

where a and b are constants representing fractionation due to diffusion (4.4‰) and carboxylation (27‰), respectively, and c_a and c_i represent the partial pressure of CO_2 ($p$$CO_2$) in the ambient atmosphere and inside the leaf, respectively [53]. The iWUE determined based on carbon isotope data was calculated as c_a minus c_i, which equals the A_n:g_s ratio, with g_s expressed for CO_2 instead of H_2O (as otherwise reported here).

2.6. Statistical Analyses

Differences among sites and among species for the different leaf structural, physiological, and chemical traits were tested using two-way ANOVA with site and species as fixed factors. Pairwise multi-comparisons among individual sites and species were conducted with the Tukey HSD test. Averages of data from different leaves on the same tree were used in these tests to treat trees as the statistical unit. Wet and dry season data were tested separately. Wet season data were very similar for the two wet season measurement campaigns and were therefore averaged across both campaigns prior to analysis. Differences in $T_{\text{leaf}} - T_{\text{air}}$ among species were tested using analysis of covariance (ANCOVA) with species as a fixed factor and PPFD as a covariate. Data were pooled across sites in this test due to the large variability in radiation among sites measured on different days. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 16.0, IBM Corporation: Armonk, NY, USA).

3. Results

First, two comments on how we evaluate elevation patterns and species differences in the following. The order of species measured were different (randomized) on different measurement days and sites (we measured one site per day). This is the main reason for the many site by species interactions in our statistical tests (Table S2). We therefore focus on the main effects of sites and species in our interpretations, not on the numerous site by species interactions. Furthermore, elevation patterns were evaluated based on the pattern of among-site differences revealed by the posthoc tests. There had to be a consistent and directional change with elevation for concluding that there was an elevation pattern.

3.1. Physiological Traits

3.1.1. Leaf Gas Exchange

In both wet and dry seasons, g_s differed significantly among species, as well as among sites at different elevations ($p < 0.001$; Figure 1a,b; Table S2). During the dry season g_s markedly increased with increasing elevation, while there was no clear relationship between g_s and elevation in the wet season. In the wet season, the only site standing out was the mid-elevation site (N1), which had significantly higher g_s compared to all other sites. This was the case in the wet season measurement campaigns of both 2017 and 2018 and was not an artefact of measuring at too low VPD inside the leaf chamber (it was ~0.5 kPa; data not shown).

Transpiration rates mostly decreased with elevation in the wet season, while there was a strong increasing trend in the dry season (Figure 1c,d). The wet season trend did not, however, hold along the entire elevation gradient since the lowest site was not significantly different compared to any other site.

Species differences were significant for g_s in both the wet and dry season, but for E, only in the wet season (Figure 1a–d; Table S2). The ES species *P. fulva* mostly had higher g_s and E than other species during both wet and dry seasons (Figure 1a–d). The difference was particularly strong for g_s in site N1 during the wet season; an observation made in both wet season campaigns. In addition, the ES species *M. kilimandscharica* had higher g_s and E than the LS species *C. grandiflora* in both seasons. In both wet and dry seasons, g_s and E decreased in the order *P. fulva*, *M. kilimandscharica*, *S. guineense*, and *C. grandiflora*. These results thus show that g_s and E are higher in ES species than in LS species.

Elevational trends in A_{sat} were also highly season dependent and there were significant differences among sites ($p \leq 0.001$) and species ($p \leq 0.005$) during both wet and dry seasons (Figure 1e,f; Table S2). During the wet season, there was no overall change with elevation as A_{sat} was highest at intermediate elevation sites. During the dry season, however, A_{sat} markedly increased with elevation. Species differences in A_{sat} were similar to those for g_s and E, i.e., decreasing in the order *P. fulva*, *M. kilimandscharica*, *S. guineense*, and *C. grandiflora*. The LS species *C. grandiflora* ($p \leq 0.002$) had lower A_{sat} than the ES species *P. fulva* and *M. kilimandscharica* during both seasons (Table S2).

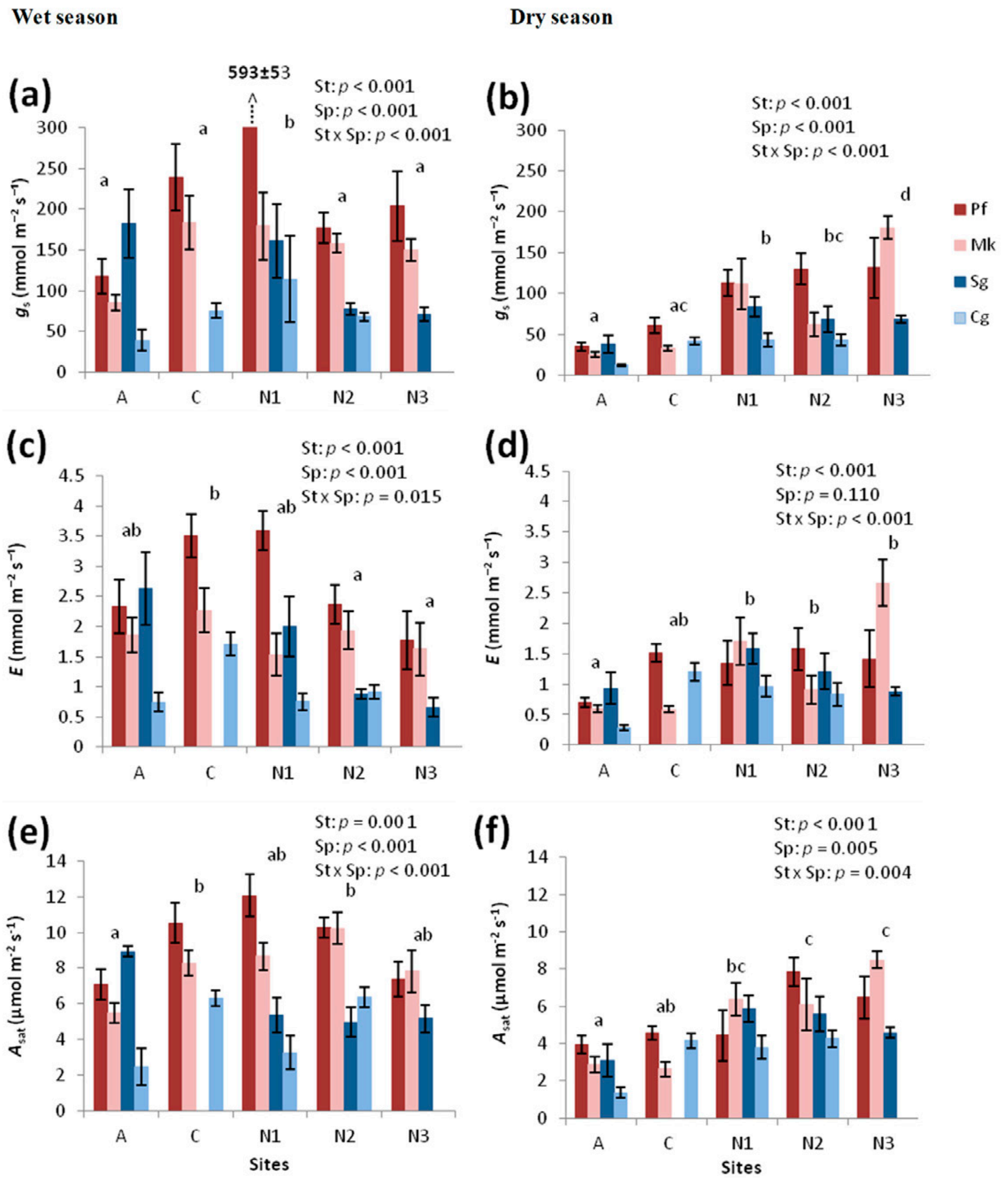

Figure 1. Leaf stomatal conductance (g_s; (**a**,**b**)), transpiration (E; (**c**,**d**)), and light saturated net photosynthesis (A_{sat}; (**e**,**f**)) of four species (Sp) at five sites (St) along an elevation gradient, measured during wet (**a**,**c**,**e**) and dry (**b**,**d**,**f**) seasons. Error bars show variation (SE) among trees within each species at each site (n = 6). P values for the effects of site (St), species (Sp), and their interaction (St × Sp) are shown in each graph. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations are provided in Table 1; the order follows increasing elevation from left to right. Species abbreviations are based on first letters in genus and species: Pf = *Polyscias fulva*; Mk = *Macaranga kilimandscharica*; Sg = *Syzygium guineense*; Cg = *Carapa grandiflora*.

The elevation trends in WUE (i.e., the A_n:E ratio) determined from gas exchange measurements were highly seasonal dependent. In the wet season, WUE increased significantly with elevation, while no clear elevation trend was present in the dry season (Figure 2a,b). The LS species *S. guineense* had higher WUE than other species in the wet season, but there were no significant differences among species in the dry season (Table S2).

There was no consistent change in iWUE (i.e., the A_n:g_s ratio) with elevation in the wet season, but iWUE strongly decreased with elevation during the dry season (Figure 2c,d). Values of iWUE decreased in the order *C. grandiflora*, *S.guineense*, *M. kilimandscharica*, and *P. fulva*; i.e., it was higher in LS compared to ES species. However, the only significant species differences were *C. grandiflora* versus *P. fulva* in the wet season and *C. grandiflora* compared to all other species in the dry season (Table S2).

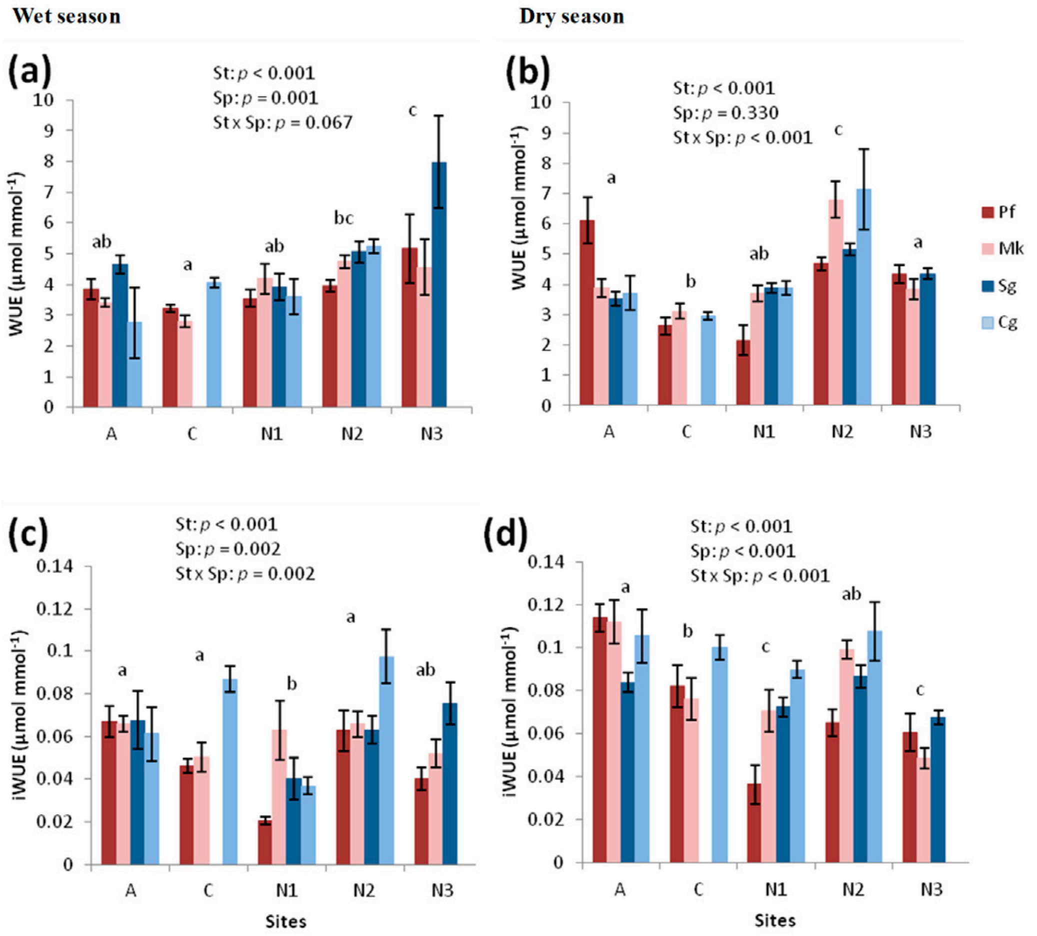

Figure 2. Water use efficiency (WUE = A_n/E; (**a**,**b**)) and intrinsic water use efficiency (iWUE = A_n/g_s; (**c**,**d**)) determined by gas exchange measurements. Data are for four species (Sp) at five sites (St) along an elevation gradient, measured during wet (**a**,**c**) and dry (**b**,**d**) seasons. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations and species names are provided in Table 1 and Figure 1, respectively; the site order follows increasing elevation from left to right.

3.1.2. iWUE from Stable Carbon Isotopes

There was no clear elevation trend in iWUE (i.e., $c_a - c_a$ = the A_n:g_s ratio with g_s expressed for CO_2) based on stable carbon isotope data, although it was higher at the lowest site compared with three out of four of the other sites (Figure 3). The ES species *P. fluva* had higher iWUE than all other species (Table S2).

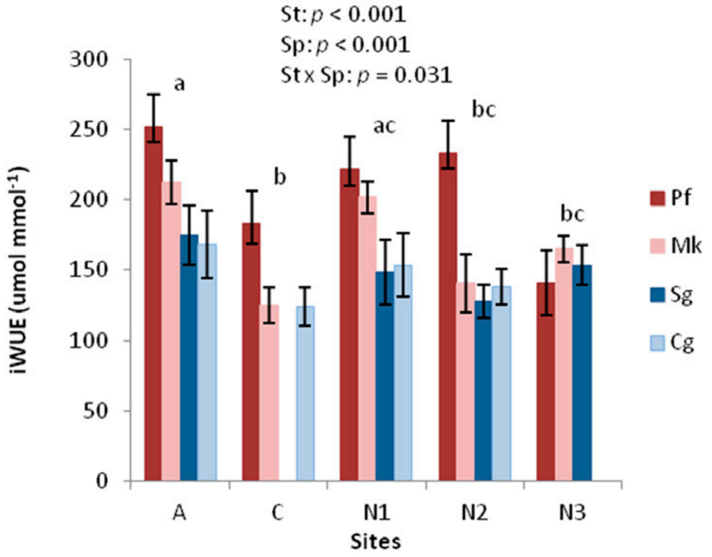

Figure 3. Intrinsic water use efficiency (iWUE = A_n/g_s) from stable carbon isotope analysis of four species (Sp) at five sites (St) along an elevation gradient. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations and species names are provided in Table 1 and Figure 1, respectively; the site order follows increasing elevation from left to right.

3.1.3. Leaf Temperature

The difference in leaf temperatures ($T_{\text{leaf}} - T_{\text{air}}$), standardized to a wind speed of 1 m s^{-1}, increased with radiation (Figure 4). The slopes of the relationships between PPFD and $T_{\text{leaf}} - T_{\text{air}}$ were markedly different among species according to the ANCOVA test ($p < 0.001$). At high PPFD, $T_{\text{leaf}} - T_{\text{air}}$ was lower in the ES species *P. fulva* than in the other species. At PPFD > 2000 µmol m^{-2} s^{-1}, T_{leaf}, on average, exceeded T_{air} by 3–5 °C in *P. fulva* compared to 7–10 °C in the other three species. This result agrees well with the higher g_s and E of this species, promoting leaf cooling. Observed T_{leaf} values at sunny conditions were frequently around 35 °C and sometimes up to 40 °C, with lower values observed at higher elevation sites and in *P. fulva* (Figure S2).

