Environmental and physiological drivers of tree growth

A pan-tropical study of stable isotopes in tree rings

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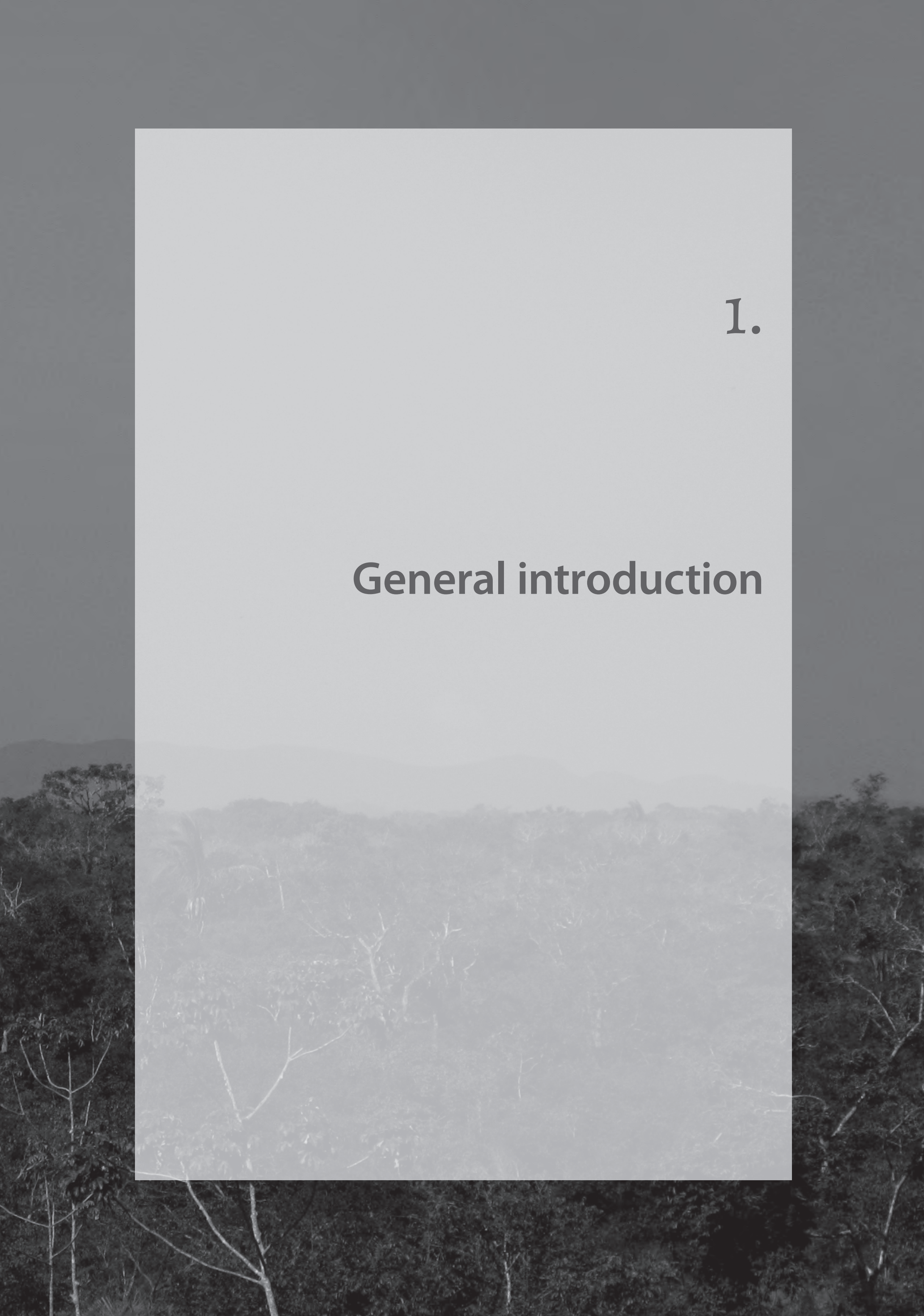
Glossary

A	photosynthetic rate
BAI	basal area increment
C_a	ambient CO_2 concentration
C_c	CO_2 concentration in the mesophyll
C_i	CO_2 concentration in the intercellular spaces in leaves
Δ	isotope discrimination (often follow by the isotope under study, e.g. $\Delta^{13}C$)
$\delta^{13}C$	stable isotope ratios of carbon ($^{13}C/^{12}C$) in a substance compared to a reference material
$\delta^{18}O$	stable isotope ratios of oxygen ($^{18}O/^{16}O$) in a substance compared to a reference material
$\delta^{15}N$	stable isotope ratios of nitrogen ($^{15}N/^{14}N$) in a substance compared to a reference material
dbh	diameter at breast height (=1.30 meter)
gs	stomatal conductance
gm	mesophyll conductance
SST	sea surface temperature
WUE	water-use efficiency
iWUE	intrinsic water-use efficiency



1.

General introduction

Forests in the wet tropics harbour an incredible biodiversity and provide many ecosystem services on which millions of people depend (Myers 1992). They are also a major component of the global carbon cycle, storing 25% of the total terrestrial carbon and accounting for a third of net primary production (Bonan 2008). Furthermore, climate models show that tropical forests decrease air temperature and increase precipitation, whereas pasturelands are associated with a drier and hotter climate (Bala et al. 2007, Bonan 2008). Therefore, any change in forest structure and forest cover in the wet tropics will not only affect local climatic conditions, but also have implications for the global carbon cycle and – as a result – may speed up or slow down global warming.

Deforestation rates are still high in the tropics and have profoundly affected the extent of forests in many countries (Hansen et al. 2013). Additionally, there are also indications that undisturbed and apparently pristine tropical forests are changing (e.g. in tree growth rates and forest biomass). These changes seem to occur without a direct human cause, as most of the forest sites studied are remote and far from human settlements. The observed changes must therefore have a more indirect cause (like climate change). In this thesis, the main focus is on changes in intact tropical forests and the potential drivers of these changes.

1.1 Changes in intact tropical forests

Evidence that intact tropical forests are changing was obtained from the repeated measurement of permanent forest plots (i.e. forest plots where size, mortality and recruitment of trees is regularly measured). The most striking findings from these studies are that there seem to have been an acceleration of tree growth and increase of forest biomass per unit of surface area over the last decades (e.g. Phillips et al. 1998, Laurance et al. 2004, Lewis et al. 2009a). Other observations include an increase of forest dynamics and of liana dominance (Phillips et al. 2002, Lewis et al. 2009a). Increased tree growth and forest biomass have been reported from permanent forest plots in South America (Lewis et al. 2004, Phillips et al. 2008), Africa (Lewis et al. 2009b) and Asia (Phillips et al. 1998, Chave et al. 2008), suggesting that this phenomenon is taking place across the tropics. If this is indeed the case, it would entail that the world's tropical forests are potentially absorbing a significant fraction of human caused CO_2 emissions and as such are mitigating climate change.

However, not all studies support these findings and some also provide evidence for constant and decreased tree growth and forest biomass (Feeley et al. 2007, Clark et al. 2010, Dong et al. 2012, Chambers et al. 2013). In addition, some researchers have warned for a potential overestimation of forest change when results from small permanent plots are extrapolated to tropical forests in general (Fisher et al. 2008, Körner 2009, Chambers et al. 2013). These uncertainties and contradicting findings have led to an ongoing debate on whether or not intact tropical forests are in a state of change.

1.2 Potential drivers of forest change

The possible alteration of tree growth rates and biomass in tropical forests has triggered a second debate on the factors that are responsible for these changes (e.g. Körner 1998, 2009, Lewis et al. 2009a). The most prominent factors in this discussion are (1) the global rise of atmospheric CO_2 concentration, (2) increased nutrient depositions, (3) climate change (global warming and changed precipitation) and (4) past disturbances.

As for the first, atmospheric CO_2 has increased by ~40% since the onset of the industrial revolution. Elevated CO_2 can directly affect plants by increasing the activity, as well as the efficiency, of the CO_2 fixing enzyme rubisco and thereby increase photosynthetic rates (Gunderson and Wullschleger 1994). Potentially more important in plant communities subjected to periods of limited water availability (like a dry season) is the reduction of stomatal conductance by elevated CO_2, which lowers evapo-transpiration and hence reduces water losses (Holtum and Winter 2010). A lowered stomatal conductance increases water-use efficiency (i.e. the amount of carbon gained through photosynthesis divided by the amount of water loss through transpiration) and might allow plants to extend their growth season and/or increase their photosynthetic activity during the hottest hours of the day when water-stress could be severe. Elevated atmospheric CO_2 is thus a very likely candidate to have stimulated tropical tree growth, provided at least that plant growth is either carbon or water limited.

The second factor that could have affected tropical forests over the last decades is anthropogenic nutrient deposition. Nutrient depositions (especially of nitrogen) have likely increased due to the enhanced industrialization, land-use intensification (e.g. the use of artificial N fertilizers) and forest fragmentation in most tropical countries (Galloway et al. 2008, Hietz et al. 2011). Nutrients can stimulate plant growth, as is well known from the positive effect of fertilizer application on crop yields.

A third potential driver of the apparent changes in intact tropical forests is climate. In the tropics, temperature has increased by ~0.26 °C per decade since 1970 and there are possibly also changes in precipitation in some regions (Malhi and Wright 2004). Increased temperature is primarily associated with a negative effect on tree growth because it can increase respiration rates and water-stress (Wright et al. 2006, Doughty and Goulden 2008). Temperature effects might be responsible for the observed decrease in tree growth found in some studies (Clark et al. 2003, Feeley et al. 2007).

An alternative explanation for the observed changes in tropical forests is the effect of large scale disturbances in the past. The origin of such a disturbance can be natural (e.g. a hurricane or a drought period related to an El Niño event) or anthropogenic (e.g. slash and burn agriculture). The occurrence of large-scale natural disturbances has been documented in scientific literature (e.g. Nelson et al. 1994, Lugo and Scatena 1996, Vandermeer et al. 2000). Furthermore, there is increasing evidence that in past centuries, human populations were large and profoundly affected the environment in most areas that are currently seen as undisturbed and pristine forests (White and Oates 1999, Clark 2007, Paz-Rivera and Putz 2009). Given the large time scale at which forest succession takes place, such past disturbances could still affect present day forest dynamics and biomass accumulation (Foster et al. 1998, Chai and Tanner 2011).

1.3 Knowledge gaps and aim of this thesis

Although the monitoring of permanent forests plots is one of the most powerful tools to study changes in forest dynamics and carbon fluxes, the time span of monitoring is still relatively short (typically less than 30 years). Thus, it is unclear whether the observed changes in tropical forests are part of a progressive long-term change (as might be expected when driven by the rise of atmospheric CO_2) or that they merely reflect decadal-scale fluctuations (e.g. caused by climatic variability). As for the possible drivers of changes in tree growth, experiments on the effect of increased temperature (Doughty 2011) and drought (Nepstad et al. 2007, Metcalfe et al. 2010) have been conducted with trees from the wet tropics, but they again are limited by a short time span studied (less than a decade). Furthermore, experiments on the effect of increased atmospheric CO_2 on tree growth and performance (so called FACE experiments) have not yet been conducted on mature tropical trees. Thus the extent and manner in which tropical trees have been affected by human-induced environmental change are still uncertain.