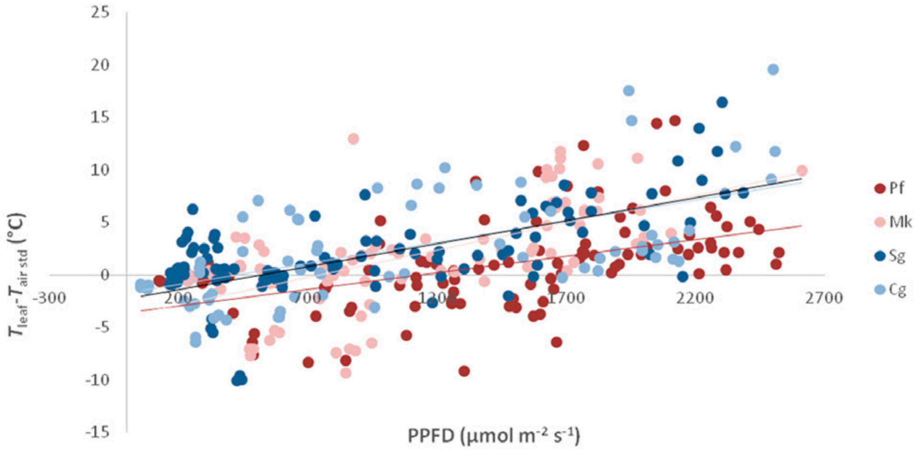

Figure 4. Relationship between leaf to-air temperature difference ($T_{\text{leaf}} - T_{\text{air}}$) and photosynthetic photon flux density (PFD) for all species in the wet season of 2017, with data pooled across sites. The slopes of the relationships were markedly different among species according to the ANCOVA test ($p < 0.001$). Values of $T_{\text{leaf}} - T_{\text{air}}$ were standardized to a wind speed of 1 m s^{-1} (see Materials and methods). Species abbreviations are explained in Figure 1.

3.2. Structural Traits

Wood density slightly declined with elevation, while LMA did not exhibit any consistent elevation trend (Figure 5). Across all sites, LMA did not vary among species, while wood density was highest in the two LS species and intermediate and lowest in the ES species *M. kilimandscharica* and *P. fulva*, respectively (Table S2).

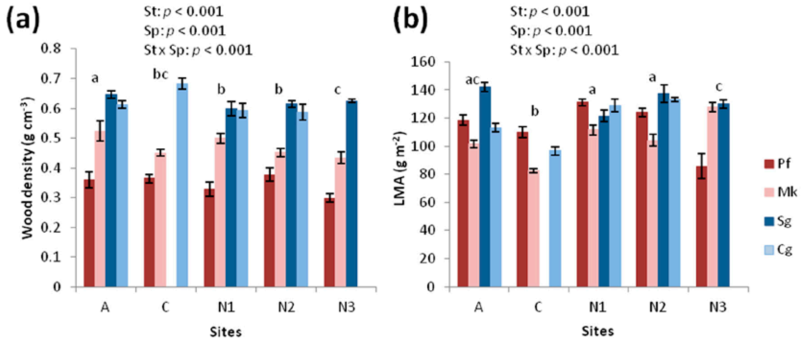

Figure 5. Leaf structural traits, wood density (**a**), and leaf mass per area (LMA; (**b**)) of four species (Sp) at five sites (St) along an elevation gradient. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations and species names are provided in Table 1 and Figure 1, respectively; the site order follows increasing elevation from left to right.

3.3. Chemical Traits

There were no clear trends with elevation for neither mass- nor area-based leaf N or P (Figure 6). Mass-based leaf N and P concentrations were overall higher in the ES species *P. fulva* than in all the other species, while area-based leaf N and P values were lower in *M. kilimandscharica* than in *P. fulva* and *C. grandiflora*.

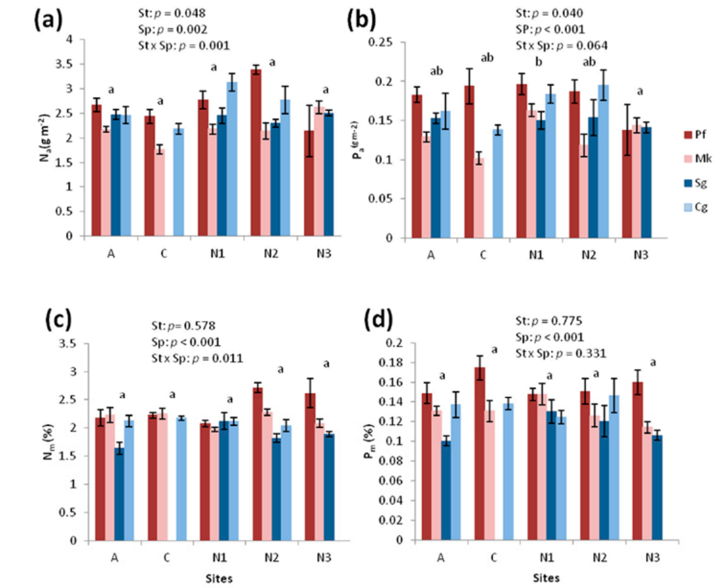

Figure 6. Leaf nitrogen per unit leaf area (N_a; (**a**)), phosphorus per unit leaf area (P_a; (**b**)), N per unit leaf mass (N_m; (**c**)), and P per unit leaf mass (P_m; (**d**)) in four species (Sp) at five sites (St) along an elevation gradient. Error bars represent standard error. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations and species names are provided in Table 1 and Figure 1, respectively; the site order follows increasing elevation from left to right.

The N:P ratio did not differ significantly among either sites ($p = 0.101$) or species ($p = 0.794$; Figure 7; Table S2). It ranged between 13–19 for all site by species combinations (Figure 7) and although site differences were not statistically significant, the average values were higher at the two highest sites (17.2 and 17.7) than at the other three sites (15.2–16.0; Table 1). The data thus clearly show that N limitation did not increase with elevation in our forests.

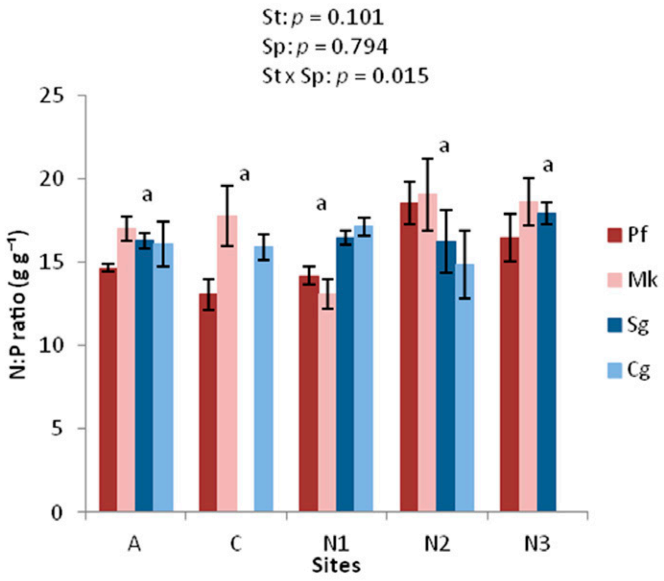

Figure 7. Nitrogen to phosphorus ratio (N:P ratio) of four species (Sp) at five sites (St) along an elevation gradient. Error bars represent standard error. Significant overall differences among sites are indicated by different letters above the bars of each site. Explanations of site abbreviations and species names are provided in Table 1 and Figure 1, respectively; the site order follows increasing elevation from left to right.

Among all other leaf nutrients, P was the only one exhibiting values below optimal ratios to N reported in the literature (Table 2). In addition, K had slightly lower mean values than the optimal ratios at the lowest and highest elevation sites. This indicates P (co-)limitation in our forests, while there seems to be no limitations by K, S, Cu, Zn, Mn, Fe, Ca, B, and Mg.

Table 2. Chemical leaf traits at all sites (mean ± SE).

Trait	A	C	N1	N2	N3	Optimal Ratios [a]
P_a (g m^{-2})	0.156 ± 0.011	0.144 ± 0.026	0.173 ± 0.010	0.163 ± 0.017	0.140 ± 0.002	
N_a (g m^{-2})	2.45 ± 0.102	2.13 ± 0.196	2.64 ± 0.203	2.65 ± 0.280	2.42 ± 0.146	
P_m (%)	0.129 ± 0.010	0.142 ± 0.011	0.137 ± 0.006	0.135 ± 0.007	0.126 ± 0.016	
N_m (%)	2.04 ± 0.135	2.09 ± 0.128	2.07 ± 0.033	2.21 ± 0.193	2.19 ± 0.218	
N/P (g g^{-1})	16.0 ± 0.493	15.6 ± 1.37	15.2 ± 0.948	17.2 ± 0.989	17.7 ± 0.619	
P/N (%)	6.34 ± 0.198	6.72 ± 0.622	6.72 ± 0.447	6.29 ± 0.449	5.83 ± 0.243	8–10
K/N (%)	29.6 ± 5.21	47.8 ± 10.0	31.6 ± 4.71	39.2 ± 3.26	27.8 ± 3.31	30–35
S/N (%)	12.6 ± 1.50	8.53 ± 1.92	10.7 ± 1.84	7.88 ± 1.57	6.99 ± 0.938	4
Cu/N (%)	0.033 ± 0.003	0.035 ± 0.003	0.053 ± 0.005	0.039 ± 0.005	0.034 ± 0.003	0.01–0.03
Zn/N (%)	0.094 ± 0.026	0.123 ± 0.029	0.128 ± 0.047	0.080 ± 0.013	0.060 ± 0.008	0.04–0.05
Mn/N (%)	3.47 ± 1.48	2.50 ± 0.807	2.82 ± 1.27	0.926 ± 0.167	2.02 ± 0.696	0.03–0.05
Fe/N (%)	2.99 ± 0.591	2.71 ± 0.499	2.52 ± 0.376	2.92 ± 0.895	3.42 ± 0.553	0.13–0.2
Ca/N (%)	47.8 ± 4.69	37.4 ± 3.84	35.6 ± 4.43	20.1 ± 2.56	28.8 ± 7.41	2–2.5
B/N (%)	0.190 ± 0.063	0.117 ± 0.004	0.090 ± 0.015	0.083 ± 0.014	0.082 ± 0.006	0.05
Mg/N (%)	22.0 ± 2.61	11.8 ± 1.03	14.9 ± 1.36	10.3 ± 0.945	12.8 ± 3.62	3.3–5
SPAD	55.0 ± 3.60	52.9 ± 5.18	57.7 ± 5.83	56.2 ± 6.92	47.9 ± 0.745	

[a] The optimal nutrient ratios are referred to here as those nutrients when all nutrients limit growth simultaneously. The values are given for nutrient to N ratios in leaves and are taken from literature [54–61].

The proxy of leaf chlorophyll content (SPAD) was lowest at the highest site, but otherwise did not show any clear trend with elevation (Table 2). Species-specific SPAD values decreased in the order *C. grandiflora* (71), *S.guineense* (52), *P. fulva* (50), and *M. kilimandscharica* (47). It was thus higher in LS species compared to ES species and there was a significant difference among all species, except between *S. guineense* and *P. fulva* (Table S2).

4. Discussion

This study investigated the climate change sensitivity of tropical montane trees by exploring spatial and temporal patterns in tree resource acquisition (carbon, water, nutrients) along an elevation gradient. While most previous tropical elevation gradient studies have measured different tree species at different elevations, we focused on the same set of species along the entire gradient. Altitudinal patterns observed here therefore reflect responses of tree species to varying climatic conditions, not changes in species composition with elevation. The advantage of our approach is that it has high relevance for ongoing climate change, where trees present today will experience substantial changes in temperature, and in some areas also in rainfall, during their lifetime.

4.1. Elevation Patterns in Gas Exchange

We observed several elevation trends in gas exchange. These were not related to leaf nutrients (Figures 6 and 7; Table 2) or structural traits (Figure 5), which did not vary with elevation. Temporal and spatial patterns in gas exchange thus reflect dynamic responses to varying climatic conditions rather than the acclimation of structural or chemical leaf and tree traits.

It turned out that our first hypothesis—predicting decreasing leaf E, constant A_{sat}, and increasing WUE with increasing elevation—was too simplistic. While it was correct for the wet season, patterns were very different in the dry season, when g_s, E, and A_n all increased with elevation (Figures 1 and 2). These results reflect that the atmospheric evaporative demand (i.e., VPD; Table 1) controls E during wet periods, while stomatal control over E dominates under drier conditions (i.e., at lower sites during the dry period). This is consistent with earlier studies in tropical lowland forests in Borneo [62], Panama [63], and central Amazonia [64].

At the three highest sites, values of A_n were quite similar in wet and dry seasons, indicating that drought stress is minor or non-existent throughout the year in these montane rainforests (Figure 1e,f). The lack of elevation trend in A_n in the wet season is consistent with earlier studies reporting no change in A_n along tropical elevation gradients in Ecuador, Hawaii, and Peru [28,29,65]. At the two lowest elevation sites, however, A_n was markedly suppressed during the dry season, as a result of large drops in g_s (Figure 1b,f). Altitudinal variation in A_n thus seem small or non-existent if soil moisture is high at both high and low elevation, while A_n is suppressed where the dry season is more pronounced at lower elevation.

Patterns in WUE and iWUE measured by gas exchange reflect those of g_s, E, and A_{sat} (Figure 2). WUE increased with elevation in the wet season (as A_n did not change but E decreased), but not in the dry season (similar increases in both A_n and E). Intrinsic WUE (iWUE) did not change with elevation in the wet season, but declined with elevation in the dry season (as A_n increased less than g_s). The results on iWUE based on stable C isotopes (Figure 3) were similar to those based on gas exchange during the wet season (Figure 2c), indicating that leaf isotopic composition is mostly determined by wet season photosynthesis. This suggests that leaf formation occurs in the wet season in our species and forests, at least at lower elevations. Taken together, the results on WUE and iWUE indicate that the photosynthetic capacity varies little with elevation, while the control over elevation patterns in E is dominated by VPD in the wet season and by g_s during the dry season.

Although there were numerous site by species interactions (Table S2), the overall trends in gas exchange were generally similar in the four species of this study (Figures 1 and 2). However, species differed greatly with respect to the magnitude of gas exchange variables. Not surprisingly, values of

g_s, E, and A_n were generally higher in ES compared to LS species, consistent with earlier studies on tropical trees (e.g., [34,66]).

Our results indicate that transpiration and CO_2 uptake of tropical montane forests may decline in a climate with more pronounced dry periods. Climate change projections for tropical forests generally indicate future warming, as well as more pronounced rainfall seasonality and increased frequency and severity of droughts [17]. In southern and eastern regions of Rwanda, both temperature and precipitation have increased from 1964 to 2010 [67]. A temperature increase has also been observed in Nyungwe, where our own measurements show a significant increase of 0.4 °C over the last 11 years (p = 0.016). These trends are projected to continue during coming decades [67]. If this will lead to an increased or decreased frequency and severity of droughts will critically depend on the balance between changes in precipitation and potential evapotranspiration (PET). In most of Rwanda, PET exceed precipitation during the main dry season. If the increase in temperature and PET is larger than the possible but uncertain increase in precipitation during this period, dry periods will become more pronounced in the future. This is the current expectation for Rwanda [67].

4.2. Leaf Temperature

The leaf-to-air temperature difference ($T_{\text{leaf}} - T_{\text{air}}$) was smallest in the ES species *P. fulva* (Figure 4), which also had the highest g_s and E in the wet season when T_{leaf} was measured (Figure 1a,c). This result provided partial support for our second hypothesis, stating that T_{leaf} greatly exceeds T_{air} in LS species, but less so in ES species with higher transpiration. It is unclear why the other ES species, *M. kilimandscharica*, did not have lower $T_{\text{leaf}} - T_{\text{air}}$ values than the LS species *C. grandiflora*, although the former species had higher E values (Table S2). It should be noted, however, that the scatter in $T_{\text{leaf}} - T_{\text{air}}$ data (although standardized to a common wind speed of 1 m s^{-1}) was rather large, making it difficult to detect possible small species differences.

Our results on T_{leaf} are in line with earlier reports of $T_{\text{leaf}} - T_{\text{air}}$ in tropical tree species. In a common garden experiment with tropical tree seedlings in Rwanda, T_{leaf} exceed T_{air} by around 10 °C in LS tropical montane tree species, while exceedances were smaller (3–6 °C) in other species with higher g_s and smaller leaves (ES tropical montane species and exotic plantation species; [23]). Similarly, $T_{\text{leaf}} - T_{\text{air}}$ values of up to 10 °C were also observed in a semi-deciduous tropical forest canopy in Panama [68]. Such large values of $T_{\text{leaf}} - T_{\text{air}}$ may have large implications for tropical tree photosynthesis. At the leaf-level, the optimum temperature for A_n may be greatly exceeded by T_{leaf}, but not by T_{air} [23]. At the canopy-level, a recent study based on Eddy Covariance data from seven tropical forests demonstrated that the declines in canopy CO_2 uptake at high temperatures were caused primarily by VPD-driven declines in g_s [69]. Such declines are likely larger when $T_{\text{leaf}} - T_{\text{air}}$, and thus also leaf-to-air VPD, is high.

Earlier controlled warming experiments [21,22], as well as the seedling common garden experiment in Rwanda [23], have indicated that LS species may be more sensitive to high T_{air} than ES species, at least at the seedling stage. The present study corroborates this for mature trees by showing that species with lower g_s and E values (which is typical in LS species) have higher T_{leaf} values than ES species with higher g_s and E values. Declines in A_n on hot days are thus likely to be larger in LS compared to ES species, regardless of whether they have lower optimum temperatures of photosynthetic biochemistry [22] or not [23]. Such negative heat effects are likely more pronounced at a low elevation, where T_{leaf} was higher (Figure S2) and perhaps also local genotypes had a higher heat sensitivity of PSII, as observed in a study comparing provenances of European beech with different altitudes of origin [70].

4.3. Nutrient Limitations

Judging from leaf nutrient ratios, N limitation did not increase with elevation in our forests. There were no significant trends in leaf N or P, expressed on neither mass- nor area-basis (Figure 6). The N/P ratio did not change significantly either (Figure 7) and it indicated P limitation rather than N limitation at all sites (Table 2). For non-P nutrients, their ratios to N were higher than the optimal values reported in the literature (Table 2), similar to the results of [45]. These results thus supported our third hypothesis that N limitation does not increase with elevation.

Previous studies in Nyungwe montane rainforest have shown that it has higher net primary productivity and trees with a higher leaf N concentration and photosynthetic capacity compared to many other tropical montane forests [34,51]. Furthermore, a study on soil N dynamics in Nyungwe reported an open and leaky N cycle [71]. These studies together demonstrate high N availability in Nyungwe. We additionally show here that there is not the slightest indication of increasing N limitations with elevation. Actually, there was an indication that the N:P ratio increased at high elevation, but this was not statistically significant (p = 0.101; Figure 7, Table S2).

Our results contrast with observations of increasing N limitation at high elevation along Neotropical elevation gradients [35,41,43], but are similar to results from a study on Borneo [44]. The general view that tropical montane forests are N rather than P limited [36,38–40] should therefore be treated with caution. Nutrient limitations of tropical montane forests vary greatly among regions and likely strongly depend on geological history.

5. Conclusions and Implications

We explored the climate sensitivity of resource use in tropical montane trees by measuring structural, physiological, and chemical traits in four tree species present along a tropical elevation gradient ranging from 1700 to 2700 m in Rwanda. Our findings highlighted a strong seasonality in altitudinal patterns. In the dry season, g_s, E, and A_n were higher at a high elevation, where drought was less pronounced, while patterns were absent or the opposite (for E) in the wet season. The leaf-to-air temperature difference was smallest in the ES species *P. fulva*, which also had the highest g_s and E values. With respect to leaf nutrient status, there was no change in leaf N, P, or N/P ratio with elevation. Moreover, nutrient ratios relative to N were higher than previously reported optimal values, except for P, indicating that tree growth is mostly P limited in Rwandan tropical forests. In summary, our results demonstrate pronounced seasonality in altitudinal patterns of tropical tree water use and CO_2 uptake, indicating decreasing transpiration and primary production in a climate with more pronounced dry periods. Furthermore, they show that N limitations do not increase with elevation, as otherwise most commonly reported for tropical montane forests.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/9/10/647/s1, Figure S1: Relationship between g_s measured by LI6400 and SC-1 instruments, Figure S2: Relationship between leaf temperature (T_{leaf}) and photosynthetic photon flux density (PFD) for all species at five sites along an elevation gradient in the wet season 2017. Species abbreviations are based on first letters in genus and species: Pf = *Polyscias fulva*; Mk = *Macaranga kilimandscharica*; Sg = *Syzygium guineense*; Cg = *Carapa grandiflora*. Explanations of site abbreviations are provided in Table 1, Table S1: Structural traits (Mean ± SD) of four species at five sites along an elevation gradient (n = 6). Explanations of site abbreviations are provided in Table 1; the order follows increasing elevation from left to right. Species abbreviations represent first letters in genus and species, Table S2: Statistical analyses for all leaf traits; physiological, structural and chemical traits as well as post hoc comparisons among sites.

Author Contributions: M.M., G.W. and J.U. planned and designed the experiment with important contributions from D.N., and B.N. (Brigitte Nyirambangutse); A.M., B.N. (Bonaventure Ntirugulirwa), M.M., E.Z., D.G., and E.N.B. conducted field measurements and data compilations; M.M. and J.U. analysed the data and wrote the paper, with G.W. providing important editorial advice. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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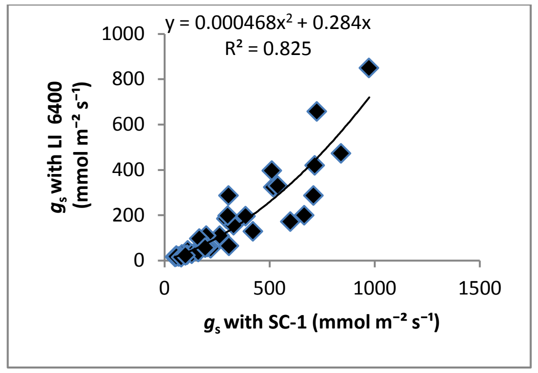

Figure S1. Relationship between g_s measured by LI6400 and SC-1 instruments

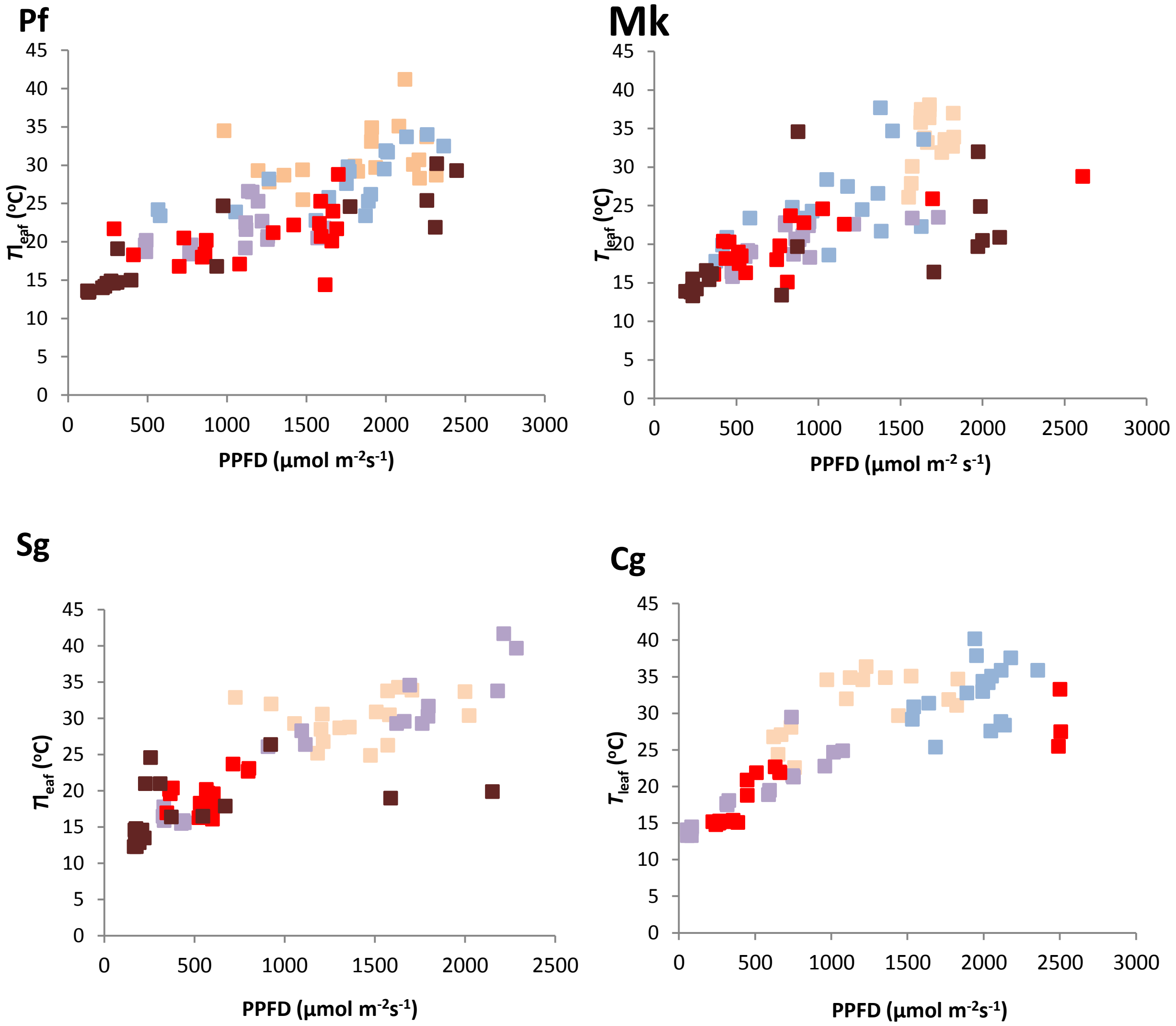

Figure S2. Relationship between leaf temperature (*T*leaf) and photosynthetic photon flux density (PFD) for all species at five sites along an elevation gradient in the wet season 2017. Species abbreviations are based on first letters in genus and species: Pf = *Polyscias fulva*; Mk = *Macaranga kilimandscharica*; Sg = *Syzygium guineense*; Cg = *Carapa grandiflora*. Explanations of site abbreviations are provided in Table 1

Table S1. Structural traits (Mean ± SD) of four species at five sites along an elevation gradient (n=6). Explanations of site abbreviations are provided in Table 1; the order follows increasing elevation from left to right. Species abbreviations represent first letters in genus and species.

Species	**Sites**	**DBH(cm)**	**Height (m)**	**Wood density (g cm^3)**	**LMA (g m^{-2})**
Pf	A	22 ± 5.2	23 ± 2	0.35 ± 0.03	118± 1
	C	39 ± 21	23 ± 2	0.36 ± 0.05	131 ± 7
	N1	27 ± 17	18 ± 5	0.32 ± 0.07	134 ± 1
	N2	48 ± 17	24 ± 4	0.37 ± 0.05	126 ± 2
	N3	8 ± 2	7 ± 7	0.29 ± 0.03	95 ± 4
Mk	A	17 ± 6	15± 3	0.52 ± 0.08	97 ± 8
	C	23 ± 9	19 ± 5	0.44 ± 0.03	93 ± 2
	N1	20 ± 4	15 ± 2	0.49 ± 0.04	111± 1
	N2	21 ± 9	19 ± 5	0.45 ± 0.03	104 ± 10
	N3	30 ± 11	19± 2	0.43 ± 0.05	135 ± 2
Cg	A	25 ± 5	19 ± 2	0.61 ± 0.03	109 ± 10
	C	29 ± 18	11 ± 4	0.68 ± 0.05	100 ± 2
	N1	17 ± 5	17 ± 6	0.59 ± 0.06	122 ± 2
	N2	25 ± 7	15 ± 2	0.58 ± 0.06	137 ± 1
	N3	-	-	-	-
Sg	A	15 ± 2	11 ± 2	0.64 ± 0.02	127 ± 2
	C				
	N1	19 ± 5	13 ± 3	0.59 ± 0.06	133 ± 3
	N2	38 ± 27	18± 7	0.61 ± 0.03	132± 2
	N3	31 ± 25	12 ± 4	0.62 ± 0.01	132± 1

Table S2. Statistical analyses for all leaf traits; physiological, structural and chemical traits as well as post hoc comparisons among sites

		Physiological traits											
		g_s (dry season)			g_s (wet season)			*E* (dry season)			*E* (wet season)		
		df	F	*P* value	df	F	*P* value	df	F	*P* value	df	F	*P* value
Site		4	18	**<0.001**	4	17	**<0.001**	4	7	**<0.001**	4	6.8	**<0.001**
Species		3	8.7	**<0.001**	3	34	**<0.001**	3	2	0.110	3	21	**<0.001**
Site*Species		10	3.6	**<0.001**	10	10	**<0.001**	10	4	**<0.001**	10	2.3	**0.015**
Post hoc multi-site comparisons													
1700m	1850m			0.555			0.090			0.113			0.164
	1950m			**<0.001**			**<0.001**			**<0.001**			0.998
	2500m			**<0.001**			0.966			**0.047**			0.568
	2700m			**<0.001**			0.560			**<0.001**			0.252
1850m	1950m			**0.004**			**0.001**			0.553			0.285
	2500m			0.080			0.301			1.000			**0.004**
	2700m			**<0.001**			0.868			0.071			**0.001**
1950m	2500m			0.787			**<0.001**			0.582			0.374
	2700m			**0.011**			**<0.001**			0.686			0.142
2500m	2700m			**<0.001**			0.893			0.066			0.964
Post hoc multi-species comparisons													
Sg	Pf			**0.026**			**<0.001**			0.778			**<0.001**
	Cg			**0.040**			0.123			0.275			0.171
	Mk			0.302			0.522			0.842			0.556
Pf	Cg			**<0.001**			**<0.001**			**0.027**			**<0.001**
	Mk			0.639			**<0.001**			0.999			**0.001**
Cg	Mk			**<0.001**			**0.002**			**0.037**			**0.004**

		A_{sat} (dry season)			A_{sat} (wet season)			WUE (dry season)			WUE (wet season)		
		df	F	*P* value	df	F	*P* value	df	F	*P* value	df	F	*P* value
Site		4	13	**<0.001**	4	5	**0.001**	4	21	**<0.001**	4	7.9	**<0.001**
Species		3	4.5	**0.005**	3	29	**<0.001**	3	1.1	0.330	3	6	**0.001**
Site*Species		10	2.8	**0.004**	10	6	**<0.001**	10	4.5	**<0.001**	10	1.8	0.067
Post hoc multi-site comparisons													
1700m	1850m			0.471			**0.009**			**0.004**			0.332
	1950m			**<0.001**			0.320			0.062			0.822
	2500m			**<0.001**			**0.033**			**<0.001**			0.591
	2700m			**<0.001**			0.887			0.997			**0.001**
1850m	1950m			0.149			0.498			0.774			0.897
	2500m			**0.003**			0.967			**<0.001**			**0.014**
	2700m			**<0.001**			0.158			**0.022**			**<0.001**
1950m	2500m			0.535			0.839			**<0.001**			0.093
	2700m			0.128			0.916			0.210			**<0.001**
2500m	2700m			0.874			0.379			**<0.001**			0.079
Post hoc multi-species comparisons													
Sg	Pf			0.555			**<0.001**			0.868			**<0.001**
	Cg			0.053			**0.029**			0.944			**0.003**
	Mk			0.756			**0.012**			0.995			**<0.001**

Pf	Cg	**0.001**	**<0.001**	0.530	0.953
	Mk	0.986	0.061	0.703	1.000
Cg	Mk	**0.002**	**<0.001**	0.987	0.922

		iWUE (dry season)			iWUE (wet season)			iWUE from isotopes		
		df	F	*P* value	df	F	*P* value	df	F	*P* value
Site		4	16	**<0.001**	4	8	**<0.001**	4	8.8	**<0.001**
Species		3	6.9	**<0.001**	3	6	**0.002**	3	15	**<0.001**
Site*Species		10	3.6	**<0.001**	10	3	**0.002**	10	2.1	**0.031**
Post hoc multi-site comparisons										
1700m	1850m			**0.035**			0.966			**<0.001**
	1950m			**<0.001**			**0.001**			0.365
	2500m			0.094			0.792			**0.005**
	2700m			**<0.001**			0.592			**0.002**
1850m	1950m			**0.021**			**0.014**			**0.044**
	2500m			0.978			0.443			0.722
	2700m			**0.001**			0.943			0.962
1950m	2500m			**0.001**			**<0.001**			0.428
	2700m			0.641			0.116			0.220
2500m	2700m			**<0.001**			0.099			0.984
Post hoc multi-species comparisons										
Sg	Pf			0.673			0.069			**<0.001**
	Cg			**0.001**			0.452			0.971
	Mk			0.900			0.980			0.447
Pf	Cg			**<0.001**			**0.001**			**<0.001**
	Mk			0.222			0.124			**0.003**
Cg	Mk			**0.003**			0.214			0.213