The paucity of long-term data can be overcome by the analysis of annual growth rings in tropical trees, which can provide information on growth rates often spanning more than a century (Zuidema et al. 2013). Tree-ring research in the tropics has long been neglected, due to the belief that trees in the wet tropics do not form reliable annual growth rings given the continuity of favourable growth conditions. However, annual growth rings in tropical trees can be formed by a cambial dormancy caused by the presence of a dry season (e.g. Worbes and Junk 1999) or by seasonal flooding (e.g. Schöngart et al. 2002). During the last decades, the presence of clear and annual growth rings has been demonstrated for many tropical tree species (e.g. Worbes 2002, Brienen and Zuidema 2005) and even for trees from the very wet tropics (Zuidema et al. 2012).

In addition to ring width as a measure of tree growth, stable isotopes in the wood of trees can be used as an archive of environmental conditions. Stable isotopes are variations of the same element that differ in the number of neutrons in the nucleus. They are stable variations with the same chemical properties, but the differences in mass allow physical, chemical and biological processes to 'discriminate' against one of them (McCarroll and Loader 2004). The result of this discrimination is a change in isotope ratio from a source (e.g. ambient CO_2) to a product (e.g. CO_2 in leaves) and is also known as 'fractionation'. Wood consists of three basic elements: carbon, oxygen and hydrogen, together with much smaller concentrations of other elements, like nitrogen. There are many points of discrimination (resulting in a fractionation), during the complex pathways that C, O, H and N make before they are built into the wood of trees. The degree of fractionation that occurs during these pathways, is affected, in various degrees, by environmental conditions (McCarroll and Loader 2004). This leads to the incorporation of environmental signals in the stable isotope ratios in wood and allows the study of long-term environmental change (see more information on stable isotopes below).

This thesis is part of a larger project on changes in tropical forests that uses growth rings in trees to obtain information covering a long temporal scale (the TroFoClim project led by Pieter Zuidema). The focus of this project is on changes in tree growth and the drivers thereof. In the project, trees were sampled at a forest site in Bolivia (by Peter van der Sleen), Cameroon (by Peter Groenendijk) and Thailand (by Mart Vlam; see locations in Fig. 1.1). At each site, more than 500 trees of five species were randomly sampled from a large plot (144-297 ha) in old-growth forest (Table 1.1 for species used in this thesis). Based on the collected tree samples, three dissertations were written: on long-term tree growth (Peter Groenendijk), on the effect of past disturbances on present-day forest dynamics (Vlam 2014) and this thesis on the human-induced environmental changes that could have affected tree growth. The main aims of this thesis are: (i) to detect temporal patterns in light, nitrogen cycling and climate variability and (ii) to assess the effects of increased atmospheric CO_2 on tree physiology and tree growth.

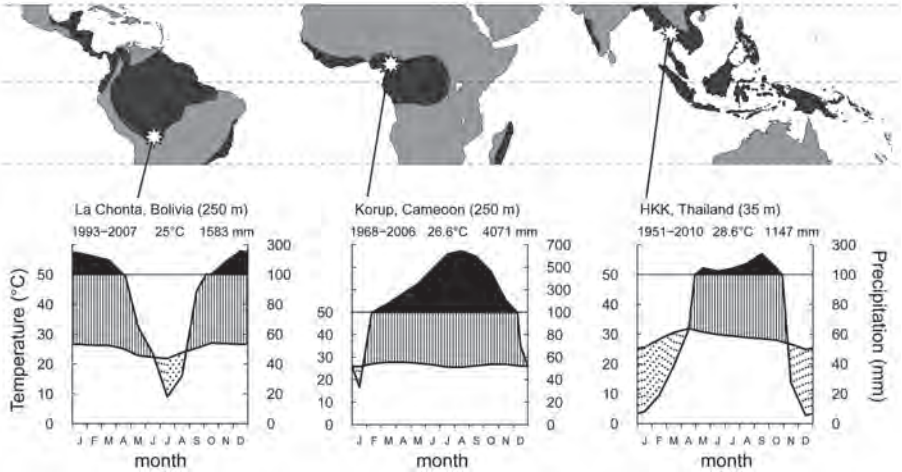


Figure 1.1 Wood samples were collected from three wet tropical forests (>1500 mm rainfall per year; dark grey areas). Bolivia: La Chonta logging concession; Cameroon: a logging concession adjacent to Korup National park and Thailand: Huai Kha Khaeng Wildlife Sanctuary (HKK). For the climate diagram of Bolivia, precipitation data were used from the La Chonta saw-mill, located 30 km north of the study site. Temperature data are from Ascención de Guarayos (1987-2006), 60 km west of the study site. For Cameroon, monthly precipitation and temperature of two nearby stations were averaged: Mamfé Airport weather station (40 km north of study site) and Bulu meteorological station (40 km south of the study site). For Thailand, precipitation and temperature data are from Nakhon Sawan, the closest station, 100 km east of the study site. The total annual precipitation at the Thai study site (HKK) is however higher than in Nakhon Sawan (around 1500 mm) and temperature lower (23.5 °C; Bunyavejchewin et al. 2009), but no long-term climate data are available for HKK. Dotted area indicates the dry season (precipitation <100 mm/month), black area the rainy season (>100 mm/month).

Table 1.1 Study species

Country	Species	Family	Func. group[1]	Phenology[2]	Ring boundary[3]	Annual rings
Bolivia	*Ampelocera ruizii*	Ulmaceae	ST	E	Marginal parenchyma	Lopez et al. (2012)
	Cariniana ianeirensis	Lecythidaceae	PST	D	Compressed fibres	Lopez et al. (2012)
	Hura crepitans	Euphorbiaceae	PST	D	Compressed fibres	Lopez et al. (2012)
	Sweetia fruticosa	Fabaceae	LLP	BD	Marginal parenchyma	Brienen and Zuidema (2003)
	Peltogyne cf. *heterophylla*	Fabaceae	ST	BD	Marginal parenchyma	Brienen and Zuidema (2005)
Cameroon	*Brachystegia cynometroides*	Fabaceae	PST	BD	Marginal parenchyma	Groenendijk et al. (2014)
	Brachystegia eurycoma	Fabaceae	PST	BD	Marginal parenchyma	Groenendijk et al. (2014)
	Daniellia ogea	Fabaceae	PST	BD	Marginal parenchyma	Groenendijk et al. (2014)
	Entandrophragma utile	Meliaceae	ST	D	Marginal parenchyma	Hummel (1946)
	Terminalia ivorensis	Combretaceae	LLP	D	Variation in wood density	Detienne et al. (1998)
Thailand	*Afzelia xylocarpa*	Fabaceae	LLP	D	Marginal parenchyma	Baker et al. (2005), Vlam et al. (2014)
	Chukrasia tabularis	Meliaceae	PST	E / BD	Marginal parenchyma	Baker et al. (2005), Vlam et al. (2014)
	Melia azedarach	Meliaceae	LLP	D	Ring porous	Baker et al. (2005), Vlam et al. (2014)
	Toona ciliata	Meliaceae	LLP	D	Ring porous	Baker et al. (2005), Vlam et al. (2014)

[1] Tree species were categorized in functional groups based on the definitions in Poorter et al. (2006): ST=shade-tolerant, PST=partial shade-tolerant, LLP=long-lived pioneer.

[2] Data on leaf phenology are from Mostacedo et al. (2003) for the Bolivian species; Williams et al. (2008) for the Thai species; Lemmens et al. (2012) and Poorter et al. (2004) for the Cameroonian species: D=deciduous, BD= brevi-deciduous, E=Evergreen

[3] Ring boundary categories after Worbes (1995), classification based on personal observations.

1.3 Theory and application of stable isotopes

To study the environmental and physiological drivers of changes in tree growth, we measured stable isotopes on trees from the three study areas. The isotope ratios of three different elements were analysed, namely those of carbon, oxygen and nitrogen. Here, a short overview is given of the basic information that these stable isotopes in wood can provide.

Carbon isotopes

Of all the carbon in the world, 98.89% has a nuclear core with six protons and six neutrons (^{12}C). A small fraction (1.1%) has an extra neutron (^{13}C) and even smaller fractions exist of none-stable isotopes, like radio carbon (^{14}C). The ratio of ^{13}C to ^{12}C in plant materials can be measured using a mass-spectrometer and is calculated as:

$$\delta^{13}C_{plant} = (R_{sample} / R_{standard} - 1)*1000 \qquad (1)$$

With, R_{sample} is the $^{13}C/^{12}C$ ratio of a plant sample (e.g. wood of a tree ring) and $R_{standard}$ the $^{13}C/^{12}C$ ratio of an internationally recognized standard material (V-PDB). The calculated $\delta^{13}C_{plant}$ is expressed in ‰.

The $\delta^{13}C$ of plant materials is affected by the general higher affinity of plants for $^{12}CO_2$ than for $^{13}CO_2$. They thus discriminate against ^{13}C, a process that contains two main parts in plants using C3 photosynthesis. First, when plant absorb CO_2 trough their stomata, CO_2 with a lighter carbon isotope diffuses more easily than those with a heavier one. This is because when molecules bounce off each other, the lighter ones bounce furthest (McCarroll and Loader 2004). As a result, the CO_2 in the intercellular spaces in leaves (C_i; Fig. 1.2) has a lower proportion of ^{13}C compared to the CO_2 outside the leaves (Ca; Fig. 1.2). A second discrimination takes place in the mesophyll by the enzyme rubisco during carboxylation, which has a higher affinity for $^{12}CO_2$. The widely used equation for these isotope discriminations is given by Farquhar et al. (1982):

$$\Delta = \delta^{13}C_a - \delta^{13}C_{plant} = -\varepsilon_D(1- C_i/C_a) - \varepsilon_C(C_i/C_a) \qquad (2)$$

$\delta^{13}C_a$ is the $\delta^{13}C$ of the atmospheric CO_2 (currently about -8‰), ε_D is the discrimination during diffusion (4.4‰) and ε_c is the discrimination by the CO_2 fixing enzyme rubisco (27‰). Although the discriminations that occur during diffusion and carboxylation are constants, the total discrimination (Δ) is not merely the sum of the individual discriminations (ε_D and ε_C), but depends on the ratio between the CO_2 concentration in the intercellular spaces in the leaves (C_i) and the atmosphere (C_a). This can be understood by evaluating two extremes. First, a case of high stomatal conductance, e.g. when wet conditions prevail. If diffusive limitations are small (C_i is high), rubisco can strongly discriminate against $^{13}CO_2$, resulting in low $\delta^{13}C$ values in the carbohydrates formed during photosynthesis. In the opposite case, e.g. when dry conditions prevail, stomatal conductance is lowered. This limits the diffusion of $^{13}CO_2$ out of the leaf and makes the internal CO_2 pool increasingly rich in ^{13}C, which in turn suppresses the discrimination by rubisco. In that case, carbohydrates will have relatively high $\delta^{13}C$ values.