	Structural traits												
		DBH			Height			Wood density			LMA		
		df	F	*P* value	df	F	*P* value	df	F	*P* value	df	F	*P* value
Site		4	3.7	**0.008**	4	8	**<0.001**	4	6	**<0.001**	4	18	**<0.001**
Species		3	1.1	0.336	3	10	**<0.001**	3	197	**<0.001**	3	25	**<0.001**
Site*Species		10	2.5	**0.007**	10	7	**<0.001**	10	3.7	**<0.001**	10	13	**<0.001**
Post hoc multi-site comparisons													
1700m	1850m			0.204			0.977			**0. 004**			**<0.001**
	1950m			0.999			0.993			**0.005**			0.518
	2500m			**0.017**			0.188			**0.005**			0.242
	2700m			0.943			**0.014**			**<0.001**			0.609
1850m	1950m			0.309			0.853			0.997			**<0.001**
	2500m			0.852			0.491			0.998			**<0.001**
	2700m			0.725			**0.003**			0.103			**<0.001**
1950m	2500m			**0.033**			0.075			1.000			0.988
	2700m			0.983			**0.043**			**0.028**			**0.038**
2500m	2700m			0.198			**<0.001**			**0.035**			**0.010**
Post hoc multi-species comparisons													
Sg	Pf			0.821			**<0.001**			**<0. 001**			**<0.001**
	Cg			0.980			0.309			0.874			**<0.001**

	Mk	0.817	**0.006**	**<0.001**	**<0.001**
Pf	Cg	0.625	**0.014**	**<0.001**	0.442
	Mk	0.306	0.306	**<0.001**	**0.007**
Cg	Mk	0.971	0.482	**<0. 001**	**<0.001**

		Chemical traits											
		P_m			N_m			P_a			N_a		
		df	F	Pvalue	df	F	Pvalue	df	F	Pvalue	df	F	Pvalue
Site		4	0.4	0.775	4	0.7	0.578	4	2.6	**0.040**	4	2.5	**0.048**
Species		3	8.6	**<0.001**	3	6.5	**<0.001**	3	6	**<0.001**	3	5.1	**0.002**
Site*Species		10	1.1	0.331	10	2.5	**0.011**	10	1.8	0.064	10	3.6	**0.001**
Post hoc multi-site comparisons													
1700m	1850m			0.783			0.996			0.696			0.165
	1950m			0.897			0.994			0.681			0.981
	2500m			1.000			0.954			1.000			0.987
	2700m			0.999			0.784			0.436			0.934
1850m	1950m			0.998			0.946			0.097			0.050
	2500m			0.810			0.998			0.747			0.057
	2700m			0.682			0.950			0.995			0.637
1950m	2500m			0.917			0.798			0.623			1.000
	2700m			0.809			0.553			**0.034**			0.693
2500m	2700m			0.998			0.988			0.488			0.721
Post hoc multi-species comparisons													
Sg	Pf			**<0.001**			**<0.001**			0.149			0.694
	Cg			0.292			0.574			0.772			0.951
	Mk			0.560			0.552			0.283			0.084
Pf	Cg			**0.017**			**0.028**			0.675			0.950
	Mk			**0.002**			**0.015**			**<0.001**			**0.002**
Cg	Mk			0.943			1.000			**0.031**			**0.020**
		N:P ratio			**B:N ratio**			**Ca:N ratio**			**Cu:N ratio**		
		df	F	Pvalue	df	F	Pvalue	df	F	Pvalue	df	F	Pvalue
Site		4	2	0.101	4	2	0.086	4	7.9	**<0.001**	4	9.4	**<0.001**
Species		3	0.3	0.794	3	0.8	0.487	3	1.6	0.184	3	2.9	**0.039**
Site*Species		10	2.4	**0.015**	10	2.2	**0.024**	10	2.7	**0.005**	10	3.5	**<0.001**
Post hoc multi-site comparisons													
1700m	1850m			0.761			0.997			0.174			0.914
	1950m			0.751			0.344			0.252			**<0.001**
	2500m			0.986			0.134			**<0.001**			0.761
	2700m			0.632			0.472			0.063			0.985
1850m	1950m			1.000			0.630			0.997			**<0.001**
	2500m			0.468			0.344			**0.039**			0.301
	2700m			0.121			0.731			0.993			0.998
1950m	2500m			0.434			0.987			**0.007**			**0.003**
	2700m			0.098			1.000			0.931			**<0.001**
2500m	2700m			0.886			0.982			0.118			0.491
Post hoc multi-species comparisons													
Sg	Pf			0.587			0.936			0.620			0.214
	Cg			0.612			1.000			0.988			0.979

	Mk			0.927			0.598			0.873			0.067
Pf	Cg			1.000			0.963			0.410			0.091
	Mk			0.901			0.896			0.164			0.938
Cg	Mk			0.901			0.662			0.976			**0.023**
		Fe:N ratio			**K:N ratio**			**Mg:N ratio**			**Mn:N ratio**		
		df	F	Pvalue	df	F	Pvalue	df	F	Pvalue	df	F	Pvalue
Site		4	2.8	**0.030**	4	5.1	**0.001**	4	12	**<0.001**	4	6.7	**<0.001**
Species		3	6.9	**<0.001**	3	17	**<0.001**	3	3.3	**0.021**	3	8.1	**<0.001**
Site*Species		10	0.7	0.733	10	2.9	**0.003**	10	3.4	**0.001**	10	7.9	**<0.001**
Post hoc multi-site comparisons													
1700m	1850m			0.373			**0.001**			**<0.001**			0.278
	1950m			0.699			0.999			**0.004**			0.547
	2500m			0.505			0.214			**<0.001**			**<0.001**
	2700m			0.352			1.000			**0.009**			0.239
1850m	1950m			0.972			**0.002**			0.125			0.978
	2500m			0.997			0.262			0.999			0.077
	2700m			**0.008**			**0.003**			0.175			1.000
1950m	2500m			0.998			0.320			**0.038**			**0.008**
	2700m			**0.025**			1.000			1.000			0.964
2500m	2700m			**0.011**			0.296			0.066			0.094
Post hoc multi-species comparisons													
Sg	Pf			**<0.001**			**<0.001**			1.000			0.376
	Cg			**<0.001**			0.583			0.102			0.312
	Mk			**0.001**			0.604			0.081			**0.036**
Pf	Cg			0.997			**0.001**			0.065			**0.005**
	Mk			0.938			**<0.001**			**0.048**			0.626
Cg	Mk			0.873			0.055			1.000			**<0.001**
		Mo:N ratio			**S:N ratio**			**Zn:N ratio**			**SPAD**		
		df	F	Pvalue	df	F	Pvalue	df	F	Pvalue	df	F	Pvalue
Site		4	4.3	**0.003**	4	13	**<0.001**	4	5	**0.001**	4	10	**<0.001**
Species		3	5.9	**0.001**	3	25	**<0.001**	3	22	**<0.001**	3	269	**<0.001**
Site*Species		10	1.4	0.187	10	1.1	0.350	10	4.2	**<0.001**	10	12.7	**<0.001**
Post hoc multi-site comparisons													
1700m	1850m			0.964			**0.004**			0.999			0.295
	1950m			**0.004**			0.071			0.199			0.073
	2500m			0.254			**<0.001**			0.731			0.652
	2700m			0.999			**<0.001**			0.263			**<0.001**
1850m	1950m			0.247			0.737			0.164			**<0.001**
	2500m			0.976			0.136			0.902			**0.015**
	2700m			0.865			**0.027**			0.468			**<0.001**
1950m	2500m			0.512			**0.002**			**0.008**			0.712
	2700m			0.021			**<0.001**			**0.001**			**<0.001**
2500m	2700m			0.478			0.909			0.903			**<0.001**
Post hoc multi-species comparisons													
Sg	Pf			0.110			0.106			**<0.001**			0.287
	Cg			0.352			**<0.001**			**0.001**			**<0.001**
	Mk			**0.001**			0.087			**<0.001**			**<0.001**
Pf	Cg			0.951			**<0.001**			**0.001**			**<0.001**
	Mk			0.247			1.000			**<0.001**			**0.002**
Cg	Mk			0.106			**<0.001**			0.114			**<0.001**

Paper IV

Canopy nutrient cycling in Afromontane tropical forests at different successional stages

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Abstract: Canopy nutrient composition and cycling control biogeochemical processes and tree growth in forests. However, the understanding of nutrient limitations and cycling in tropical montane forests (TMF) is limited, in particular for Afromontane forests. We investigated leaf nutrient composition and resorption, canopy nutrient cycling and soil carbon and nutrient content in 15 permanent plots at different successional stages in a TMF in Rwanda. Macro- and micronutrients were analysed in attached green leaves as well as in shed leaves of 10 early (ES) and 10 late (LS) successional tree species. Leaf nutrient concentrations mostly did not differ between ES and LS species (exception: K was 20% higher in ES), but the ratios of P, K and Mg to N were significantly higher in ES species. Nutrient resorption efficiency exhibited large interspecific variation, but did not differ between ES and LS species. It was much higher for N (37%), P (48%) and K (46%) than for other nutrients. Total leaf litterfall was on average 4.9 t ha^{-1} yr^{-1} and ground litter turnover rates of C and N were 0.98 and 0.78 y^{-1}, respectively. Neither litterfall, litter turnover or the total content of C, N, P and K in leaf litterfall differed between ES and LS stands. In conclusion, high leaf N concentrations, intermediate N:P ratios and low resorption efficiencies in this Afromontane forest compared to results reported for other TMFs together indicate high fertility and likely co-limitation by N and P, however progressively increasing towards P limitation during the course of succession.

Keywords: Nitrogen, Phosphorus, Potassium, Resorption, Litterfall, Africa

1. Introduction

Plant productivity and the global carbon cycle are strongly constrained by nutrient availability (Fernández-Martínez et al., 2014). Nitrogen (N) and phosphorus (P) are the two major elements typically limiting net primary production (NPP) in terrestrial ecosystems (Chapin, 1980; Vitousek et al., 2010), where most boreal and temperate forests are limited by N (Hyvönen et al., 2007), and most tropical forests (mainly in lowlands) by P (Cleveland et al., 2011; Reich & Oleksyn, 2004). Two factors that may explain this difference in limiting element between boreal/temperate and tropical forest are that many tropical soils are highly weathered (e.g. oxisols and ultisols) with low availability of P, and that N turnover rate is faster in tropical forests compared to temperate and boreal forests, due to faster mineralization in warmer climate. Compared to tropical lowland forests, tropical montane forests (TMF) are suggested to have lower mineralisation rates due to lower temperature (Kirschbaum, 1995; Salinas et al., 2011; van de Weg et al., 2009), which may constrain the availability of especially N as most of it is tightly bound to soil organic matter (Attiwill & Adams, 1993). Furthermore, landslides are more common in montane areas which may affect mineral nutrient availability, especially of N, due to soil mixing (Crausbay & Martin, 2016; Fetcher et al., 1996; Wilcke et al., 2003). It has therefore been suggested that TMF to a large extent are limited by N rather than P (Gay et al., 2022; Vitousek & Sanford, 1986), which is supported by observations of a gradual decrease in leaf N content and N:P ratios with increasing elevation in the Andes (Bahar et al., 2017; Fisher et al., 2013; van de Weg et al., 2009) as well as by results from fertilisation experiments (Tanner et al., 1992). However, other studies have proposed that TMFs likely are co-limited by multiple nutrients (Dalling et al., 2016).

The nutrient dynamics of TMFs are poorly known (Cai & Bongers, 2007; Dalling et al., 2016; Giambelluca & Gerold, 2011; Scatena; et al., 2010), in particular for Africa, the world's second largest tropical forest region (Dalling et al., 2016; Malhi et al., 2013). Since the nutrient conditions may differ between Afromontane forests and the more well-studied montane forests of the Andes, further information on nutrient cycling in African TMF is required to better understand their ecosystem processes, including biomass production.

Plants require a number of macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Mn, Fe, Zn, Cu, B, Mo etc.) for growth and other vital plant functions (Ågren, 2008; White & Brown, 2010). The plant demand for these elements is often evaluated by their stoichiometric relationships with N in the leaves (Ågren, 2008). Knecht and Göransson (2004) (Knecht & Göransson, 2004) suggested that these relationships are similar across all terrestrial plants. Thus, leaf nutrient ratios combined with information about required ratios can be used as a diagnostic tool to determine nutrient limitations in ecosystems (Linder, 1995). Therefore, focusing on the full set of mineral nutrients seems imperative (Townsend et al., 2011).

Nutrients in leaves originate from direct acquisition of new nutrients from the soil as well as from the internal recycling of nutrients from old leaves (i.e. nutrient resorption) during senescence (Killingbeck, 1996). Nutrient resorption efficiency (NuRE; i.e. the percentage of the leaf nutrient pool that is resorbed before the leaf is lost through abscission) for N (NRE) and P (PRE) has been observed to be in the range between 30 and 93%, where PRE typically was slightly higher than NRE (Aerts, 1996; Chen et al., 2021; Cleveland et al., 2013; Liu et al., 2014; Tsujii et al., 2017; Vergutz et al., 2012). Resorption is thus a key conservation mechanism for these potentially growth limiting elements (Drenovsky et al., 2019; Vitousek, 1982). There are few studies on resorption efficiency for potassium (KRE), despite its high leaf concentration and critical role in osmoregulation and stomatal function (Larcher 2003), but KRE as high as 70% has been reported (Urbina et al., 2021). Even less information is available for non NPK-macronutrients and micronutrients (Brant & Chen, 2015; Liu et al., 2014; Vergutz et al., 2012).

There have been several attempts to elucidate patterns in the very large variation in NuRE values reported in the literature, and their underlying determinants, e.g.Wood et al., (2011). The nutrient status of soil and green leaves seem not to be strongly linked to NuRE. The relationship between soil nutrient availability and NuRE was negative in some studies (Aerts & de Caluwe, 1994; Boerner, 1984); Stachurski and Zimka 1975), non-significant in other studies (Aerts, 1996; Bowman et al., 1995; Chapin & Moilanen, 1991; Schlesinger et al., 1989; Wright & Westoby, 2003), and positive in a third group of studies (Boerner, 1986; Nambiar & Fife, 1991; Sabaté et al., 1995). With respect to foliar nutrient status, two recent global studies (Kobe et al., 2005; Vergutz et al., 2012) found that NRE decreases with increasing green leaf nutrient concentration although some earlier studies did not support this pattern (Enoki & Kawaguchi, 1999; Lal et al., 2001). Recent suggest NuRE is controlled by stoichiometry and nutrient concentration at community level and by nutrient limitation at species level (Chen et al., 2021; Ji et al., 2018; Sun et al., 2023; Wang et al., 2021; Zeng et al., 2017).

Meta-analyses comparing NuRE of different plant functional types have found that NRE is higher in deciduous compared to evergreen species (Aerts, 1996; Yuan & Chen, 2009) and lower in trees compared to shrubs (Yuan & Chen, 2009). These studies also found that evergreen trees had higher PRE than deciduous trees while there was no difference in PRE between trees and shrubs. Differences were also related to life-form, with successively decreasing NRE from herbs, shrubs, trees, graminoids to N-fixers (Yuan et al., 2005). With respect to phylogeny, both NRE and PRE were reported to be lower in angiosperms compared to gymnosperms (Tang et al., 2013).

Little is known regarding possible differences in NuRE between early (ES) and late successional (LS) tree species and results are contrasting. NRE did not change with overstory succession and PRE and KRE decreased with overstory succession (Chen & Chen, 2022). Contrary, ES species showed higher NRE and PRE than LS (Zeng et al., 2017) while other studies reported significantly lower NRE and PRE in ES compared to LS species (Fahey et al., 1998; Urbina et al., 2021).

Trees which appear early in the succession after disturbance are favoured by comparatively high availability of resources, due to e.g. enhanced N mineralisation and abundant light. At later successional stages, progressively more mineral nutrients will be locked up in biomass, which may cause increasing nutrient limitations (Grime et al. 2001). ES tree species are thus likely to have a rather competitive strategy (sensu Grime) in which nutrient savings are of limited importance, while at later stages of forest succession the species composition will change towards an increasing abundance of more stress tolerant species with more efficient nutrient use including resorption from senescing leaves (Grime et al. 2001). However, NuRE has been found to decrease with stand age (Killingbeck et al., 1990; Y. Li et al., 2013; Nambiar & Fife, 1991; Ye et al., 2012), but it is unknown if this is due to tree ontogeny or altered tree species composition. Since tropical secondary forests are expected to become progressively more common due to intensified land use (Lewis et al., 2015), it is important to know if NuRE is significantly different in ES compared to LS species.

In several past estimations of NuRE, the changes in leaf mass and leaf area occurring during senescence have often been neglected. Both leaf mass and area decline substantially during senescence (Cai & Bongers, 2007; Güsewell, 2005; Van Heerwaarden et al., 2003), and failure to account for these effects may cause severe underestimations of NuRE (Tang et al., 2013; Van Heerwaarden et al., 2003; Vergutz et al., 2012). Knowledge regarding leaf mass and area loss during senescence in different types of plants and ecosystems is thus needed for robust correction of earlier estimates of nutrient resorption efficiency.

Here, we examined leaf nutrient composition, resorption efficiency, canopy nutrient cycling and soil carbon and nutrient content in ES and LS forest stands in the Afromontane tropical forest of Nyungwe National Park, Rwanda. We tested the hypotheses that:

(i) N is more growth limiting than P and other macro- and micronutrients, as judged by leaf elemental composition.
(ii) ES species have higher leaf N content and N:P ratios but lower nutrient resorption efficiencies compared to LS species.
(iii) Resorption efficiency is generally coordinated among different nutrients, but higher for P and K compared to enzyme-bound N and S, and for macro- compared to micronutrients.

2. Materials and Methods

Study site

The study site was located in the Nyungwe tropical montane rainforest which is a National Park in South-western Rwanda (2º17´ - 2º50´S, 29º07´ - 29º26´E) ranging from 1600 to 2950 m a.s.l. and covering an area of 1013 km^2. The Nyungwe forest has historically been disturbed and consists today of a mixture of late successional (LS) and early successional (ES) forest with patches of savannas, bamboo groves and marshes. The dominating soil type is ultisols with

patches of inceptisols (Nzeyimana et al., 2014) developed on quartzite schist, mica schist, schist and granite as parent material (Verdoodt & Ranst, 2003). The mineral top soil consists of clay, sand and silt ranging between 2-71%, 9-61% and 5-61% with averages of 34, 43 and 23%, respectively (Gharahi Ghehi et al., 2014). At a meteorological station located at Uwinka (2° 28′ 43″ S, 29° 12′ 00″ E, 2465 m a.s.l. elevation; (Nsabimana, 2009; Nyirambangutse et al. 2017), the average day and night air temperatures were 15.7 °C and 13.5 °C, respectively, the average relative humidity was 81%, and annual precipitation was 1867 mm during 2007 - 2015. The seasonal variation in temperature was small (1.1 °C between warmest and coldest month), but precipitation varied seasonally, with a dry period of two months, normally occurring from mid-June to mid-August. Precipitation also varied with location, from c. 1650 to 3000 mm y^{-1} (Nyirambangutse et al., 2017) .

Plots and species

The study was conducted in 15 permanent plots with a planimetric area of 100 m x 50 m (0.5 ha) established in 2011/2012 along an east-west transect of 32 km at elevations of 1950 to 2550 m a.s.l., ranging from a dominance of early successional (ES) to a dominance of late successional (LS) forest stands, both having closed canopies interspersed with small open areas (Nyirambangutse et al., 2017). Plot stem density varied between 220 and 958 stems ha^{-1} (> 10 cm diameter at breast height) and above ground dry biomass (AGB) between 142 and 793 t ha^{-1}. Stem density was not significantly different between successional stages while LS stands had a 35% higher AGB than ES stands. Each plot was subdivided into eight subplots with the size of 25 m x 25 m, resulting in 120 subplots in total. All stems with a breast height diameter (*D*) of ≥ 5 cm were positioned on plot maps, identified to species when possible and measured for *D*. For this study the four most abundant species (based on basal area) within each plot was selected. This selection resulted in the following 20 species (out of a total of 83 identified species) representing 78% of the total basal area of all plots: *Afrocrania volkensii* (Harms) Hutch., *Agauria salicifolia* Hook.f.ex Oliv., *Carapa grandiflora* Sprague, *Chionanthus africanus* (Knoblauch) Stearn, *Cleistanthus polystachyus* Hook.f.ex Oliv., *Faurea saligna* Harv., *Ficalhoa laurifolia* Hiern, *Harungana montana* Spirl., *Ilex mitis* Radlk., *Macaranga kilimandscharica* Pax, *Maytenus acuminata* (L.f.) Loes., *Ocotea kenyensis* (Chiov.) Robyns & R.Wilczek, *Ocotea usambarensis* A.Juss., *Olinia rochetiana* A.Juss., *Polyscias fulva* (Hiern) Harms, *Prunus africana* (Hook.f.) Kalkman, *Psychotria mahonii* C.H. Wright, *Rapanea melanophloeos* Mez, *Strombosia scheffleri* Engl., and *Syzygium guineense* (Willd.) DC. Ten species were classified as ES and ten as LS, see Table S1. For further details of the plots and species see Nyirambangutse et al. (2017) (Nyirambangutse et al., 2017).

Meteorological measurements

Data on air temperature, relative humidity, solar radiation, and precipitation were collected every 30 min from four meteorology stations installed along the plot transect. One major station was established at the Uwinka research site in February 2007 (Nsabimana, 2009; Nyirambangutse et al., 2017) and three small stations were established in June 2013. The Uwinka station was installed in a 15 m tower at a hilltop to reach above the canopy while the others were installed at open areas at 3 m height (1.5 - 2 m above ground vegetation). The small stations were equipped with sensors for measurements of temperature, relative humidity, solar radiation and precipitation. For details see Nyirambangutse et al. (2017).

Green leaf properties

Attached green leaves were sampled from branches up to a height of 20 m in the canopy, by cutting branches using a telescopic pole made of carbon fibre (GeoData management system Inc, Berea, OH, USA) with a saw-blade at the top. The sampling was conducted in August to December 2013. On each plot, one branch mostly from the mid to upper canopy at an open or semi-open position (i.e. sun exposed at least part of the day) from three randomly selected individual trees with a dbh > 20 cm of the four most abundant species were collected. From each branch, five mature and non-senescent leaves were sampled from different positions of the branch. From each leaf sample, six to eight leaf discs of a fixed area was obtained by using a hole punch (10 or 18 mm diameter, depending of leaf size). Both discs and remaining leaves were oven-dried at 70ºC to constant mass. The discs were weighted to calculate the leaf mass per unit area (LMA). To estimate the shrinkage of dry compared to fresh green leaves, 15 discs from fresh green leaves of known size were dried and measured for area using a scanner (Epson 1600+ scanner equipped with a transparency unit for dual scanning) and image analysing software (WinSEEDLE Pro 5.1a, Regent Instruments Inc., Canada).

Litterfall collection

Litterfall was collected twice per month over a period of 24 months (i.e. from January 2013 to December 2014) using ninety litter traps evenly distributed over all plots. In each plot, six of the subplots were randomly assigned one trap that was randomly placed at one of 16 grid points within each subplot using a 5 x 5 m grid. The litter traps consisted of nylon mesh bags suspended from a circular wire frame of aluminum (0.3 m^2; Jädraås skog och mark, Jädraås, Sweden) and mounted horizontally on wooden poles ca 0.8 m above ground level. The litter from each trap was collected separately, placed in paper bags, and sent to the laboratory where it was oven-dried at 70ºC to constant mass. After drying, each sample was divided into five fractions (leaves, reproductive organs, twigs, epiphytes, and unidentified fine debris), and weighed. Thereafter the litter collected during four consecutive three-month periods in 2013 was combined and mixed for each trap, from which about 10 leaves were collected randomly for the species that were sampled for green leaves. These combined samples were used for both litter nutrient flux and leaf NuRE calculations. The maximum number of samples for a species was thus 360 (6 traps, 4 three-month periods, 15 plots), but the actual number of samples was typically considerably less as most species were only found in a few litter traps per plot and not in all plots. A hole punch was used to obtain discs of a fixed area from each dried sample. The discs were weighted to calculate the litter leaf mass per unit area (LiMA).

To assess whether or not substantial leaching losses occurred during our bi-monthly sampling intervals, we compared the nutrient concentrations of litter collected during periods with low compared high precipitation. The third quarter of the year had the markedly lowest precipitation but exhibited no significant differences in litter nutrient concentrations compared to the three quarters with plentiful rain, indicating negligible leaching losses.

Soil samples

The litter layer and soil were sampled from the centre of each subplot quadrant (4 per subplot, i.e. 480 samples in total), where the litter and organic (O) soil were separately excavated from a 0.5 x 0.5 m horizontal ground area. Below the O-horizon, three consecutive cores (8 cm diameter and 15 cm depth each) of mineral soil down to a depth of 45 cm were sampled using a root auger (Ejkelkamp Soil & Water, Giesbeek, The Netherlands), thereafter the four mineral samples within each subplot and depth were mixed. A subsample of 20% based on the fresh mass was taken from each O-horizon sample and mixed mineral samples, thereafter roots were extracted from each subsample. All samples were then brought to the lab for drying to constant mass in oven set to 70 °C.

Chemical analysis of green leaf, litter and soil samples

The green leaf samples were grinded to a fine powder using a ball mill (model MM 301, Retsch, Haan, Germany) equipped with grinding jars and balls made of wolfram. Litter samples where quickly pre-grinded using a grinder for coffee beans and soil samples were pre-grinded using a mortar and pestle and both were further grinded to a fine powder using the ball mill above, but with jars made of stainless steel. The C and N concentrations of all samples were determined by dry combustion using an elemental analyser (EA 1108, Fison Instruments, Rodano, Italy). A subset of leaf and litter samples was analysed for P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo using inductively coupled plasma mass spectrometry (Basic Suite 1VE1; ACME Analytical Laboratories, Vancouver, BC, Canada). However, we refrain to report the concentration of Fe and Mn in the litter, as there might be contamination of these elements due to the use of stainless steel material in the grinding.

N was analysed on 249 green leaf samples (each sample from different trees) and 601 species specific leaf litter samples. To test for differences between plots, both leaves and litter from *M. kilimandscharica* and *S. guineense* were analysed for N, and samples from these species therefore constituted about 30% and 50% of the leaf and litter samples, respectively. The subset for which all nutrients were analysed consisted of 76 green leaf and 118 leaf litter samples.

Nutrient resorption calculations

Nutrient resorption efficiency was defined as the proportion of mature leaf nutrients that was withdrawn prior to abscission (Killingbeck, 1996; Li et al., 2016; Reed et al., 2012). During senescence the leaf area shrinks and the leaf mass declines. These effects need to be accounted for when calculating resorption efficiency based on the nutrient content of green leaves and leaf litter (Van Heerwaarden et al., 2003). We therefore established species-specific correction factors based on leaf shrinkage and leaf mass loss which were used when calculating the mineral resorption efficiency (NuRE) of the leaves. The fraction of shrinkage in area of senesced ($A_{Shrinkage}$) leaves was estimated by:

$$A_{Shrinkage} = 1 - \left(\frac{L_{A,dry}}{L_{A,fresh}}\right) \qquad (1)$$

where $L_{A,fresh}$ and $L_{A,dry}$ are the areas of fresh green and dry leaf discs, respectively, assuming that the shrinkage during senescence mainly is due to drying. The fraction of mass loss (M_{Loss}) during senescence was estimated by:

$$M_{Loss} = \left(\frac{LiMA \times (1 - A_{Shrinkage})}{LMA}\right) \qquad (2)$$

where *LMA* and *LiMA* represent the leaf mass per unit area and the litter dry mass per unit area, respectively. The NuRE in percent was then calculated as:

$$\mathrm{NuRE} = \left(1 - \frac{[\mathrm{Nu}]_{LT} \times (1 - M_{Loss})}{[\mathrm{Nu}]_{L}}\right) \times 100 \qquad (3)$$

where $[\mathrm{Nu}]_L$ and $[\mathrm{Nu}]_{LT}$ area the nutrient concentration in the green leaves and litter, respectively. The calculation of species-specific mean NuRE was based on subplot data, except for *M. kilimandscharica* and *S. guineense* for which plot level data was used.

Nutrient leaf litter flux calculations

For the nutrient litter flux we only considered the leaf fraction which is the largest component of the litterfall and normally has the highest concentrations of nutrients (e.g. Dent et al. 2006). The leaf litter flux in each plot was based on an estimate of the mean leaf litter nutrient concentration from the species specific concentration, the plot basal area of each species and the plot annual leaf litterfall. Leaf litter C or N turnover rate (LLiT_i) was estimated from the C and N content in the leaf litter fall (LLiF_i), the amount of C and N in ground litter (above the O-horizon, L_i) and the fraction of leaf litter to total litter fraction (f_{LL}) according to Hou et al. (2015) where *i* represents C or N:

$$\mathrm{LLiT}_i = \frac{\mathrm{LLiF}_i}{\mathrm{L}_i \cdot f_{LL}} \qquad (4)$$

Statistical analysis

Differences in leaf nutrient concentrations and resorption efficiency among species were analysed by one-way ANOVA and differences between ES and LS species or stands were analysed using two-tailed independent-samples t-test using SPSS software (IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY: IBM Corp.). Data that violated the assumption of normality or that contained outliers were log-transformed before the statistical analysis. The significance of the relationship between concentration of nutrients and resorption of nutrients were determined using the regression analysis tool in SigmaPlot 12.5 (Systat Software Inc., San José, CA, USA).

3. Results

Leaf morphology and nutrient concentrations

Data on LMA and N content showed that the subset of leaf (30%) and litter (20%) samples, for which we analysed all macro- and micronutrients, was highly representative of the full dataset for which LMA and N were analysed (Fig. S1). When the subset data was plotted against the full data set, strongly significant ($P < 0.0001$) relationships were found for LMA ($R^2 = 0.95$), leaf N concentration ($R^2 = 0.95$) and NRE ($R^2 = 0.90$, Fig. S1). We further tested if LMA and leaf nutrient concentrations varied due to possible differences in nutrient availability among plots at different successional stages by comparing plots where *M. kilimandscharica* (dominant in ES plots) and *S. guineense* (dominant in LS plots) abundantly co-occurred (6 plots) with plots where only one of the species was abundant (4 and 5 plots, respectively). The levels of LMA and nutrient concentrations as well as the differences between species was similar between groups of plots, except for Cu in *M. kilimandscharica* (Table S1), indicating that there were no consistent differences in nutrient availability among plots at different successional stages.

Mean LMA varied approximately by a factor of two (79 to 168 g m^{-2}) among species. There was a significant difference in LMA between species ($P < 0.001$; Table 1), but no significant differences between the two successional groups ($P = 0.35$). The estimated leaf shrinkage and leaf mass losses during senescence were on average 9.5% and 17%, respectively, with significant differences among species for both variables ($P < 0.001$), but not between successional groups ($P = 0.66$ and $P = 0.10$, respectively; Table 1).

There were significant ($P < 0.028$) differences among species regarding leaf concentrations of all macro- and micronutrients except for Fe ($P = 0.92$; only N, P and K data shown in Table 1). Only leaf concentrations of K and Mg were significantly different between ES and LS species (K: -21%, $P = 0.042$ and Mg: -61%, $P = 0.021$ in LS compared to ES). When relating the nutrient content to N, in addition to K and Mg, P was also significantly lower (-23%) in LS compared to ES species ($P \leq 0.017$; Table 2). The N:P ratio, used as diagnostic tool for identifying N and P limitations, ranged between 10.9 and 20.5 (average value 16.2 ± 2.6).

Table 1. Leaf properties related to resorption of N, P and K in 10 early successional (ES) and 10 late successional (LS) tree species. SG, successional group; #, number of replicates; shrink, the area shrinkage of dried in relation to fresh leaves; LMA, leaf mass per unit area; M-loss, the estimated loss of mass during senescence; Leaf N_m, P_m, K_m, the dry mass concentration of N, P, K in attached green leaves; NRE, PRE, KRE, the resorption efficiency of N, P and K in senesced and shed leaves in relation to the element content of attached green leaves; Diff, the difference between LS and ES species; *P*-values (SG), the results of t-test between ES and LS species; *P*-values (species), the results of one-way ANOVA testing differences among species. The replicated unit (#) for *M. kilimandscharica* and *S. guineense* is plots and for other species sub-plots. Bold value, *P* < 0.05.