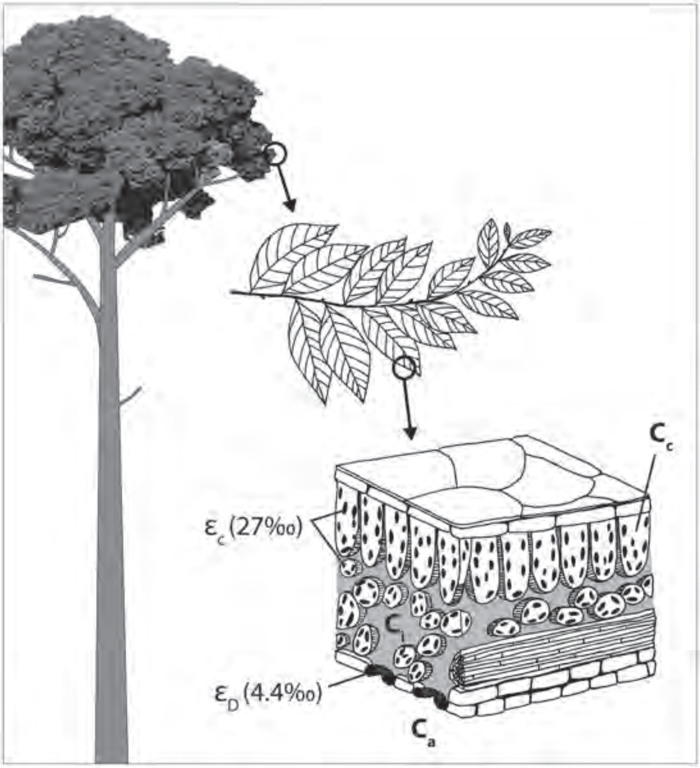


Figure 1.2 From a tree to a cross-section of a leaf; with C_a referring to the CO_2 concentration of the atmosphere, C_i to the CO_2 concentration in the intercellular space in leaves (grey area in cross-section), C_c to the CO_2 concentration in the mesophyll. The ε_D is the discrimination against $^{13}CO_2$ during diffusion (4.4‰) and ε_c is the discrimination by the CO_2 fixing enzyme rubisco (27‰). Illustration of cross-section adapted from Winterborne (2005).

Following the Farquhar model, $\delta^{13}C$ in plant materials can be used as a proxy for C_i and its derivative intrinsic water-use efficiency (iWUE; see Box 1). C_i is an important parameter that has long been studied to assess tree physiological responses (changes in stomatal conductance and photosynthetic rates) to environmental variability. But, although equation 2 is commonly used for the interpretation of $\delta^{13}C$ values in plant materials, it does not include two additional discrimination processes. Firstly, the Farquhar model assumes an infinite mesophyll conductance. Mesophyll conductance is the diffusion of CO_2 from the intercellular space to the mesophyll, where CO_2 is eventually fixed. In the case of infinite mesophyll conductance, the CO_2 concentration in the mesophyll (*Cc*; Fig. 1.2) is in equilibrium with C_i and the C_i-C_c gradient is more or less constant. This would cause only an offset between the C_i calculated from $\delta^{13}C$ and actual C_i. As long as this offset remains the same, it would not hinder the interpretation of changes in $\delta^{13}C$ as being caused by changes in C_i. There is however, increasing evidence that mesophyll conductance can change in response to irradiance and CO_2 (Douthe et al. 2012, Flexas et al. 2012), thereby leading to a variable difference between C_i and C_c (but these results are not generally supported, e.g. Tazoe et al. 2009, Yamori et al. 2010). Because mesophyll conductance is difficult to measure (Pons et al. 2009), it is generally ignored when interpreting carbon isotopes in plant material. Changes in mesophyll conductance are also not examined in this thesis, but it should thus be noted that $\delta^{13}C$ is a measure of C_c and possibly not always a good reflection of C_i.

Secondly, when $\delta^{13}C$ is measured in tree rings (as was done in this thesis), additional discrimination against ^{13}C takes place when simple leaf sugars are converted to more complex chemical structures that are used to build cellulose molecules. This causes the $\delta^{13}C$ values of cellulose in growth rings to be lower than the $\delta^{13}C$ of leaf sugars (about 1-2‰; Gleixner et al. 1993, Badeck et al. 2005). In this thesis, we assumed that this discrimination remains constant and only causes an offset between the leaf $\delta^{13}C$ and tree-ring $\delta^{13}C$, and therefore does not bias estimates of relative changes in C_i and its derivative iWUE.

BOX 1: Water-Use Efficiency - Description based on Ehleringer et al. (1993) and references therein.

Plant water-use efficiency is generally defined as the ratio of water loss compared to carbon gain or, in agricultural terms, between water consumption and productivity. At the leaf level, carbon and water fluxes involve net photosynthesis (*A*), measured as CO_2 uptake, and transpiration (*E*) measured as water loss. In steady state, these fluxes can be quantified by:

$$E = vg \tag{b1}$$

and

$$A = (C_a - C_i)g/1.6 \tag{b2}$$

With *v* being the gradient in humidity from the leaf's intercellular spaces to the outside air, *g*: the stomatal conductance for water vapour, C_a: the CO_2 concentration of the atmosphere and C_i: the CO_2 in the intercellular space of leaves. The '1.6' in equation b2 is included because stomatal conductance for water vapour is 1.6 times lower than the conductance for CO_2. The gradient in water-vapour pressure between the leaf and atmosphere (*v*) can be determined by:

$$v = e_i - e_a / P \tag{b3}$$

With, e_i: the vapour pressure inside the leaves (assumed to be at saturation, i.e. relative humidity in the intercellular spaces of leaves is assumed to be 100%), e_a: the vapour pressure of the atmosphere and *P* is the total atmospheric pressure. $e_i - e_a$ is also called the vapour pressure difference.

The instantaneous water-use efficiency is defined as the ratio of the fluxes of net photosynthesis and transpiration. Because *A* and *E* share a diffusion pathway through the stomata, the instantaneous water-use efficiency can be determined without knowing *g*:

$$\mathrm{A/E} = C_a(1 - C_i/C_a) / 1.6v = (C_a - C_i) / 1.6v \tag{b4}$$

Published data can be used for the atmospheric CO_2 concentration (e.g. in McCarroll and Loader 2004), whereas an estimated of C_i can be derived from carbon stable isotopes in plant materials. When the vapour pressure gradient between the leaves and the atmosphere (*v*) is ignored, or assumed constant, the ratio of net CO_2 assimilation (*A*) to stomatal conductance (*gs*) is compared. This is called the intrinsic water-use efficiency (iWUE):

$$\mathrm{iWUE} = (C_a - C_i) / 1.6 = A/gs \tag{b5}$$

Oxygen isotopes

Most oxygen in the world is ^{16}O (99.76%) and has eight neutrons and eight protons in the nucleus. A small proportion (0.2%) is ^{18}O and has two extra neutrons. The oxygen isotope composition of plant material is calculated as:

$$\delta^{18}O_{plant} = (R_{sample} / R_{standard} - 1) \tag{3}$$

where R_{sample} is the $^{18}O/^{16}O$ ratio of a plant sample (e.g. wood in tree rings) and $R_{standard}$ the $^{18}O/^{16}O$ ratio of an internationally recognized standard material (Vienna-Standard Mean Oceanic Water; VSMOW).

The analysis of oxygen isotopes in wood of tropical tree species is a relatively new development that has primarily been used for the study of climate. Oxygen isotopes in tropical tree rings seem to have a high potential to record the amount of rainfall on a regional scale. The underlying mechanism is that the $\delta^{18}O$ ratio of newly formed wood is strongly determined by the isotopic signature of the water absorbed at the time of wood formation (McCarroll and Loader 2004, Barbour 2007). During water uptake by roots no fractionation of oxygen isotopes occurs, thus water in the xylem has the same $\delta^{18}O$ signature as the soil water surrounding the roots (Dawson and Ehleringer 1991). At leaf level, water is lost to the atmosphere in the process of evaporation (Fig. 1.3b). This causes an enrichment of ^{18}O in the leaves, because water with the light ^{16}O-isotope needs less energy to evaporate. As a consequence more light than heavy isotopes are lost from the leaves. The resulting ^{18}O enrichment of leaf water can be as much as 20‰ (Saurer et al. 1998). This process is also aided by faster diffusion of H_2O with ^{16}O than with ^{18}O. Thus, the $\delta^{18}O$ signature of water in the leaves is the combination of the $\delta^{18}O$ signature of xylem water and the evaporative enrichment of ^{18}O in leaves.

Carbon dioxide is the substrate for photosynthesis, but the oxygen in CO_2 exchanges completely with water prior to photosynthesis in the reaction $CO_2 + H_2O = CHO_3^- + H^+$ (Deniro and Epstein 1979, Roden et al. 2000), which is catalysed by a carbonic-anhydrase. As a consequence, the triose-phosphate that is formed in the chloroplast and that forms the basis of more complex sugars, is strongly imprinted with the $\delta^{18}O$ signature of leaf water instead of the $\delta^{18}O$ signature of the absorbed CO_2 (Deniro and Epstein 1979). No further alteration of the isotope composition of leaf sugars occurs during phloem transport from the leaves to the site of cellulose synthesis in the stem. However, during cellulose formation (the basic component of wood), 20% of the oxygen atoms of sugars exchange with oxygen atoms from xylem water, due to the formation of carbonyl groups which exchange their oxygen atom with the oxygen atom of surrounding water molecules (Cernusak et al. 2005, Barbour 2007; Fig. 1.3c). This dampens the enrichment of ^{18}O that occurred in the leaves and enforces the $\delta^{18}O$ signature of xylem water just before oxygen molecules are fixed in the wood of growth rings.

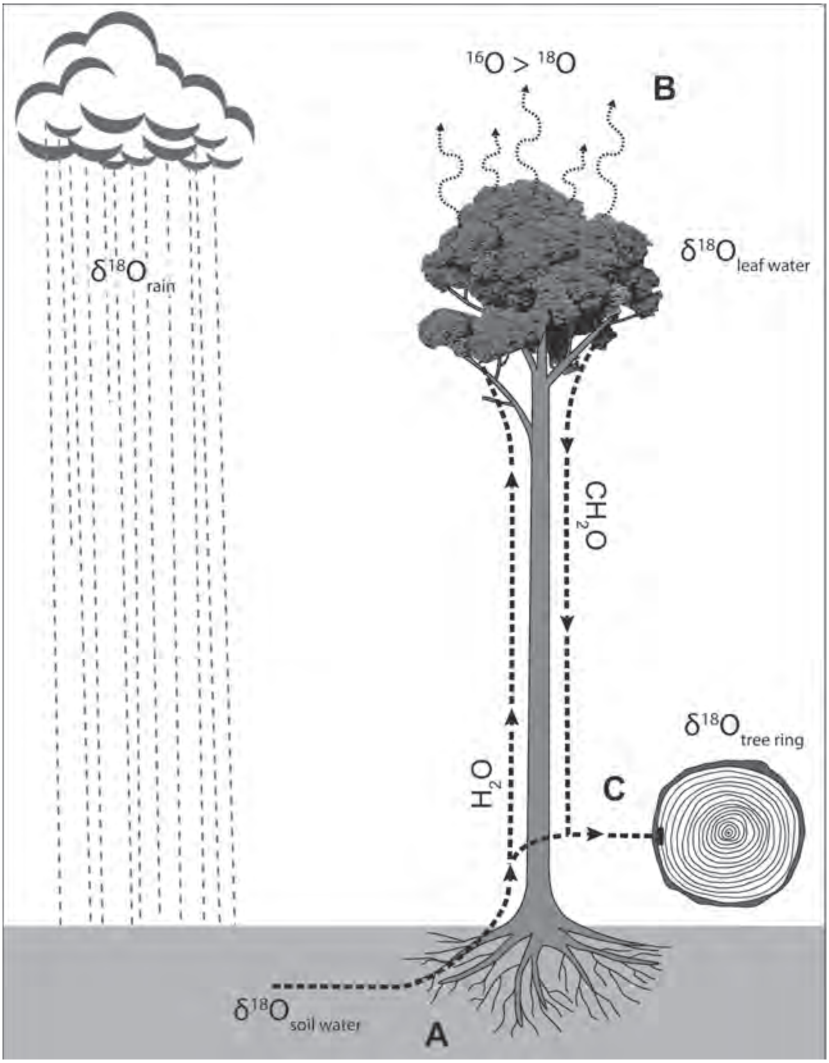


Figure 1.3 Graphic representation of the main controls of tree-ring $\delta^{18}O$ values. **(A)** Plants absorb water with a certain isotope signature ($\delta^{18}O_{soil\ water}$). No fractionation takes place during water absorption and transport to the leaves. Thus the $\delta^{18}O$ signature of xylem water is the same as $\delta^{18}O_{soil\ water}$. **(B)** Evaporation causes an enrichment of ^{18}O in the leaves, changing $\delta^{18}O_{soil\ water}$ into $\delta^{18}O_{leaf\ water}$. CH_2O refers to the sugars that are transported downwards in the phloem; these sugars are imprinted with the $\delta^{18}O$ signature of leaf water. **(C)** During cellulose formation, 20% of the oxygen atoms of sugars can undergo isotope exchange with xylem water. This dampens the enrichment of ^{18}O that occurred in the leaves and enforces the xylem (soil) water signal just before oxygen molecules are fixed in the wood of growth rings.

As xylem water is the same as soil water, trees can thus record the $\delta^{18}O$ signature of soil water in their cellulose. When trees are rooting superficially, this soil water originates from rainwater that recently fell. In the sub-tropics and tropics the $\delta^{18}O$ signature of rainwater is strongly determined by condensation processes in clouds (Dansgaard 1964). Water molecules with a heavy ^{18}O condense more easily, making the isotopic content of the remaining vapour increasingly depleted in ^{18}O. As clouds rain out and move inland, the remaining water vapour becomes increasingly lighter, a process that is more dominant in years with intense rainfall (Risi et al. 2008). This causes a close relation between precipitation amount and the isotopic composition of rain (Villacís et al. 2008, Kurita et al. 2009). Because of the capacity of trees to record the $\delta^{18}O$ signature of rain, tree-ring $\delta^{18}O$ has been used to study paleo-climatic variability on large (regional) scales. Tree-ring $\delta^{18}O$ has been correlated to basin-wide precipitation in the Amazon (Ballantyne et al. 2011, Brienen et al. 2012b), regional precipitation in Thailand (Poussart and Schrag 2005) and Indonesia (Schollaen et al. 2013b) and to El Niño Southern Oscillation (ENSO) variability in Laos (Xu et al. 2011). When the $\delta^{18}O$ of the absorbed soil water is known, tree-ring $\delta^{18}O$ can further be used to determine the evaporative enrichment in the leaves (Barbour 2007). As this enrichment is strongly affected by relative humidity, $\delta^{18}O$ has been used as a proxy for relative humidity (Cullen and Grierson 2007) and vapour pressure difference between the leaves and the air (Kahmen et al. 2011).

Nitrogen isotopes

There are two stable isotopes of nitrogen: ^{14}N (around 99.6% of all natural nitrogen) and ^{15}N (0.4% of all nitrogen). Nitrogen isotopes can also be measured in the wood of trees and are expressed in the same way as carbon isotopes in eq. 1 and oxygen isotopes in eq. 3, but then against a standard material ($R_{standard}$) relevant for nitrogen isotopes (e.g. L-glutamic acid).

In plants, direct discrimination against ^{15}N during nitrogen uptake by the roots only seems to occur if nitrogen demand is low compared with supply (Evans et al. 1996, Högberg et al. 1999). Thus, plant $\delta^{15}N$ reflects the $\delta^{15}N$ signature of the nitrogen sources exploited by plants under most field conditions (Evans et al. 1996, Högberg et al. 1999). The $\delta^{15}N$ of these nitrogen sources (NO_3^- and NH_4^+) is in turn shaped by various soil processes that can characterize the local nitrogen cycle. As such, tree-ring $\delta^{15}N$ has the potential to record changes in the nitrogen cycle of terrestrial ecosystems.

In temperate and boreal forests, nitrogen isotopes in tree rings have been studied on various tree species and have been shown to record the effect of elevated natural and experimental nitrogen input (Bukata and Kyser 2005, Elhani et al. 2005, McLauchlan et al. 2007, Guerrieri et al. 2011). The effect of increased nitrogen depositions can be reflected in tree-ring $\delta^{15}N$ because high rates of nitrogen deposition and high ambient nitrogen availability lead to an increase of nitrification in the soil (Högberg 1997). During nitrification, ^{15}N-depleted nitrate (NO_3^-) is formed, leaving behind ^{15}N-rich ammonium (NH4+; Hogberg and Johannisson 1993, Högberg 1997). If not directly absorbed by plants, a fraction of this nitrate is lost due to downward leaching (Fig. 1.4). This process may lead to a gradual enrichment of the remaining soil N pool. This enrichment may be enforced by an increased denitrification with elevated N input (Mariotti et al. 1981). During denitrification, nitrate is reduced to gaseous N_2 that is ultimately lost to the atmosphere (Fig. 1.4) and a relatively large fractionation against ^{15}N occurs during this process, enriching the remaining nitrate with ^{15}N (Mariotti et al. 1981, Hobbie and Ouimette 2009).

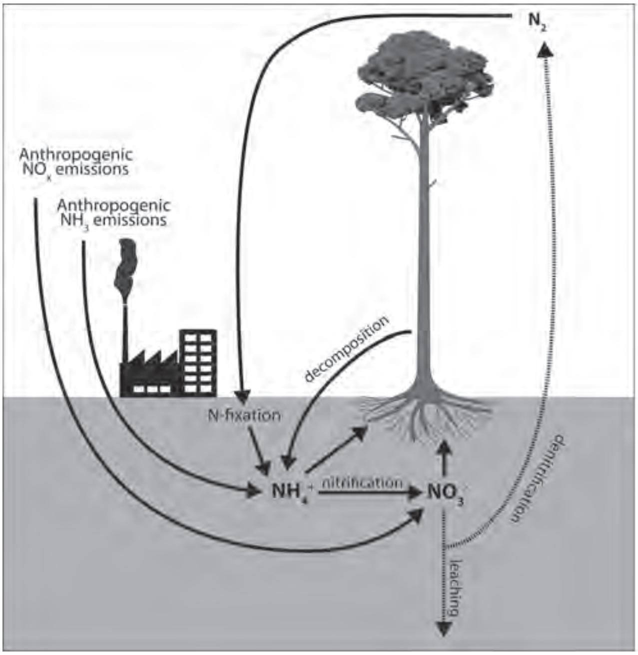


Figure 1.4 A simple representation of a nitrogen cycle. Trees absorb nitrogen in the form of NO_3^- and NH_4^+. Increased anthropogenic nitrogen inputs can lead to an increased downward leaching of ^{15}N-depleted NO_3^- and possibly also to an increased loss of ^{15}N-depleted nitrogen during denitrification. These processes (dashed lines) may cause an enrichment of ^{15}N in the remaining soil N pool.

Elevated anthropogenic nitrogen input can lead to an enrichment of ^{15}N in the soil and could thus be recorded as an increase of tree-ring $\delta^{15}N$ values over time. In field studies however, several uncertainties still hinder a clear interpretation of temporal changes in tree-ring $\delta^{15}N$. Two important factors that complicate the use of $\delta^{15}N$ as an indicator of nitrogen cycle change, are the effect of nitrogen translocation across ring boundaries (e.g. Elhani et al. 2003, Hart and Classen 2003) and ontogenetic (i.e. size developmental) effects on tree-ring $\delta^{15}N$ values (e.g. Hobbie and Högberg 2012). In this thesis we explicitly take these two factors into account.

1.4 Thesis outline

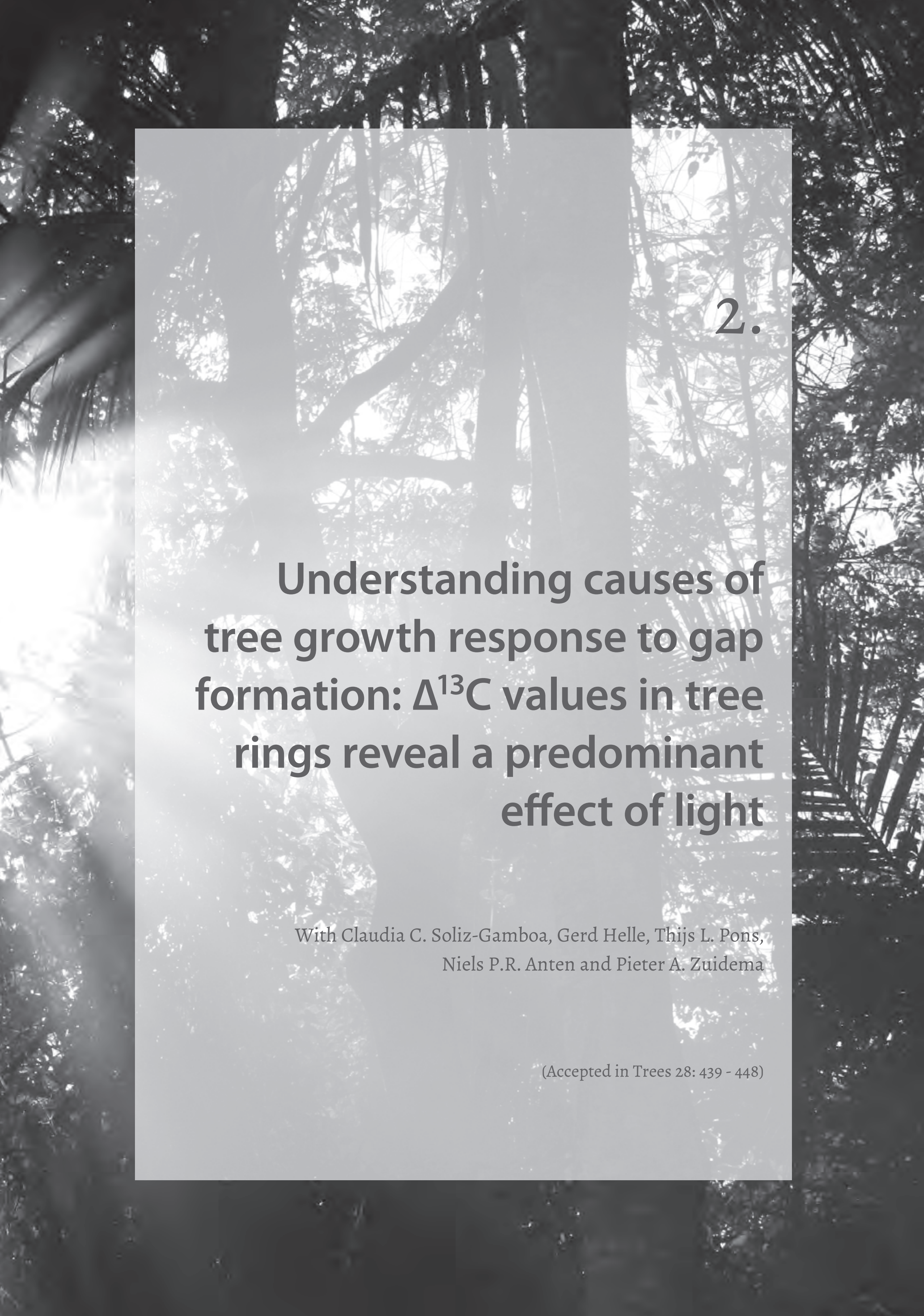
The main focus of this thesis is on long-term changes in environmental conditions and tree growth. However, to assess the occurrence of such long-term changes, it is also important to understand shorter-term fluctuations due to forest dynamics, because these interfere with changes on a longer temporal scale. In CHAPTER 2 we therefore evaluate how forest dynamics affects tree growth and tree-ring $\delta^{13}C$ values and which environmental signals can be derived from the combined study of growth and $\delta^{13}C$. To this end, growth rates and $\delta^{13}C$ values of 42 *Peltogyne* cf. *heterophylla* trees from North-eastern Bolivia were studied before and after the formation of a single tree-fall gap in their vicinity. The main aim is to understand the reasons for the highly variable growth responses after gap formation and to disentangle the effect of changes in light and nutrient availability from those in water availability.