Species	SG	#	Shrink (%)	M-loss (%)	LMA ($g\ m^{-2}$)	Leaf N_m ($mg\ g^{-1}$)	Leaf P_m ($mg\ g^{-1}$)	Leaf K_m ($mg\ g^{-1}$)	NRE (%)	PRE (%)	KRE (%)
					Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
Afrocrania volkensii	ES	3	15.6	29.3	92 ± 12	22.4 ± 3.5	2.07 ± 0.4	10.5 ± 1.1	59.4 ± 6.1	59.4 ± 9.4	45.1 ± 14.6
Agauria salicifolia	ES	2	5.1	16.2	134 ± 18	11.3 ± 0.1	0.76 ± 0.0	9.1 ± 1.0	34.8 ± 1.5	42.7 ± 21.0	20.2 ± 5.7
Harungana montana	ES	4	16.4	22.4	114 ± 13	20.7 ± 2.0	1.45 ± 0.2	8.6 ± 1.6	36.9 ± 16.1	48.5 ± 6.1	68.1 ± 17.1
Ilex mitis	ES	4	8.6	21.9	168 ± 12	13.9 ± 1.0	0.79 ± 0.0	12.5 ± 4.5	15.5 ± 10.4	31.0 ± 5.3	38.3 ± 37.7
Macaranga kilimandscharica	ES	10	9.8	15.5	117 ± 14	19.8 ± 2.2	1.42 ± 0.1	8.8 ± 1.7	51.6 ± 8.4	63.7 ± 7.5	69.3 ± 16.9
Maytenus acuminata	ES	3	8.4	10.5	84 ± 19	19.9 ± 1.1	1.47 ± 0.1	11.4 ± 2.4	12.4 ± 10.1	31.9 ± 13.5	21.4 ± 14.1
Polyscias fulva	ES	3	12.0	20.7	159 ± 29	20.2 ± 2.7	1.36 ± 0.2	8.5 ± 7.1	53.8 ± 9.6	58.9 ± 8.5	51.8 ± 35.3
Prunus africana	ES	2	9.8	23.0	112 ± 15	17.4 ± 0.9	1.56 ± 0.6	8.9 ± 1.7	26.0 ± 3.9	48.9 ± 20.2	9.2 ± 17.3
Psychotria mahonii	ES	3	7.1	14.7	100 ± 13	27.6 ± 6.5	1.42 ± 0.2	8.5 ± 4.1	46.8 ± 6.6	59.2 ± 13.7	78.9 ± 6.2
Rapanea melanophloeos	ES	3	6.3	20.8	126 ± 4	20.9 ± 3.1	1.38 ± 0.2	7.5 ± 0.6	60.4 ± 3.6	74.5 ± 3.7	51.2 ± 11.8
Carapa grandiflora	LS	4	5.1	12.5	152 ± 12	19.1 ± 1.9	1.18 ± 0.1	7.5 ± 1.4	37.6 ± 7.8	48.9 ± 23.6	42.8 ± 35.8
Chionanthus africanus	LS	3	6.3	6.8	98 ± 16	18.2 ± 2.7	1.32 ± 0.2	7.5 ± 0.9	4.0 ± 16.4	29.2 ± 13.2	19.3 ± 9.9
Cleistanthus polystachyus	LS	3	9.0	18.5	108 ± 28	20.3 ± 1.7	1.21 ± 0.1	6.4 ± 0.6	40.2 ± 9.6	33.6 ± 25.3	37.5 ± 24.3
Faurea saligna	LS	4	7.6	5.3	101 ± 28	14.4 ± 2.4	0.77 ± 0.1	6.4 ± 1.4	40.1 ± 4.1	49.5 ± 11.5	56.9 ± 22.2
Ficalhoa laurifolia	LS	2	11.9	27.3	90 ± 12	21.1 ± 2.9	1.08 ± 0.1	6.3 ± 0.4	44.5 ± 2.0	49.3 ± 7.6	60.3 ± 10.8
Ocotea kenyensis	LS	3	4.8	7.6	124 ± 7	23.6 ± 1.6	1.15 ± 0.1	10.9 ± 2.0	35.4 ± 18.7	48.2 ± 16.2	53.7 ± 6.5
Ocotea usambarensis	LS	3	6.9	7.8	97 ± 17	21.2 ± 2.0	1.15 ± 0.1	8.1 ± 5.0	24.7 ± 1.7	36.6 ± 10.5	55.1 ± 15.1
Olinia rochetiana	LS	3	14.7	18.8	79 ± 17	26.7 ± 0.4	1.57 ± 0.2	10.2 ± 1.5	48.4 ± 3.0	54.2 ± 4.4	54.4 ± 10.6
Strombosia scheffleri	LS	3	13.2	22.9	111 ± 3	33.3 ± 3.2	1.76 ± 0.3	8.9 ± 1.0	35.5 ± 9.0	33.7 ± 12.4	29.1 ± 21.1
Syzygium guineense	LS	11	12.2	16.5	138 ± 18	15.6 ± 2.2	0.97 ± 0.1	6.2 ± 1.7	40.5 ± 10.9	56.4 ± 10.9	58.9 ± 9.8
All species		76	9.5	17.0	115 ± 25	20.4 ± 5.0	1.3 ± 0.3	8.6 ± 1.8	37.4 ± 15.0	47.9 ± 12.4	46.1 ± 18.7
ES mean			9.9	19.5	121 ± 27	19.4 ± 4.5	1.4 ± 0.4	9.4 ± 1.5	39.8 ± 17.5	51.9 ± 13.9	45.4 ± 23.2
LS mean			9.2	14.4	110 ± 22	21.3 ± 5.5	1.2 ± 0.3	7.8 ± 1.7	35.1 ± 12.6	43.9 ± 9.7	46.8 ± 14.1
Diff %			-7	-26	-9	10	-11	-17	-12	-15	3.2
P-values (SG)			0.66	0.10	0.35	0.40	0.32	**0.04**	0.50	0.16	0.87
P-values (Species)			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.028**	**0.024**	**<0.001**	**<0.001**	**<0.001**

The N:P ratio was significantly lower in ES (14.7 ± 2.4) compared to LS (17.7 ± 2.0) species (*P* = 0.013). For most nutrients, the leaf concentration to N ratio was above the optimal ratios defined in the literature (Table 2). However, for P the ratio was below optimal in 18 of 20 species, while for K and B only 1 and 2 species, respectively, were below the optimal ratio. We refrain to compare the data on Mo to N ratio as we did not find any reliably optimal ratios for this element in the literature. P and S were the only two nutrients for which leaf concentrations were significantly correlated to leaf N concentration (*P* < 0.001; Fig. 1a, c, e) across species. Including only data for the most abundant ES and LS species *M. kilimandscharica* and *S. guineense*, respectively, plot-specific values of leaf P, K and S concentrations were significantly related to leaf N concentration (*P* ≤ 0.0002; Fig. S2).

Table 2. The ratio of macro and micro nutrients relative to N in attached green leaves and the resorption efficiency (RE) of these elements during senescence (means ± SD and range) of all plots (1-15) and plots classified as early (ES; n = 5) or late (LS; n = 5) successional. The last column shows optimal nutrient ratios[a]. Diff, represents the mean % differences of LS in relation to ES plots and *P*-values are the results of a t-test of the difference between ES and LS plots. Contrary to Table 1, the resorption values include only 18 species because of errors in the micronutrient analysis of 2 species. 'NRE - all' represents NRE based on the larger set of samples for which only N was analysed. Bold value, *P* < 0.05.

	All species (1-20)		Species of different successional groups				Optimal ratios[a]
Leaf nutrient			ES	LS			
ratios	Mean ± SD	Range	Mean ± SD	Mean ± SD	Diff (%)	*P*-value	
N:P ($g\ g^{-1}$)	16.2 ± 2.6	10.9 - 20.5	14.7 ± 2.4	17.7 ± 2.0	17	**0.013**	
P:N (%)	6.4 ± 1.2	4.9 - 9.3	7.1 ± 1.3	5.8 ± 0.7	-23	**0.013**	8-10
K:N (%)	44.8 ± 15.6	27.0 - 88.6	51.9 ± 19.0	37.6 ± 6.3	-38	**0.017**	30-35
Ca:N (%)	44.0 ± 27.6	12.7 - 104.2	52.9 ± 27.5	35.1 ± 26.0	-51	0.13	2-2.5
Mg:N (%)	13.4 ± 8.3	5.5 - 34.3	17.5 ± 9.8	9.4 ± 3.4	-86	**0.014**	3.3-5
S:N (%)	15.1 ± 4.0	9.5 - 25.7	15.6 ± 3.7	14.6 ± 4.4	-7	0.53	4
Fe:N (%)	1.4 ± 0.5	0.8 - 2.7	1.5 ± 0.5	1.3 ± 0.3	-16	0.25	0.13-0.2
Mn:N (%)	2.4 ± 1.9	0.2 - 6.9	2.5 ± 2.0	2.3 ± 1.8	-9	0.65	0.03-0.05
Zn:N (%)	0.107 ± 0.070	0.053 - 0.369	0.130 ± 0.092	0.084 ± 0.028	-54	0.11	0.04-0.05
B:N (%)	0.095 ± 0.079	0.040 - 0.338	0.122 ± 0.106	0.069 ± 0.016	-77	0.069	0.05
Cu:N (%)	0.041 ± 0.014	0.025 - 0.079	0.041 ± 0.016	0.041 ± 0.013	-1	0.68	0.01-0.03
Mo:N (%)	0.0012 ± 0.001	0.0002 - 0.006	0.001 ± 0.001	0.001 ± 0.002	43	0.23	
NRE - all (%)	40 ± 11	19 - 62	42 ± 14	37 ± 8	-15	0.71	
NRE (%)	40 ± 13	12 - 60	41 ± 18	39 ± 7	-7	0.78	
PRE (%)	49 ± 12	31 - 74	52 ± 15	46 ± 9	-15	0.87	
KRE (%)	50 ± 16	20 - 79	49 ± 21	50 ± 11	1	0.91	
CaRE (%)	4 ± 29	-59 - 64	10 ± 29	-1 ± 31	1095	0.87	
MgRE (%)	20 ± 22	-14 - 62	22 ± 24	18 ± 21	-26	0.65	
SRE (%)	22 ± 15	-7 - 44	21 ± 16	23 ± 14	9	0.52	
ZnRE (%)	15 ± 30	-55 - 53	7 ± 37	22 ± 21	67	0.10	
BRE (%)	0 ± 27	-67 - 39	2 ± 25	-0.9 ± 29	298	0.98	
CuRE (%)	6 ± 21	-38 - 35	7 ± 20	5 ± 23	-43	0.81	
MoRE (%)	26 ± 53	-90 - 85	35 ± 52	17 ± 56	-101	0.86	

[a] The optimal nutrient ratios are defined as those when all nutrients limit growth simultaneously. The values are given for nutrient to N ratios in leaves and are derived from: Ericsson and Kähr (1993, 1995), Göransson (1993), Göransson (1994), Göransson (1998), Göransson (1999), Knecht and Göransson (2004) and Linder (1995).

Nutrient resorption

NuRE differed significantly among species (Table 1), but was independent of successional status (Table 2). Three nutrients (N, P, K) revealed substantial resorption (average 40-50%), while the other macro- and micronutrients had rather low (≤ 26%) and highly variable NuRE. Species-specific values of NRE ranged between 12 – 60% (mean = 37%) while values of PRE were higher and ranged between 31 – 74% (mean = 48%) and KRE between 9 – 79% (mean

46%). Three species had very low NRE (≤ 16%; *C. africanus, I. mitis* and *M. acuminata)*, of which one was classified as LS and two as ES.

Across species, resorption efficiencies and leaf concentrations were not significantly related for N, P, and K, suggesting that mature leaf concentration was not a good predictor of the interspecific variation in NuRE (Fig. S3). However, analysing plot-data for the dominant species *M. kilimandscharica* and *S. guineense* only, there were significant positive relationships between leaf N concentration and NRE for both species and for leaf K concentration and KRE in *S. guineense* (Fig. S4; Table S4). The interspecific variation in PRE, KRE and SRE was significantly related to variation in NRE (R^2 = 0.34-0.73; $P \leq 0.0067$; Fig. 1b, d, f). However, both PRE ($P < 0.0001$) and KRE ($P = 0.064$) were generally higher than NRE at low levels of NRE (Fig. 1b, Table1).

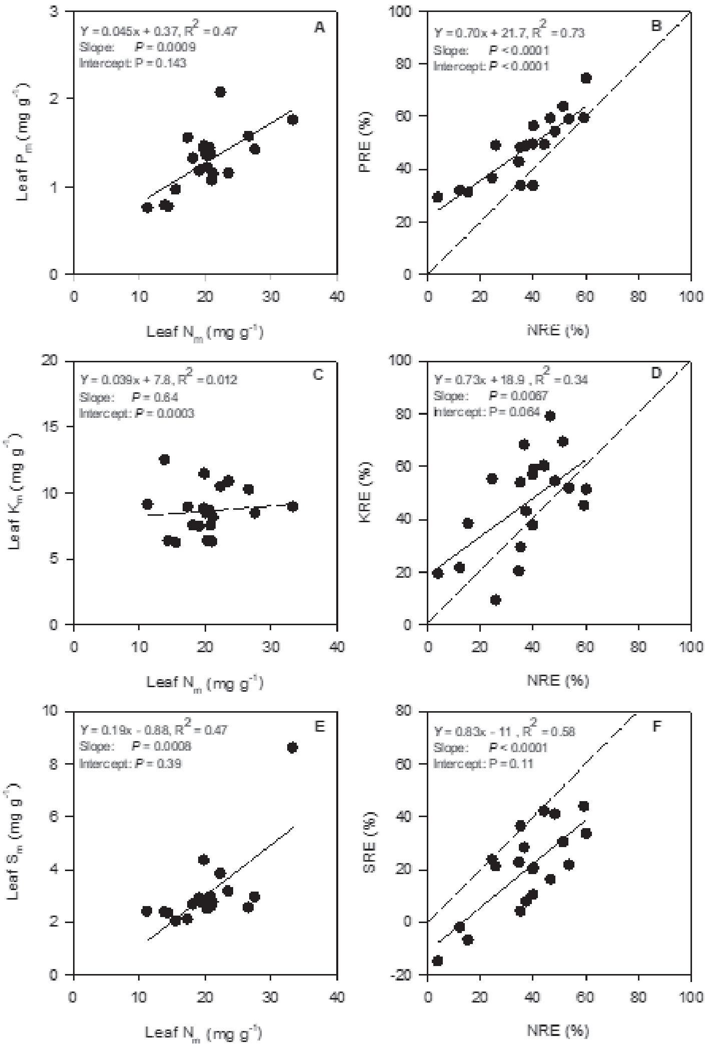

Figure 1. Leaf concentration of (a) P (Pm), (c) K (Km) and (e) S (Sm) in relation to leaf concentration of nitrogen (Nm) as well as resorption efficiency of (b) P (PRE) and (d) K (KRE) and (f) S (SRE) in relation to the resorption efficiency of N (NRE). Each data point represents the mean for one species. Equations, regression lines and R2 values are given for each relationship, with P-values given for both slope and y-intercept. The diagonal line in b, d and f represents the 1:1 line.

Table 3. Annual litterfall of different fractions collected during 2013 and 2014 (means ± SD and range) of all plots (1-15) and plots classified as early (ES; n = 5) or late (LS; n = 5) successional. Diff represents the mean % differences of LS in relation to ES plots and P-values are the results of a t-test of the difference between ES and LS plots.

	All species (1-20)		Plots at different successional stages			
			ES	LS		
Litter fraction	Mean SD	Range	Mean SD	Mean SD	Diff (%)	*P*-value
Leaf litter (Mg ha^{-1} yr^{-1})	4.94 ± 1.14	3.06 - 6.91	4.61 ± 0.83	4.84 ± 1.5	5	0.77
Repr. litter (Mg ha^{-1} yr^{-1})	1.09 ± 0.84	0.34 - 3.1	0.69 ± 0.25	1.13 ± 1.01	63	0.38
Wood litter (Mg ha^{-1} yr^{-1})	1 ± 0.43	0.47 - 1.97	0.84 ± 0.28	1.16 ± 0.51	39	0.25
Epiphyte litter (Mg ha^{-1} yr^{-1})	0.4 ± 0.56	0.02 - 1.96	0.34 ± 0.6	0.59 ± 0.77	73	0.58
Other litter (Mg ha^{-1} yr^{-1})	0.08 ± 0.08	0.03 - 0.35	0.11 ± 0.13	0.05 ± 0.02	-52	0.35
Total litter (Mg ha^{-1} yr^{-1})	7.51 ± 1.8	4.5 - 11.2	6.6 ± 0.84	7.78 ± 2.52	18	0.35
Fraction of leaf litter (%)	66 ± 9	54 - 80	70 ± 11	63 ± 6	-11	0.22

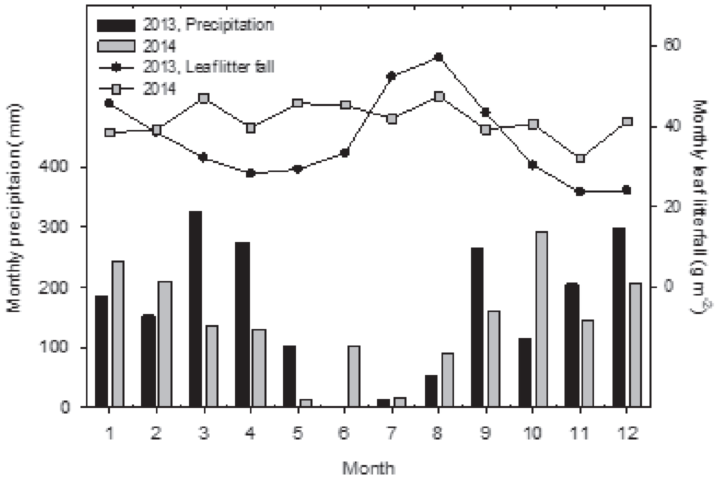

Figure 2. Monthly leaf litterfall and precipitation during 2013 and 2014. Precipitation was measured at the Uwinka station.