In CHAPTER 3 we investigate the use of $\delta^{18}O$ as a tool for climate reconstructions in West and Central Africa, an area where the availability of instrumental climatic data is very limited. We relate $\delta^{18}O$ in growth rings of five large *Entandrophragma utile* trees from Cameroon to precipitation and sea surface temperatures on a regional scale and over almost a century.

In CHAPTER 4 tree-ring $\delta^{15}N$ values from around 400 trees belonging to six species are used to assess the effect of increased nitrogen depositions on nitrogen cycles in the three study sites (Fig. 1.1). We use a strict control for ontogenetic effects (i.e. during size development) as well as potential nitrogen translocation effects on tree-ring $\delta^{15}N$ values.

In CHAPTER 5 the effect of increased atmospheric CO_2 levels on tree physiology (photosynthesis and stomatal conductance) and tree growth is studied. Around 1100 trees of 12 species (Table 1.1) from the three sites were measured. Long-term CO_2 effects are assessed by the measurement of ring widths and $\delta^{13}C$. We again employ a sampling design that corrects for confounding ontogenetic changes in growth and $\delta^{13}C$.

CHAPTER 6 provides an overview of stable isotope studies on tropical trees and integrates the findings of this thesis. In the second part of chapter 6, a synthesis is given of what stable isotope research has taught us about environmental changes in tropical forests.



2.

Understanding causes of tree growth response to gap formation: $\Delta^{13}C$ values in tree rings reveal a predominant effect of light

With Claudia C. Soliz-Gamboa, Gerd Helle, Thijs L. Pons,
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Abstract

The formation of tree-fall gaps in closed canopy forests usually entails considerable increases in light and nutrient availability for remaining trees, as well as altered plant water availability, and is considered to play a key role in tree demography. The effects of gaps on tree growth are highly variable and while usually stimulatory they may also include growth reductions. In most studies, the causes of changes in tree growth rates after gap formation remain unknown. We used changes in carbon isotope ^{13}C discrimination (Δ^{13}C) in annual growth rings to understand growth responses after gap formation of *Peltogyne* cf. *heterophylla*, in a moist forest of Northern Bolivia. We compared growth and Δ^{13}C of the seven years before and after gap formation. Forty-two trees of different sizes were studied, half of which grew close (<10m) to single-tree fall gaps (gap trees), the other half more than 40 m away from gaps (controls). We found variable responses among gap trees in growth and Δ^{13}C. Increased growth was mainly associated with decreased Δ^{13}C, suggesting that the growth response was driven by increased light availability, possibly in combination with improved nutrient availability. Most trees showing zero or negative growth change after gap formation had increased Δ^{13}C, suggesting that increased water stress did not play a role, but rather that light conditions had not changed much or nutrient availability was insufficient to support increased growth. Combining growth rates with Δ^{13}C proved to be a valuable tool to identify the causes of temporal variation in tree growth.

2.1 Introduction

The formation of tree-fall gaps in closed canopy forests can cause a radical change in environmental conditions for neighbouring trees, and is generally believed to play a key role in tree demography (Whitmore 1989, Clark and Clark 1992, Condit 1995). Gap formation can strongly increase light availability for juvenile and suppressed trees (Canham 1988, Canham et al. 1990) and improve nutrient availability (Denslow et al. 1998, Burgess and Wetzel 2000, Muscolo et al. 2007). In addition, water availability close to canopy gaps may be improved by reduced transpiration of canopy trees and reduced root competition (Breda et al. 1995, Bladon et al. 2006). However, increased irradiance and temperature in and around gaps can also increase evaporation from the soil surface and thus reduce moisture content of the upper soil (Barg and Edmonds 1999, Olander et al. 2005). Furthermore, increased crown exposure to light and wind in and close to a gap may increase transpirational demand and thus increase water stress even if gap formation itself does not change soil water availability (Bladon et al. 2006, Bladon et al. 2007). Thus, the changes of environmental conditions after gap formation can be complex and may affect tree growth in complex ways.

Empirical studies in tropical forests show that gap formation often increases diameter growth rates of nearby trees (Brokaw 1985, Brown 1996, Hérault et al. 2010). However, not all trees exhibit growth stimulation after gap formation: growth may remain unchanged or even decline in the years following gap formation (Poorter 1999, Miya et al. 2009, Hérault et al. 2010, Soliz-Gamboa et al. 2012). Increased growth rates of understory trees are often assumed to be caused by increased light availability (Hartshorn 1978, Martinez-Ramos et al. 1988, Clark and Clark 1992), whereas a positive growth response of adult trees that have reached the forest canopy may also result from improved water and nutrient availability. While these are the generally expected causes, the actual drivers of growth changes after gap formation remain unknown in most studies.

The measurement of stable carbon isotopes in tree rings may make it possible to determine whether growth changes after gap formation are mostly due to changes in light conditions or changes in water availability (Saurer et al. 1997, Hartmann et al. 2008, Powers et al. 2009a, 2010). From the carbon isotope composition of plant material ($\delta^{13}C$), the discrimination against $^{13}CO_2$ during CO_2 assimilation can be derived ($\Delta^{13}C$). This can be used as a proxy for the intercellular CO_2 concentration (C_i) in the absence of changes in the atmospheric

CO_2 concentration (C_a, currently ca. 395 ppm) using the model of Farquhar et al. (1982):

$$\Delta = \delta^{13}C_a - \delta^{13}C_{plant} = a + (b - a)\, C_i/C_a \qquad (1)$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ of atmospheric CO_2 (currently ca. -8.3‰), a (4.4‰) refers to the slower diffusion of $^{13}CO_2$ compared to $^{12}CO_2$ through the stomata, and b (27‰) to the discrimination by the CO_2 fixing enzyme Rubisco. A change in C_i can be caused by changes in photosynthetic rates (A) and/or stomatal conductance (gs). Increased light intensity increases A more than g_s, whereas decreased water availability decreases gs more than A. Nutrient availability relative to the growth potential of the plant has an effect similar to light intensity and is thus difficult to disentangle from light effects (Ehleringer et al. 1986, Cernusak et al. 2009).

Although changes in $\Delta^{13}C$ alone are not conclusive with respect to the causes of tree responses to altered environmental conditions, their combined analysis with tree growth data that can be used as a proxy for A, allow the identification of the main drivers of growth changes. For example, if light availability increases after gap formation, it may increase the growth potential of a remaining tree by increasing photosynthetic rates. This can lead to increased growth when the availability of water and nutrients is sufficient. Increased growth is then associated with decreased $\Delta^{13}C$. If water stress hampers a growth increase after gap formation or even reduces growth, then $\Delta^{13}C$ is expected to decrease. An absence of changes in growth and $\Delta^{13}C$ after gap formation indicates either insufficient increase of light availability or a simultaneous decrease in nutrient availability and an increased growth potential due to higher light availability.

In a previous publication, we reported on a highly variable growth response to gap formation for the shade-tolerant tree *Peltogyne* cf. *heterophylla* in a Bolivian moist forest (Soliz-Gamboa et al. 2012). Growth rates of some trees increased after gap formation while growth of others showed a negative or no change (mostly small juvenile trees). In the current study, we aimed to identify the causes of observed diameter growth responses using the combined analyses of changes in growth and stable carbon isotopes. Specifically, we use $\Delta^{13}C$ values obtained from tree rings to investigate (1) whether increased tree growth after gap formation is a consequence of increased light (and/or nutrients) or water availability and (2) whether increased water stress after gap formation causes a reduction of growth rates in some trees. We measured tree-ring width and carbon isotope ratios in growth rings in trees of different sizes before and after gap formation.

2.2 Material and methods

Study area and species

The study was carried out in the logging concession "Los Indios" (10°26'S, 65°33'W) located 86 kilometres northeast of the town of Riberalta, Bolivia. Forests in the region are semi-deciduous with a dense canopy at 30-35 m high and some emergent trees up to 45 m. Density of stems >10 cm diameter at breast height (dbh) is 423 ha^{-1} (Toledo et al. 2008). The study area was selectively logged from May to September 2001, at an intensity of 1.4 trees ha^{-1}. After logging no further disturbances took place.

Mean annual precipitation is 1690 mm and mean annual temperature is 27 °C (Soliz-Gamboa et al. 2012). We checked if average annual precipitation in the growing season (October – May) differed between the 7-year periods before and after logging (the study period) using climatic data for Riberalta from the database of the KNMI Climate Explorer (Trouet and Van Oldenborgh 2013). No significant difference in average annual precipitation was found between the years before and after gap formation (ANOVA: F=0.670, p=0.428). Unfortunately, no temperature data were available to determine if there were differences in growth season temperatures.

Our study species, *Peltogyne* cf. *heterophylla* (Fabaceae, Caesalpinoideae; hereafter referred to by its genus name), is a shade tolerant tree, which is evergreen as a juvenile and brevi-deciduous as an adult (Soliz-Gamboa et al. 2012). Tree-ring studies have been conducted on this species and the annual nature of growth rings has been established (Brienen and Zuidema 2005, Soliz-Gamboa et al. 2011). *Peltogyne* is a relatively slow growing species, especially in the juvenile phase. Sampled trees of ca. 25 cm dbh were often more than 100 years old.

Data collection and tree-ring measurements

We sampled 21 trees at less than 10 meters from single tree fall gaps or from similar-sized canopy openings formed by skidder trails (gap trees) and 21 trees at more than 40 meters away from gaps (control trees). The trees ranged in size from 5 to 25 cm dbh, with a similar size distribution in gap and control trees. The average age of the control trees was 104 years (range: 55 - 158 years) and 102 years in the gap trees (range: 32 - 162 years). Even though *Peltogyne* trees can attain sizes of >100 cm dbh, we did not collect trees larger than 25 cm, because gap effects were expected to be stronger for juvenile – sub-adult trees. We avoided sampling trees that were badly damaged or severely liana-infested.

For each tree sampled, we determined the Canopy Closure Index (CCI) (Lieberman et al. 1989, Lieberman et al. 1995). The CCI is an estimate of the level of shade cast upon the crown of a focal tree based on the density and heights of neighbouring trees that are taller than the focal tree. A low CCI indicates a low density of taller trees surrounding the focal tree and thus, a higher light availability. For all trees up to 20 cm dbh, cross-sectional stem discs were collected using a chain saw. Five of the larger trees were sampled in three different directions using a 22-mm increment borer mounted on a motor (Stihl BT42). All samples were collected at 50 cm height. Cores and discs were air-dried and polished using grits up to 1000. Tree rings in the discs and cores were dated and ring widths were measured using a LINTAB 6 and TSAPWin software (Rinntech, Germany). Tree-ring widths (annual increment in cm year^{-1}) where converted to Basal Area Increment (BAI; cm^{2} year^{-1}) as this gives a good estimate of biomass growth (Baker et al. 2004). Individual tree growth responses were determined by comparing the average annual BAI in the seven years after (2001-2007) to the average annual BAI in the seven years before (1994-2000) gap formation. Periods of seven years were chosen as logging took place seven years before wood samples were taken (in 2008). The year 2001 was included in the period after gap formation because logging occurred prior to the onset of the growing season.