Nutrient flux in litterfall

The average annual total litterfall was 7.5 ± 1.8 Mg ha^{-1} yr^{-1}. It was not significantly different between ES and LS forest stands (Table 3). Similar results were obtained for all five components, leaves, reproduction organs, twigs (wood), epiphytes and other. The leaf litterfall was on average 66 ± 9% of total litterfall (Table 3), amounting to 4.9 Mg ha^{-1} yr^{-1} (ranging from 3.1 to 6.9 Mg ha^{-1} yr^{-1} in the plot with lowest and highest, respectively). In 2013, we observed a pronounced seasonal variation in leaf litterfall with a peak in the later part of the dry season (July - August). No such seasonal pattern was observed in 2014, when the dry season was less pronounced due to unusual rain events in June (Fig. 2).

Table 4. The flux of C, N, P, K in leaf litter and content of C and N in different soil layers (means ± SD and range) of all plots (1-15) and plots classified as early (ES; n = 5) or late (LS; n = 5) successional. L, litter layer; O, organic layer; M, mineral layer (0-45 cm depth below O-layer); LLF, leaf litterfall. Diff represents the mean % differences of LS in relation to ES plots and *P*-values are the results of a t-test of the difference between ES and LS stands. Bold value, $P < 0.05$.

	All species (1-20)			Plots at different successional stages					
				ES		LS		Diff	
Parameter	Mean	SD	Range	Mean	SD	Mean	SD	(%)	*P*-value
Leaf litter flux (kg ha^{-1} y^{-1})									
C	2437 ±	565	1508 - 3461	2232 ±	399	2441 ±	759	9	0.60
N	61.6 ±	13.2	41.7 - 85.2	57.4 ±	8.7	60.7 ±	16.3	5	0.73
P	3.2 ±	0.7	2.0 - 4.3	3.0 ±	0.4	3.0 ±	0.6	-2	0.84
K	19.3 ±	4.8	11.7 - 29.1	17.5 ±	4.6	18.7 ±	2.7	9	0.52
Soil C (t ha^{-1})									
L	4.3 ±	1.4	1.6 - 6.3	4.8 ±	1.0	3.5 ±	1.6	-28	0.15
O	31.4 ±	12.8	9.1 - 50.8	26.4 ±	6.7	35.8 ±	15.3	35	0.25
M	157 ±	37	86 - 196	173 ±	13	139 ±	45	-20	0.14
Soil N (t ha^{-1})									
L	0.14 ±	0.05	0.05 - 0.23	0.17 ±	0.04	0.10 ±	0.04	-38	**0.045**
O	1.7 ±	0.65	0.47 - 2.75	1.49 ±	0.31	1.81 ±	0.85	22	0.45
M	10.8 ±	2.47	6.15 - 14.02	12.56 ±	1.06	9.14 ±	2.53	-27	**0.024**
C/N ratio									
LLF	39.6 ±	2.7	33.7 - 44.5	38.8 ±	1.4	40.1 ±	4.2	3	0.53
L	31.1 ±	4.7	26.4 - 44.5	29.5 ±	2.0	34.0 ±	6.9	15	0.20
O	18.8 ±	1.9	16.0 - 24.0	17.6 ±	1.0	20.1 ±	2.2	14	0.053
M	14.1 ±	1.0	12.4 - 16.3	13.5 ±	0.7	14.5 ±	0.7	8	**0.047**
Litter turnover rate (y^{-1})*									
C	0.98 ±	0.48	0.43 - 2.14	0.74 ±	0.24	1.2 ±	0.56	64	0.12
N	0.78 ±	0.42	0.31 - 1.76	0.56 ±	0.18	1.0 ±	0.43	79	0.067
Annual leaf litter flux in relation to total soil content (%)									
C	1.3 ±	0.44	0.77 - 2.72	1.10 ±	0.19	1.37 ±	0.04	25	**0.014**
N	0.5 ±	0.16	0.28 - 0.99	0.40 ±	0.05	0.55 ±	0.04	37	**0.001**

*Assuming the same fraction of leaf in the litter layer as in the litter fall (66%).

The plot level fluxes of N, P and K to the forest floor through leaf litterfall ranged 42 – 85, 2.0 – 4.3 and 12 – 29 kg ha^{-1} y^{-1}, respectively (Table 4). None of these fluxes differed between ES and LS forest stands. However, the N content of the ground litter was significantly higher (+70%; $P = 0.045$) in ES plots compared to the LS plots (Table 4), mainly as a result of larger litter mass (37%, expressed as C) with lower C/N ratio (-13%) in ES plots (Table 4).

The annual leaf litter flux of C and N resulted in a ground litter turnover rate of 0.98 and 0.78 y^{-1}, respectively, and constituted on average 1.3% and 0.5%, respectively, of the total soil content of these elements (in ground litter, organic layer and down to 45 cm in the mineral soil). In relation to the total content of the soil, the proportion of the annual leaf litter flux of N was significantly lower (-27%; $P = 0.001$) in the ES compared to the LS plots, due significantly higher N content in both ground litter and mineral soil.

4. Discussion

This study presents data on leaf concentrations and resorption efficiencies of a dozen nutrient elements in 20 abundant tree species to assess potential nutrient limitations, and explore canopy nutrient cycling in tropical montane forests at different successional stages.

Nutrient limitations in Nyungwe TMF

Nutrient availability is a major factor limiting tree growth and NPP in TMFs (Benner et al. 2010). We used the concept of optimal stoichiometric relationships of nutrients to identify potentially limiting macro- and micronutrients (Ågren, 2008), developed for plants in growth chamber experiments and validated by boreal and temperate forest experiments (Ingestad, 1987; Linder, 1995). The optimal nutrient ratios commonly quantified in relation to N, have been defined as those "when all nutrients limit growth simultaneously" (Knecht & Göransson, 2004). As a consequence of how the optimal nutrient ratios were determined experimentally, they also represent the minimum requirement of individual nutrients supply to support maximum growth (Ingestad & Agren, 1992). As it has been suggested that these ratios are similar across all terrestrial plants (Knecht & Göransson, 2004) we used these ratios to indicate limitations by individual nutrients.

We found that for all macro- and micronutrients except P, their ratios to N were mostly higher than or similar to the suggested optimal ratios, which indicates that none of K, Ca, Mg, S, Fe, Mn, Zn or B were limiting tree growth in Nyungwe TMF. While B and K were just slightly below the limiting ratio in three species, P was below the suggested optimal ratio range for P (8-10% of N) in 18 of the 20 studied species. Koerselman and Meuleman (1996) suggested somewhat different limits for the transition between N and P limitation, in the range of N:P ratios between 14 and 16 (corresponding to P:N ratios of 6.3-7.1%) (Koerselman & Meuleman, 1996). According to these limits, 11 species were P limited, 5 species N limited and 4 species co-limited by N and P, indicating overall co-limitation by N and P in Nyungwe forest, but with P being somewhat more limiting than N. Although TMF has been considered to be N limited (Vitousek & Sanford, 1986) the results from fertilisation experiments in TMF indicate that some forest are limited by N, some by P, and other co-limited by both Tanner et al. 1998; Benner et al. 2011 and references therein (Benner et al., 2011; Tanner et al., 1998).

The Nyungwe forest seems to be richer in N than other TMF, as we observed mean leaf N concentrations of 20.4 mg g^{-1} (Table 1) while other TMF studies found average values ranging from 10 to 18 mg g^{-1} (Homeier et al., 2012; Kitayama & Aiba, 2002; Soethe et al., 2008; van de Weg et al., 2009; Vitousek & Sanford, 1986) . For leaf P, however, values in our study are within the range found in these earlier studies (1.3 mg g^{-1} in our study compared to 0.5 – 2.1 mg g^{-1} in (Homeier et al., 2012; Kitayama & Aiba, 2002; Soethe et al., 2008; van de Weg et al., 2009; Vitousek & Sanford, 1986).

There were significantly higher N:P ratios in LS compared to ES species (Table 2), indicating that the degree of P limitation is greater in LS than in ES stands. This significant difference was the combined result of opposite but small and individually non-significant differences in leaf N and P content in the two species groups (Table 1). Revisiting the first two hypotheses, we thus did not find support for N being the most growth limiting nutrient (rejecting our first hypothesis, except for some ES species), nor for ES species having higher leaf N content and N:P ratios than LS species (rejecting the first part of the second hypothesis). Instead, the results indicate an increasing P deficiency stress during succession in this TMF.

Patterns of nutrient resorption

The average N, P and K resorption efficiencies (37%, 48 %, 46%; Table 1) were lower than the global averages across plant types (62%, 65%, 70%) estimated by Vergutz et al., (2012). Global average for only evergreens of NRE and PRE for evergreen trees (52%, 60%; He et al., 2020), but still higher than our study. However, our results corroborate the general observation that NuRE for nutrients other than N, P and K is lower but also more variable (Liu et al., 2014; Vergutz et al., 2012; Yan et al., 2016). Furthermore, our findings the resorption efficiencies among nutrients are coordinated (Fig. 1b, d, e) and that PRE and KRE are higher than NRE (Table 1) agree with the observation of NuRE among different nutrients across different plant functional types (He et al., 2020; Vergutz et al. 2012) which confirms our third hypothesis. The very large and significant inter-specific variation in NRE (4 – 60%), PRE (29-75%) and KRE (9-79%) found in the present study is also consistent with previous studies (Aerts, 1996; Aerts & Chapin, 2000; Killingbeck, 1996; Wood et al., 2011). Since there were no systematic within-species differences in leaf nutrient concentrations and resorption efficiencies of N, P and K among plots at different successional stages and locations (Table 1, S2), this inter-specific variability was rather caused by inherent differences among species rather than by variation in environmental conditions.

It has been proposed that NuRE is higher at lower nutrient availability (Li et al., 2013; Mao et al., 2013), supported by the observation that NuRE decreases with increasing leaf nutrient concentrations in a global meta-analysis (Kobe et al., 2005). However, we found no significant relationships between the NuRE and leaf concentrations of N, P or K across all species (Fig. S3) which is consistent with findings by Enoki & Kawaguchi (1999) and Kazakou et al. (2007). However, we observed significant positive within-species relationships between NuRE and leaf concentrations of N, P and K in the two dominant species *M. kilimandscharica* and *S. guineense* (Fig. S2, Table S3) which is in line with the observations in a studies of two tropical forest in French Guiana (Urbina et al., 2021). While the species dominating ES stands (*M. kilimandscharica*) had higher NuRE and leaf nutrient content than the species dominating LS stands (*S. guineense,* Table S2), this does not reflect a general species difference between the two successional groups as ES and LS species mostly did not differ (Table 1, 2). Thus, the second part of our second hypothesis, that LS species have higher nutrient resorption efficiency than ES species, was not supported. However, further investigations are warranted to be conclusive as this is in contrasts with other studies reporting significantly lower NRE and PRE in ES compared to LS species (Fahey et al., 1998; Urbina et al., 2021).

A potential bias in our NuRE estimates is that green leaves were all collected in the mid to upper canopy while senesced leaves collected in litter traps came from all parts of the canopy. However, the potential error caused by this was probably minor since a previous study in Nyungwe found no significant differences in mass-based leaf N or P concentrations between upper and lower canopy leaves across six tree species (three ES species, three LS species; Dusenge et al., 2015). There was, however, a significant difference in LMA between upper and lower canopy leaves in that study, which may have somewhat affected the estimates of mass loss and, hence, NuRE made in the present study.

Both leaf mass loss (mean 17%, ranging from 4.3% to 29.3%) and leaf area shrinkage (mean 9.5%, ranging from 4.8% to 16.4 %; c.f. eq. 2) during senescence were substantial in our species and ecosystems. If not accounted for, the NuRE underestimation in the present study would have been on average 11%-units, but up to 23%-units in one species were recorded.

Canopy nutrient cycling

The annual litterfall (4.5-11.2 Mg ha^{-1} yr^{-1}) was in the range of earlier observations in tropical montane forests (3.6 - 11 Mg ha^{-1} yr^{-1}; Vitousek and Sandford 1986; Veneklaas 1991; Kitayama and Aiba 2002; Cizungu et al. 2014; Becker et al. 2015; Paudel et al. 2015), as was also the proportion of leaf mass litterfall (66% in our study compared to the common range 60-70%). Like other studies, we observed a peak in the litterfall during the dry season (Cizungu et al., 2014; Köhler et al., 2008; Paudel et al., 2015) of the first year, but not during the second year. The lack of peak in the second year was likely because the dry period was interrupted by heavy rain events that year (Figur 2), making the drought less prominent. Litterfall reflects the canopy NPP and is an important pathway of nutrient cycling in forests (Bray & Gorham, 1964; Malhi et al., 2011; Vitousek, 1984). The link between NuRE and tree growth is not straightforward since high NuRE directly improves tree nutrient economy but indirectly leads to lower soil nutrient availability due to lower litter quality and therefore lower mineralisation rate (Aerts, 1997). Conversely, low NuRE causes higher quality litter (i.e. with high N concentration) which may result in a positive plant-soil feedback (Lü et al., 2012). The rather low NRE observed in this forest combined with generally high leaf N concentrations may therefore benefit N mineralisation and the overall nutrient cycling.

In earlier studies, leaf litter accounted for 56-71% of the total N, P and K content of litterfall in various tropical forests (Paudel et al., 2015; Veneklaas, 1991; Yang et al., 2004) . Assuming our study has a median proportion of this interval (where N, P and K content was analysed for leaf litter only) would give a nutrient return in all litter of c. 90, 5 and 30 kg ha^{-1} yr^{-1} for N, P and K, respectively. This is in the upper range observed in several other TMF in South America and Africa (28 - 90, 1 - 5, 6 - 39 kg ha^{-1} yr^{-1} of N, P and K, respectively; Becker et al., 2015; Vitousek & Sanford, 1986), but much lower than Paudel et al. (2015) observed in a lower mature and regenerating TMF in south China (147, 13, 59 kg ha^{-1} yr^{-1} of N, P and K, respectively). We also found that the average litter N concentration in the Nyungwe forest (average 15 mg g^{-1} and range 8 - 28 mg g^{-1}) was high compared to most other TMF (6 – 1.2 mg g^{-1}; Becker et al., 2015; Veneklaas, 1991; P. M. Vitousek & Sanford, 1986), suggesting high N mineralisation rates and nutrient availability in this forest. Overall this study suggests that Nyungwe forest is more fertile than many other TMF, which is also supported by earlier studies showing high photosynthetic capacity (Dusenge et al., 2015) and NPP in both ES and LS stands in Nyungwe forest (Nyirambangutse et al., 2017).

5. Conclusion

This study reduces major knowledge gaps regarding nutrient limitations and cycling in Afromontane tropical forests by demonstrating that that in the largest remaining African TMF, Nyungwe: (i) P limitation is more common than limitations by N or other nutrients, especially at late successional stages; (ii) nutrient concentrations and resorption efficiencies were mostly similar in ES and LS species, but ES species had lower N:P ratios; and (iii) resorption efficiency is generally coordinated among different nutrients, but higher for P and K compared to enzyme-bound N and S, and for macro- compared to micronutrients. While the third finding was as hypothesized, the first two show that African TMFs are distinctly different compared to Neotropical counterparts, in which N limitation is the rule and fast-growing ES species have higher N concentrations and N:P ratios than LS species (Poorter et al., 2004; Schönbeck et al., 2015). Leaf N concentrations were higher in our forest compared to previously studied TMFs. In addition, an earlier study at the same plots in Nyungwe found that our forest also has higher productivity and aboveground biomass compared to Neotropical TMFs (Nyirambangutse et al., 2017), and another Nyungwe study reported high soil N transformation and leaching rates (Rütting et al., 2015). We thus conclude that our African TMF is more fertile, and less N limited than Neotropical counterparts. Moreover, while nutrient concentrations and resorption efficiencies were mostly similar in ES and LS species, relative P limitation rather than relative N increased increased during succession. This is also indicative of a forest with plenty available soil N.