Isotope analyses

Wood material was cut from each of the 14 annual rings (in accordance with the above-mentioned periods 1994-2000 before and 2001-2007 after gap formation) of every tree. Complete growth rings were sampled. Cellulose extraction was performed on the raw wood samples using the adapted Jayme-Wise method (Wieloch et al. 2011). Holocellulose samples were subsequently homogenized in a demi-water solution by a mixer mill (Retsch MM301, Germany) and oven-dried at 70 °C. This novel homogenization procedure gave good results as multiple $\delta^{13}C$ measurements on subsamples from the same cellulose gave very similar results (maximum difference of 0.04‰). All cellulose samples were analysed in a continuous flow mode with an element analyzer coupled to a mass spectrometer at the Geo Forschungs Zentrum in Potsdam, Germany. The carbon isotope composition ($\delta^{13}C$, in ‰) was then calculated as:

$$\delta^{13}C_{\text{tree-ring}} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 \qquad (2)$$

where, R_{sample} is the $^{13}C/^{12}C$ ratio of a sample and R_{standard} the $^{13}C/^{12}C$ ratio of an internationally recognized standard material (V-PDB). Discrimination (Δ) against the heavier ^{13}C can be calculated by:

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_{\text{tree-ring}}) / (1 + \delta^{13}C_{\text{tree-ring}}) \qquad (3)$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ of atmospheric CO_2. As $\delta^{13}C_a$ decreased by 1.5‰ since the onset of the industrial revolution due to the burning of fossil fuels that are depleted in ^{13}C, we used year-specific values for each of the 14 years of ring formation (McCarroll and Loader 2004). We excluded one large control tree from the analyses, which exhibited significantly lower $\Delta^{13}C$ values over the entire 14-year period studied.

We examined $\Delta^{13}C$ instead of the estimate of the intercellular CO_2 concentration in leaves (C_i), which can be derived from $\Delta^{13}C$, because in the period covered by our study (1994-2007), atmospheric CO_2 concentration (C_a) increased by 7% (from 359 ppm to 384 ppm). If plants do not respond by changing stomatal conductance and/or assimilation, a 7% increases in atmospheric CO_2 will result in a similar 7% increase of C_i. This so-called passive response (McCarroll et al. 2009) may obscure effects on C_i caused by gaps. We therefore used $\Delta^{13}C$ which is based on the ratio between C_i and C_a, and has been shown to be less influenced by the rise of C_a than C_i (e.g. Hietz et al. 2005, Loader et al. 2011, Nock et al. 2011). In addition, our experimental setup corrects for this potential confounding factor by including proper controls.

Statistical analyses

Growth rates (BAI) and $\Delta^{13}C$ often vary over the size range of trees (e.g. Clark and Clark 1992, Gagen et al. 2007). This ontogenetic variation could potentially obscure any gap-related effects and might be especially problematic in our data set as we collected trees of different sizes (5 - 25 cm dbh). We therefore standardized BAI and $\Delta^{13}C$ series for each tree by dividing annual values by their 14-year average value. In this way, annual BAI and $\Delta^{13}C$ values are expressed as proportional deviations from the 14-year average. We subsequently analysed the overall effect of gap formation on standardized BAI and $\Delta^{13}C$ with a mixed-effects model. Mixed-effects models are well suited for the repeated measures structure of tree-ring and isotope data series. We included 'gap-formation' as a fixed factor and 'individual tree' as a random factor. Tree size (dbh at the time a gap was formed) was included as a covariate. We also tested if there was an interaction between the effect of gap formation and tree size. Gap and control trees were analysed separately.

Pearson's correlations were used to test the relation between changes in BAI and changes in $\Delta^{13}C$. Proportional change (in %) in BAI and $\Delta^{13}C$ were calculated as this allows for a better comparison of the responses of individual trees than absolute changes, which are, especially for BAI, much greater in large than small trees. We used an ANCOVA to test if there was a difference between gap and control trees in light levels (the inverse of CCI), including tree size as a covariate. Log-transformed CCI was taken to obtain linear relations. We analysed the relation between CCI and BAI and between CCI and $\Delta^{13}C$ with a multiple linear regression, using log-transformed BAI and $\Delta^{13}C$ (again to obtain linear relationships). We also included trees size (dbh) as a covariate in the regression analysis, because BAI and $\Delta^{13}C$ are strongly size dependent, but furthermore, because CCI also decreases with tree size. All analyses were performed using IBM SPSS Statistics, version 19.0.

2.3 Results

Effect of gap formation on growth and $\Delta^{13}C$

Among the individual *Peltogyne* trees, we found both increased, decreased and unchanged growth in basal area (BAI) after gap formation (Fig. 2.1). Of these gap trees, 14 out of 21 trees showed an increased growth of >25 to 700% (Fig. 2.2). Among control trees, which were located far away from gaps, 8 out of 21 trees showed an increased BAI of >25 to 200% (Fig. 2.2). Given the high variability of responses in the group of gap trees, it is not surprising that we did not find an overall effect of gap formation on standardized BAI in a linear mixed-effects model (Table 2.1). Nevertheless, we did find a significant interaction between dbh and gap formation ($p<0.001$; Table 2.1), suggesting that the response to gap formation depends on tree size. In particular, several medium-sized trees (dbh ca. 15cm) showed strong positive growth changes after gap formation, which were larger than most of the growth changes in small trees (Fig. 2.2).

No significant effect of gap formation was found on $\Delta^{13}C$ in a mixed-effects model analysis (Table 2.1). Again, the response of the $\Delta^{13}C$ values to gap formation was highly variable (Fig. 2.1 & 2.2). It is possible that very old trees do not respond as vigorously to gap formation as similar-sized but younger trees. We checked if age is a stronger driver of the responses to gap formation than size. However, this does not seem to be the case, as there is no significant relation between tree age and its capacity to respond (as for tree size, we only found a significant interaction between gap-formation and tree age in the gap trees: F=7.988, p=0.005).

Table 2.1 Results of the mixed-effects model analyses with standardized Basal Area Increment (BAI) and ^{13}C discrimination ($\Delta^{13}C$) as dependent variables and gap formation, tree size (dbh at the time of gap formation; inserted as covariate) and the interaction between gap-formation and dbh (gap*dbh) as factors. Separate analyses were performed for control and gap trees. d.f.: denominator degrees of freedom (numerator d.f. = 1 in all cases).

		Control trees			**Gap trees**		
BAI	**Fixed effects**	**d.f.**	**F-value**	**p-value**	**d.f.**	**F-value**	**p-value**
	gap formation	258.438	0.023	0.879	238.066	1.487	0.224
	dbh	233.512	2.536	0.113	250.492	0.358	0.550
	gap*dbh	233.512	0.051	0.821	250.492	18.390	**>0.001**
$\Delta^{13}C$	**Fixed effects**	**d.f.**	**F-value**	**p-value**	**d.f.**	**F-value**	**p-value**
	gap formation	226.851	0.196	0.658	250.057	0.041	0.839
	dbh	210.051	0.185	0.667	231.386	0.796	0.373
	gap*dbh	210.051	0.026	0.871	231.386	2.930	0.088

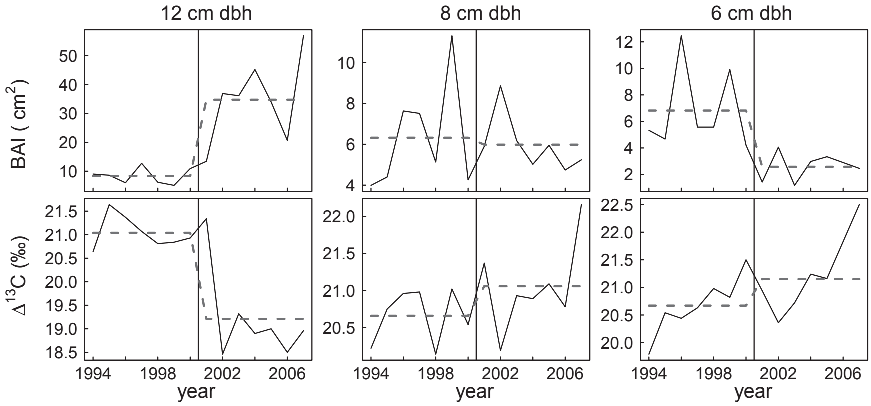


Figure 2.1 Three examples of annual Basal Area Increment (BAI) and ^{13}C discrimination (Δ^{13}C) for individuals of *Peltogyne* cf. *heterophylla* that grew < 10 meters from a logging gap. The black vertical reference line shows the time of gap formation, the dashed grey lines the average BAI and Δ^{13}C pre- and post-gap formation

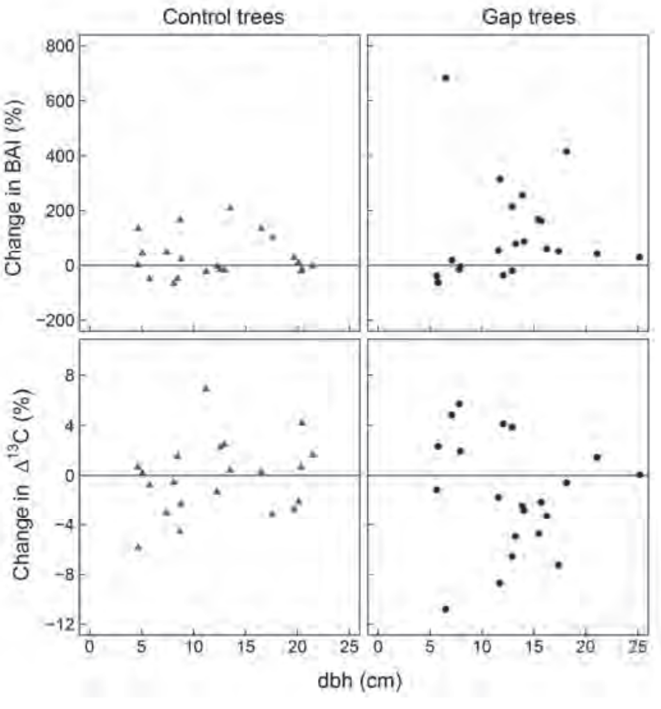


Figure 2.2 Changes in Basal Area Increment (BAI) [top panel] and ^{13}C discrimination (Δ^{13}C) [bottom panel] for *Peltogyne* cf. *heterophylla* after gap formation with respect to tree size (dbh) at the time of gap formation. Proportional changes (in %) were taken to account for potential size effects on growth and Δ^{13}C.

Understanding growth changes

To interpret the causes of growth changes, we related the proportional change (in %) in BAI to the change in $\Delta^{13}C$ (Fig. 2.3). In both control- and gap trees, changes in BAI correlated significantly with changes in $\Delta^{13}C$ (Pearson's $r^2=0.237$ ($p=0.013$) and 0.469 ($p<0.001$), respectively). Among the trees (both gap and control) showing increased growth, 16 out of 22 were associated with a decrease in $\Delta^{13}C$ (Fig. 2.3), whereas 12 out of 19 trees with no or a negative growth change showed an increase in $\Delta^{13}C$ (Fig. 2.3).

Because our study is retrospective, we have no data on the changes in light, nutrient and water availability occurring during gap formation. However, we measured canopy closure index (CCI: the inverse of light availability) once in 2007, seven years after gap formation. We found that, even seven years after logging, gap trees were, on average, growing at higher light conditions than control trees (ANCOVA: $F=10.804$, $p=0.002$; Fig. 2.4). For both gap and control trees, CCI decreased with tree size (Pearson's $r^2=0.301$ ($p=0.009$) and 0.740 ($p<0.001$), respectively). Logically, larger trees experienced, in general, higher light levels. We also related CCI to the averaged BAI and $\Delta^{13}C$ of the last 5 years (2003-2007) using a linear regression analysis. As tree size also has a strong effect on BAI and $\Delta^{13}C$, we included tree size as a covariate in the analyses. We found a highly significant increase of BAI with decreasing CCI ($r^2=0.680$, $p<0.001$; Fig. 2.5) and a significant increase of $\Delta^{13}C$ with increasing CCI ($r^2=0.480$, $p<0.001$; Fig. 2.5). Hence, trees with a low CCI, meaning a low density of taller neighbouring trees and thus, receiving abundant light, grew faster and had a lower $\Delta^{13}C$.

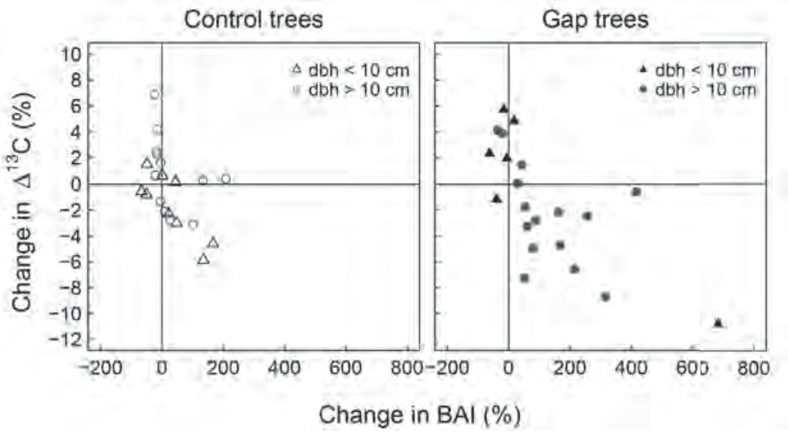


Figure 2.3 Changes in Basal Area Increment (BAI) with respect to ^{13}C discrimination (Δ^{13}C) for *Peltogyne* cf. *heterophylla* after gap formation for control trees [left panel] and gap trees [right panel]. In both control and gap trees, changes in BAI correlated significantly with changes in Δ^{13}C (Pearson's r^2=0.237 (p=0.013) and 0.469 ($p<0.001$), respectively).

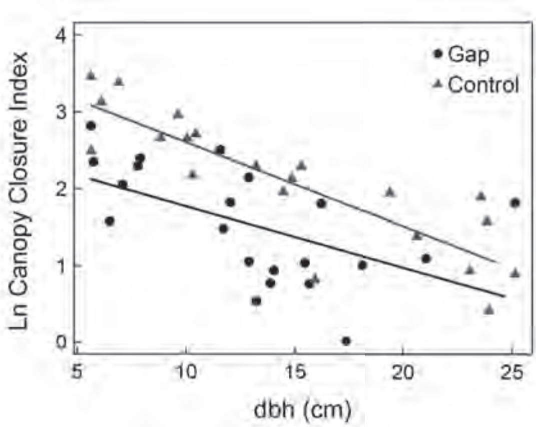


Figure 2.4 Light availability (the inverse of Canopy Closure Index; CCI) for gap and control trees in relation to tree size (dbh). Log-transformed CCI was taken to obtain linear relations. Gap trees grew, on average, in higher light conditions than control trees even after seven years of gap formation (ANCOVA: F=10.804, p=0.002). CCI significantly decreases with tree size in both gap and control trees (Pearson's r^2=0.301 (p=0.009) and 0.740 ($p<0.001$), respectively).

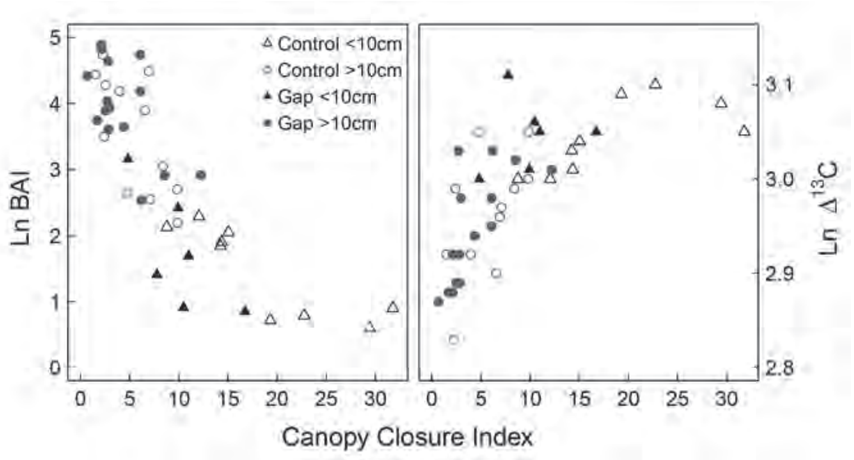


Figure 2.5 The relationship between of Basal Area Increment (BAI) and Canopy Closure Index (CCI) [left panel] and between ^{13}C discrimination (Δ^{13}C) and CCI [right panel] for *Peltogyne* cf. *heterophylla*. For BAI and Δ^{13}C we arbitrarily took the average of the last 5 years (2003-2007), CCI was measured only once in 2007. Log-transformed BAI and Δ^{13}C were used in order to obtain linear relationships. Data were analysed with a multiple linear regression (including tree size as a covariate); r^2=0.680 ($p<0.001$) and 0.480 ($p<0.001$), respectively.

2.4 Discussion

We used stable carbon isotope ratios in annual growth rings to study the causes of growth changes after gap formation in a common shade-tolerant tree, *Peltogyne cf. heterophylla,* in North-eastern Bolivia. No general effect of gap formation on growth rates (BAI) and ^{13}C discrimination was found (Δ^{13}C; see Table 2.1). The lack of an overall effect of gap formation can be explained by the large variability in responses: BAI and Δ^{13}C showed increased, zero and negative changes after gap formation (Fig. 2.1). However, for BAI, but not for Δ^{13}C, we did find a significant interaction between gap formation and tree size (Table 2.1), suggesting that the growth response to gap formation depends on tree size. In particular, the medium-sized trees (dbh ca. 15 cm) seem to respond to gap formation by increasing growth rates, but most of the small trees showed negative to zero growth responses (Fig. 2.2).

What explains the variation in responses to gap formation? Trees showing increased growth rates after gap formation were mainly associated with a decrease in ^{13}C discrimination (Fig. 2.3). Such a decrease in plant Δ^{13}C can be caused by both changes in light/nutrient- and water availability (Ehleringer et al. 1986, Cernusak et al. 2009). Improved light and, to a lesser extent, increased nutrient availability, can increase assimilation rates relative to stomatal conductance and therefore, decrease $\Delta^{13}C$ (Ehleringer et al. 1986, Cernusak et al. 2009). Indeed, we found a significant negative relation between light availability (the inverse of CCI) and $\Delta^{13}C$ (Fig. 2.5), a relationship that has already been well documented (Ehleringer et al. 1987, Zimmerman and Ehleringer 1990). The observed decrease in $\Delta^{13}C$ can also point to increased water stress because reduced water availability decreases stomatal conductance and therefore, C_i (McNulty and Swank 1995, Saurer et al. 1995, Cernusak et al. 2009). But in the latter case, one would expect a concomitant decrease in growth (as reduced water availability slows down growth). Hence, the increase of growth rates in combination with reduced $\Delta^{13}C$ after gap formation, as observed in our study, is likely caused by increased light availability, possibly in combination with a proportionally increased nutrient availability.

Growth rates can also increase by a lowering of water stress after gap formation, e.g. by reduced root competition. However, if increased growth rates are the results of improved water availability, we expect to find increased C_i and hence $\Delta^{13}C$, or no change in C_i and $\Delta^{13}C$ when occurring in synchrony with increased light/nutrient levels. This is opposite to what we found. Our results thus suggest that changes in water availability after gap formation were less important than changes in light and nutrients for the stimulation of growth.

It was surprising that we did not find a growth response to gap formation in most of the smaller tees (Fig. 2.2). These trees are expected to benefit the most from increased light availability after gap formation given their shaded position in the forest understory. Poorter (1999), working in the same region, found no strong growth response of seedlings of some species in high light conditions and suggested this might be a result of increased water stress. This does not seem to apply to our study system, as we found no indications of increased water stress, i.e. no significant decrease in $\Delta^{13}C$ was observed in combination with decreased growth rates. On the contrary, growth reduction was mainly associated with increased $\Delta^{13}C$ (Fig. 2.3), which suggests decreasing light availability. When comparing the gap trees with the control trees (Fig. 2.2 & 2.3), it is noteworthy that the latter show a temporal variation in growth and $\Delta^{13}C$ that is not very different from the gap trees. This indeed suggests that the effect of gap formation (e.g. on understory light levels) might not have been very strong for most trees. However, even seven years after gap formation, gap trees were, on average, still growing in higher light condition than control trees (Fig. 2.4). This was especially the case for the medium-sized trees.

A lack of growth stimulation after gap formation is also possible if excessive irradiance during the first years after gap formation causes a strong photo-inhibition, which reduces photosynthetic rates and thereby, increased $\Delta^{13}C$. For shade-tolerant species, like *Peltogyne* cf. *heterophylla*, photo-inhibition might be a severe problem in high light environments (Krause et al. 1995, Krause and Winter 1996, Houter and Pons 2005). Still, the greatest increase in growth after gap formation (nearly 700%) in combination with the strongest observed decrease in $\Delta^{13}C$ (-11%) was found in one of the smallest trees (Fig. 2.3). This tree also had a very high light availability (CCI = 4.83) when measured in 2007, suggesting that at least some small *Peltogyne* trees are able to take advantage of gap formation and do not experience strong photo-inhibition. Long-term tree-ring studies also indicate that small trees of this species are able to undergo strong growth spurts (Brienen and Zuidema 2006, Soliz-Gamboa et al. 2011).

Growth stimulation in response to gap formation may also have been hampered by a lowered nutrient availability, which may be caused by the increased growth and nutrient absorption of nearby larger trees or by a reduced leaf litter fall (lower nutrient input) due to the removal of a dominant tree from the canopy. A reduced nutrient availability can lower photosynthetic rates and as such, increases C_i and $\Delta^{13}C$. Although we do not fully understand why several small trees did not respond to gap formation, our data show that this lack of response was not caused by prolonged water stress, but rather that light availability did not sufficiently increase or that, particularly when $\Delta^{13}C$ increased, nutrient availability decreased.