Our results clearly demonstrate that our forest exhibits nutrient limitations that substantially differ from those of Andean TMFs. This is perhaps not surprising, given their distinct geological and climatic history, but highlights the need for explicit consideration of these differences in efforts to quantify tropical carbon and nutrient pools and fluxes.

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Supplementary material

The following Tables and Figures are supplementary for this article:

Table S1. Characteristics of studied species.

Table S2. Leaf mass per unit area, leaf nutrient concentrations and resorption efficiency in *Macaranga kilimandscharica* and *Syzygium guineense* from different plots.

Figure S1. Leaf mass per unit area, leaf nutrient concentrations and nitrogen resorption efficiency of a subsample versus all samples.

Figure S2. Leaf P, K and S concentration in relation to N concentration in *Macaranga kilimandscharica* and *Syzygium guineense* from different plots.

Table S3. Results from statistical analysis of leaf P, K and S concentration in relation to N concentration in *Macaranga kilimandscharica* and *Syzygium guineense* from different plots.

Figure S3. The resorption efficiency of N, P and K in relation to the leaf concentration of each element.

Figure S4. Resorption efficiency of N, P and K in relation to the leaf concentration of each element in *Macaranga kilimandscharica* and *Syzygium guineense* from different plots.

Table S4. Results from statsitical analysis of resorption efficiency of N, P and K in relation to the leaf concentration of each element in *Macaranga kilimandscharica* and *Syzygium guineense* from different plots.

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Table S1. Characteristics of the studied tree species[a]. Successional groups (SG)[b], no of stems ha^{-1} in plots, basal area (BA) in plots, no of trees (#) and mean diameter at breast height (*D*) of trees from which leaves were sampled.

			Plot means		Sample trees	
Species	Family	SG	No of stems *D* > 5 cm (ha^{-1})	BA (m^2 ha^{-1})	#	*D* (cm)
Afrocrania volkensii (Harms) Hutch.	Cornaceae	ES	16	0.50	3	31
Agauria salicifolia Hook.f. ex Oliv.	Ericaceae	ES	11	0.56	3	34
Carapa grandiflora Sprague	Meliaceae	LS	34	0.78	21	36
Chionanthus africanus (Knoblauch) Stearn	Oleaceae	LS	10	0.11	3	31
Cleistanthus polystachyus Hook.f. ex Planch.	Euphorbiaceae	LS	9	0.79	11	37
Faurea saligna Harv.	Proteaceae	LS	7	1.80	12	27
Ficalhoa laurifolia Hiern	Theaceae	LS	10	0.73	11	35
Harungana montana Spirl.	Clusiaceae	ES	5	0.70	12	54
Ilex mitis Radlk.	Aquifoliaceae	ES	10	0.36	8	50
Macaranga kilimandscharica Pax	Euphorbiaceae	ES	239	7.40	38	29
Maytenus acuminata (L.f.) Loes.	Celastraceae	ES	31	0.37	4	22
Ocotea kenyensis (Chiov.) Robyns & R.Wilczek	Lauraceae	LS	9	0.90	15	55
Ocotea usambarensis Engl.	Lauraceae	LS	25	0.97	12	52
Olinia rochetiana A.Juss.	Oliniaceae	LS	8	0.54	6	31
Polyscias fulva (Hiern) Harms	Araliaceae	ES	11	1.00	14	43
Prunus africana (Hook.f.) Kalkman	Rosaceae	ES	3	0.17	11	28
Psychotria mahonii C.H.Wright	Rubiaceae	ES	34	0.36	7	25
Rapanea melanophloeos Mez	Myrsinaceae	ES	19	0.29	5	25
Strombosia scheffleri Engl.	Olacaceae	LS	5	0.36	13	30
Syzygium guineense (Willd.) DC.	Myrtaceae	LS	93	7.94	38	47

[a] Plant and family names are in accordance with The International Plant Names Index database (http://www.ipni.org/).

[b] The classification of the species into successional groups was based on information in the following publications in combination with our own observations on the degree of occurrence in ES and LS stands of the present study: Bussman (2002), Tesfaye et al. (2002), Bloesch et al. (2009),Fashing (2004), Eilu and Obua (2005), Fisher and Killmann (2008), Kindt et al. (2014), Rutten et al. (2015).

Table S2. Leaf mass per unit area (LMA), leaf nutrient concentrations (X_m) and resorption efficiency of N, P and K in *Macaranga kilimandscharica* and *Syzygium guineense* in plots where they co-occur abundantly and therefore both sampled (CP) and where only one of the species was abundant and sampled (SP). There are 6 CP (plot no 4, 5, 7, 8, 9 & 12), 4 SP (1, 2, 3 & 6) for *M. kilimandscharica* and 5 SP (10, 11, 13, 14, 15) for *S. guineense.* Bold value, $P < 0.05$.

	Plot	LMA	Leaf nutrient ratio or concentration (mg g^{-1})													Resorption (%)		
Species	group	(g m^{-2})	N:P	N_m	P_m	K_m	Ca_m	Mg_m	S_m	Fe_m	Mn_m	Zn_m	B_m	Cu_m	Mo_m	NRE	PRE	KRE
Macaranga kilimandscharica	CP	115	14	19	1.4	8.3	4.9	2.0	2.6	0.2	415	13	19	8	0.10	52	67	76
	SP	122	15	21	1.5	9.3	6.0	2.2	3.0	0.2	451	14	17	9	0.14	56	61	60
Diff. between plot groups (%)		6	7	13	5	13	22	8	12	15	9	5	-7	16	40	8	-8	-20
P-values (between plot groups)		0.48	0.22	0.055	0.41	0.38	0.39	0.65	0.28	0.40	0.78	0.57	0.74	0.04	0.49	0.27	0.32	0.19
Syzygium guineense	CP	137	17	15	0.9	5.4	5.2	2.3	1.8	0.2	642	9.1	13	4	0.06	37	49	52
	SP	136	15	15	1.0	6.2	6.8	1.9	2.1	0.2	350	9.4	15	9	0.10	41	61	59
Diff between plot groups (%)		-1	-7	2	10	15	32	-18	15	20	-45	3	15	101	67	9	25	14
P-values (between plot groups)		0.73	0.23	0.70	0.59	0.97	0.08	0.17	0.52	0.27	0.15	0.95	0.87	0.11	0.64	0.92	0.23	0.89
Diff between species (%)	CP	19	22	-21	-35	-35	4	13	-31	9	54	-32	-31	-43	-41	-27	-27	-31
	All	17	14	-22	-32	-29	7	-3	-27	10	6	-30	-20	-19	-22	-22	-11	-14
P-values (between species)	CP	**0.012**	**0.002**	**0.019**	**<0.001**	0.067	0.97	0.51	**0.024**	0.82	0.18	**0.002**	0.18	**0.013**	0.64	**0.017**	**0.031**	**0.011**
	All	**0.009**	**0.005**	**<0.001**	**<0.001**	**0.004**	0.62	0.76	**<0.001**	0.49	0.76	**<0.001**	0.09	0.24	0.41	**0.008**	0.10	0.12

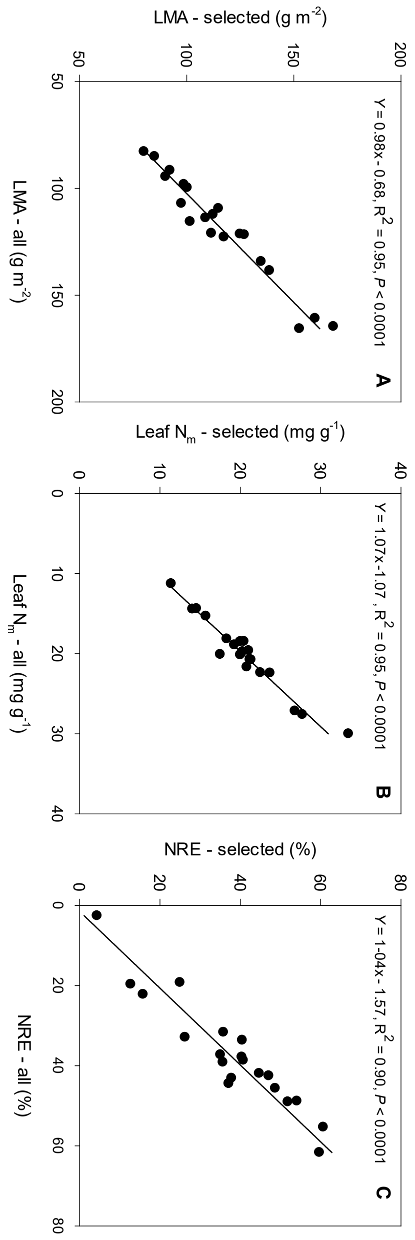

Figure S1. The (a) leaf mass per unit area (LMA), (b) leaf N concentration (N_m) and (c) N resorption efficiency (NRE) of leaf samples where only N was analysed versus samples selected for analysis of all macro and micro nutrients. Each data point represents the mean for one species. The regression equation, R^2 and *P* value is given for each relationship. All nutrients were analysed for 76 leaf samples and 118 leaf litter samples, while N concentration only was analysed in 249 leaf samples and 601 leaf litter samples.

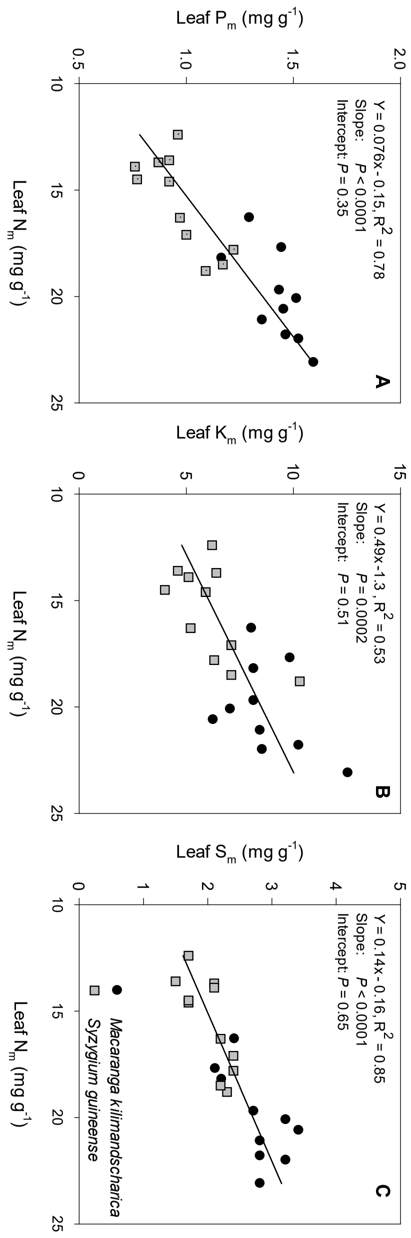

Figure S2. Leaf concentration of (a) P (P_m), (b) K (K_m) and (c) S (S_m) in relation to the concentration of N (N_m) in *M. kilimandscharica* and *S. guineense.* Each data point represents the species-specific mean of trees from one plot. Equations, regression lines and R^2 values across both species are given in the figure, including *P*-values for both slope and *y*-intercept. Individual species parameters are given in Table S3.

Table S3. Species-specific parameters for the relation between leaf concentration of P (P_m), K (K_m) and S (S_m) versus N (N_m) in *M. kilimandscharica* (*Mk*) and *S. guineense* (*Sg*). Data points are shown in Fig. S2. Bold value, $P < 0.05$.

	Leaf P_m vs Leaf N_m		Leaf K_m vs Leaf N_m		Leaf S_m vs Leaf N_m	
	Mk	*Sg*	*Mk*	*Sg*	*Mk*	*Sg*
Slope	0.041	0.051	0.33	0.49	0.13	0.11
y-intercept	0.61	0.17	2.0	-1.4	0.09	0.32
R^2	0.48	0.59	0.16	0.42	0.43	0.58
P-value slope	**0.027**	**0.006**	0.26	**0.032**	**0.040**	**0.006**
P-value y-intercept	0.078	0.45	0.71	0.65	0.94	0.53

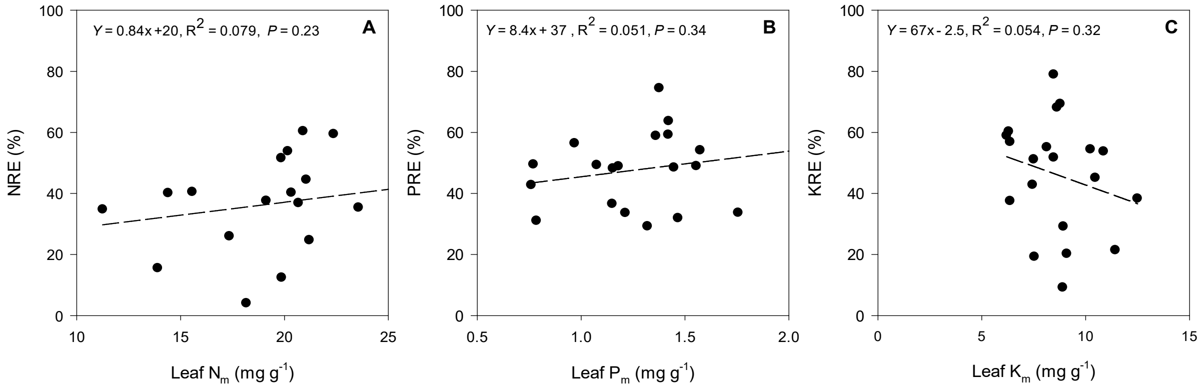

Figure S3. The resorption efficiency of N (NRE, a), P (P_m, b) and K (K_m, c) in relation to the leaf concentration of each of these elements. Each data point represents the mean of one species (n = 20). Equations, regression lines and R^2 values *P*-values are given for each relationship.

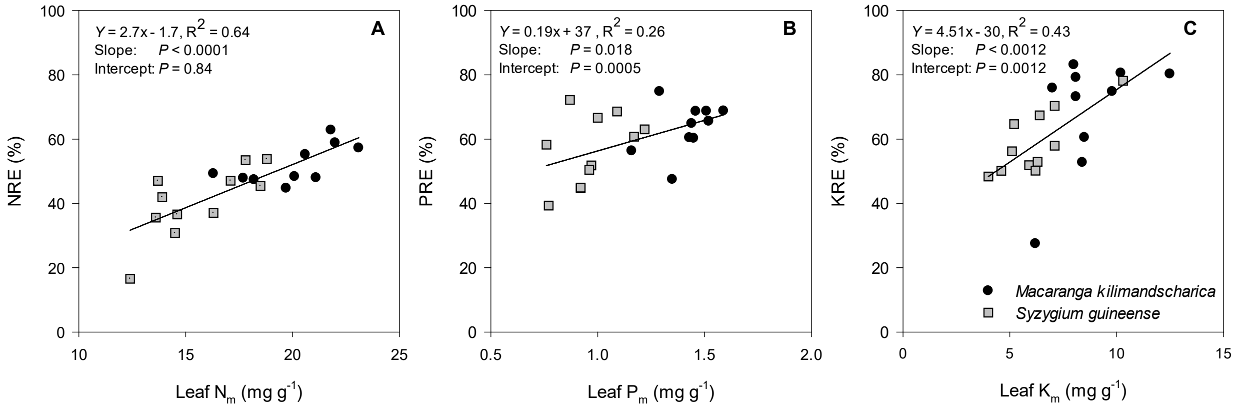

Figure S4. The resorption efficiency of N (NRE, a), P (PRE, b) and K (KRE, c) in relation to the concentration of each of these elements in *M. kilimandscharica* and *S. guineense.* Each data point represents the species-specific mean of trees from one plot. Equations, regression lines and R^2 values across both species are given in the figure, including *P*-values for both slope and *y*-intercept. Individual species parameters are given in Table S4.

Table S4. Species-specific parameters for the relation between resorption efficiency of N (NRE), P (PRE) and K (KRE) versus leaf concentration of N (N_m) in *M. kilimandscharica* (*Mk*) and *S. guineense* (*Sg*). Data points are shown in Fig. S4. Bold value, *P* < 0.05.

	NRE vs Leaf N_m		PRE vs Leaf P_m		KRE vs Leaf K_m	
	Mk	*Sg*	*Mk*	*Sg*	*Mk*	*Sg*
Slope	1.9	3.6	22.9	32.3	5.2	4.5
y-intercept	14.0	-16.3	31.0	25.3	24.0	31.2
R^2	0.44	0.55	0.14	0.19	0.28	0.58
P-value slope	**0.037**	**0.009**	0.30	0.18	0.12	**0.006**
P-value y-intercept	0.38	0.37	0.32	0.27	0.38	**0.004**

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