We exclude the possibility that the results presented here are strongly affected by ontogenetic (i.e. developmental) changes in growth and tree-ring $\delta^{13}C$. For carbon isotopes, a 'juvenile effect' has often been observed in the early years of tree growth (Freyer 1979, McCarroll and Loader 2004) and has been attributed to ^{13}C-depleted CO_2 near the ground from soil respiration, as well as to other environmental changes (e.g. light) during development (Leavitt 2010). Such ontogenetic changes can potentially interfere with changes caused by gap formation. We, however, expect that ontogenetic effects are very small to absent in our data because: (1) the studied trees were relatively old (minimum tree age was 31 years), (2) the small trees studied had their leaves at least several meters above the forest floor, excluding an effect of ^{13}C-depleted CO_2 near the ground,

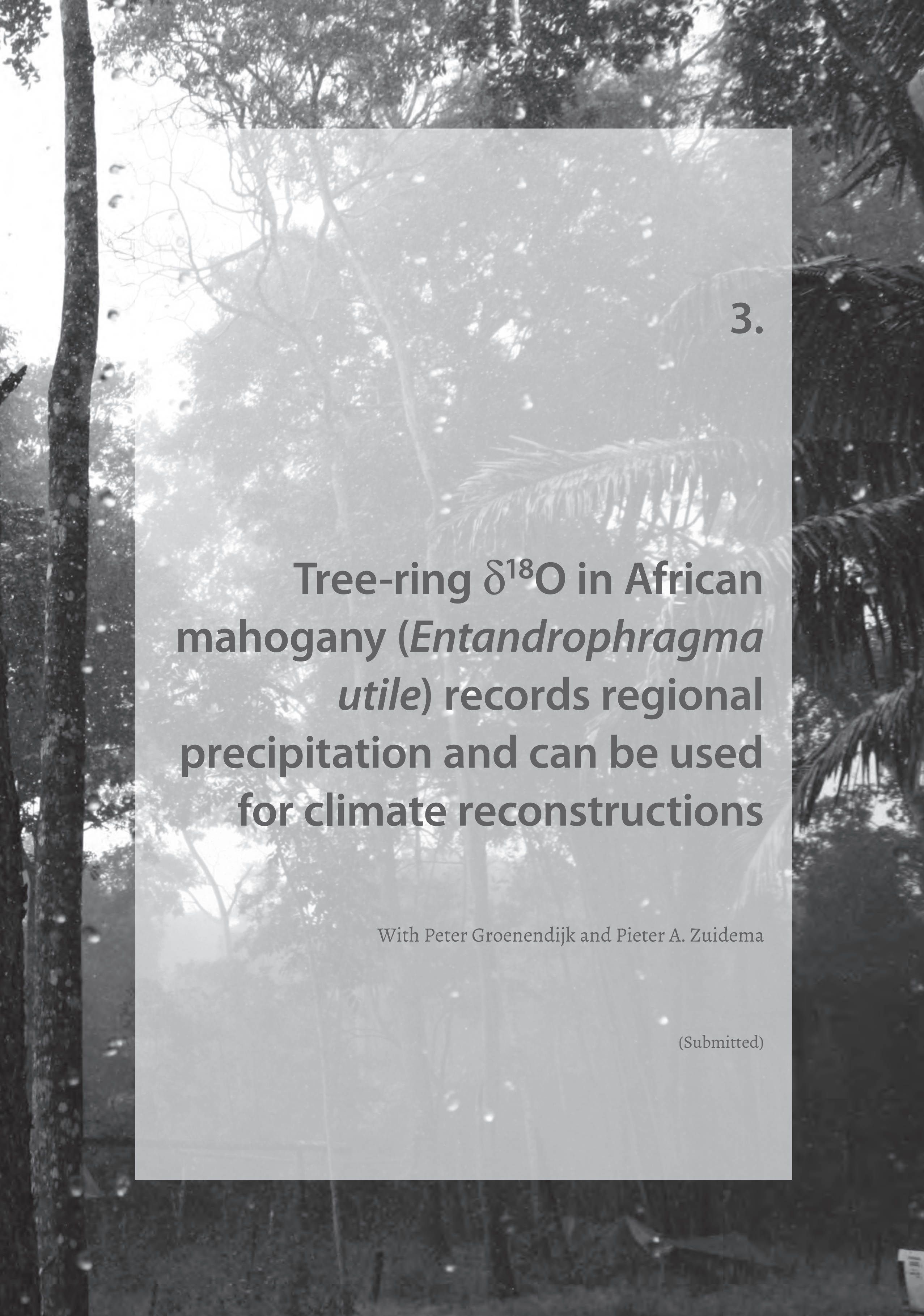
(3) the study period was relatively short and ontogenetic changes in growth are gradual for this species (Rozendaal et al. 2010b, 2011) and thus cannot explain the abrupt growth changes observed in some trees (Fig. 2.1), and (4) our experimental setup includes proper controls, i.e. gap trees were compared to control trees that, if present, have similar ontogenetic patterns in growth and $\delta^{13}C$, but did not experience nearby gap formation (Fig. 2.2 & 2.3).

The combined analyses of tree growth and carbon isotopes can help to identify some of the possible causes of strong growth decreases (suppressions) or increases (releases) in tree-ring series. It is often assumed that these patterns are mainly caused by periods of low and high light, respectively, and therefore reflect canopy dynamics (Wright et al. 2000, Baker and Bunyavejchewin 2006, Brienen and Zuidema 2006). However, in a Mexican dry forest, some releases could also be related to reduced temperatures, likely causing reduced water stress (Brienen et al. 2010). Carbon isotope ratios in growth suppressions and releases can reveal whether growth changes are mainly driven by shifts in water or light availability and hence, distinguish between the effects of climate and forest dynamics.

We conclude that for *Peltogyne*, the shade-tolerant species studied, growth increase after gap formation is mainly governed by increased light availability and not by increased water availability. Although such light-mediated gap effects have often been proposed, our analyses of carbon isotope ratios indeed point to an effect of light, probably in combination with increased nutrient availability.

Acknowledgements

We thank Carmen Bürger of GeoForschungsZentrum in Potsdam, Germany, for her help with the analysis of the wood samples, Mart Vlam for his assistance during the sampling of wood in growth rings and two anonymous reviewers for their constructive and helpful comments.



3.

Tree-ring δ^{18}O in African mahogany (*Entandrophragma utile*) records regional precipitation and can be used for climate reconstructions

With Peter Groenendijk and Pieter A. Zuidema

(Submitted)

Abstract

The availability of instrumental climate data in West and Central Africa is very restricted, both in space and time. To allow the reconstruction of paleoclimatic variability in the region, proxies are increasingly used. Growth rings in the stems of trees and stable isotopes in their wood are often strongly influenced by climate variability. The tree species *Entandrophragma utile* is potentially useful for paleoclimatic studies because it presents clear annual growth rings, has a wide-spread distribution in tropical Africa and is long lived (>300 years). Here we show that oxygen isotope values ($\delta^{18}O$) in tree-rings of *E. utile* from North-western Cameroon correlated to regional precipitation (1930-2009). All correlations found were negative, following the proposed recording of the 'amount effect' by trees in the tropics. We also found tree-ring $\delta^{18}O$ to track sea surface temperatures (SST) in the Gulf of Guinea (1930-2009). It seems likely that *E. utile* at our study site records a direct effect of SST on the $\delta^{18}O$ of water vapour, in addition to the fractionation of ^{18}O in rain that occurs during transportation to the site and which depends on the precipitation amount. Years with high SST in the Gulf of Guinea coincide with high precipitation over vast areas in West and Central Africa during the peak of the growing season and are recorded in tree rings as years with relatively low $\delta^{18}O$ values. Our results suggest that oxygen isotope values in tree-rings of this species are a reliable proxy for regional precipitation. Oxygen isotope analysis of *E. utile* therefore is a promising tool to reconstruct paleoclimatic variability during the last centuries in the West and Central Africa.

3.1 Introduction

West and Central Africa form one of the core convection areas in the tropics, but the regional climate is surprisingly little studied (Washington et al. 2013). This paucity is mainly caused by the lack of instrumental climate data in the area, which limits the understanding of the regional climate and thus the ability to predict future changes (Washington et al. 2013). The lacuna of instrumental data can be filled by proxies that allow the reconstruction of the climatic variability of the past.

A promising new tool for such climate reconstructions is the study of stable oxygen isotopes in growth ring of (tropical) trees (Zuidema et al. 2013). From these oxygen isotopes ($\delta^{18}O$), information on large-scale atmospheric processes and precipitation over vast areas has been derived (Brienen et al. 2012b, Schollaen et al. 2013b). The relation between tree-ring $\delta^{18}O$ and precipitation is based on the fact that trees can record the $\delta^{18}O$ variability of rainwater, which in the sub-tropics and tropics, is strongly determined by condensation processes in clouds (Dansgaard 1964). Water molecules with a heavy ^{18}O condense more easily, making the isotopic content of the remaining vapour increasingly depleted in ^{18}O. As clouds rain out and move inland, the remaining water vapour becomes increasingly lighter, a process that is more dominant in years with intense rainfall (Risi et al. 2008, Kurita et al. 2009). This so called 'amount effect' takes place at a regional level, generating a negative correlation between the amount of precipitation and the fraction of ^{18}O in rainwater (Kurita et al. 2009).

Additional factors however, like the isotopic signal of the source water from which moisture is formed and the recycling of rain water through evapo-transpiration in plants during moisture transport also affect the $\delta^{18}O$ signature of rainwater (Salati et al. 1979, Bony et al. 2008). Furthermore, evapo-transpiration in leaves can lead to an enrichment in ^{18}O of the absorbed water (Barbour 2007). The interpretation of tree-ring $\delta^{18}O$ values can therefore be complex and the dominant factor controlling tree-ring $\delta^{18}O$ values will most likely depend on the species studied as well as the location of sampling (Brienen et al. 2013).

Previous studies have related tree-ring $\delta^{18}O$ to basin-wide precipitation in the Amazon (Ballantyne et al. 2011, Brienen et al. 2012b), regional precipitation in Thailand (Poussart and Schrag 2005) and Indonesia (Schollaen et al. 2013b) and to El Niño Southern Oscillation (ENSO) variability in Laos (Xu et al. 2011). To our knowledge, no tree-ring $\delta^{18}O$ studies have been conducted in tropical Africa.

Here, we evaluated the climate signals in tree-ring $\delta^{18}O$ of an African mahogany species (*Entandrophragma utile*) from North-western Cameroon. To this end, we assessed (1) if growth rings in *E. utile* can be reliably dated, (2) if $\delta^{18}O$ values of multiple trees show a strong common signal and (3) whether a common $\delta^{18}O$ signal can be correlated to precipitation, preferably on a regional scale. We consider *E. utile* to be a species with a high potential for usage in climate reconstructions, because of its wide-spread distribution in tropical Africa, the presence of clear annual growth rings and a long lifespan (>300 years).

3.2 Material and methods

Regional setting

Trees were sampled inside the FSC-certified Forest Management Unit 11.001, of Transformation REEF Cameroon. This area is adjacent to the northwest border of Korup National park, in the Southwest Region of Cameroon, between 5°23'N, 9°09'E and 5°23'N, 9°12'E (Fig. 3.1a). The vegetation in the region consists of semi-deciduous lowland rainforest. The regional climate is equatorial, with a unimodal rainfall distribution and a dry season (with monthly rainfall <100mm) from December to February (Fig. 3.1b). Total annual precipitation varies strongly in the region. From 1968 to 2010, average annual precipitation amounted 2920mm (range: 2070-3690mm) at the Mamfé Airport weather station, ~40km north of the study area, whereas at Mundemba meteorological station, ~40km south of the study area, annual precipitation averaged 5220mm (range: 3151-9345mm). From these nearby records we estimate that at our site, annual precipitation is approximately 4000 mm and mean annual temperature approximately 26.5°C (Fig. 3.1b).

Study species

The study species, *Entandrophragma utile* (also known as Sipo), is a shade-tolerant and deciduous tree species from the family Meliaceae. It is an emergent tree species, which can reach a maximum age of around 260 years (Nzogang 2009), a maximum dbh of 250 cm and a total canopy height of up to 60 m (Voorhoeve 1965). It is found in tropical Africa from Guinea to Togo and from Benin to Uganda and Angola, preferring fertile and well-drained soils in both upland evergreen forests and semi-deciduous moist- and dry forests (Poorter et al. 2004). The annual nature of ring formation in this species has been demonstrated by Hummel

(1946) and Mariaux (1981). Ring boundaries are characterized by the formation of a band of terminal parenchyma (Fig. 3.1c).

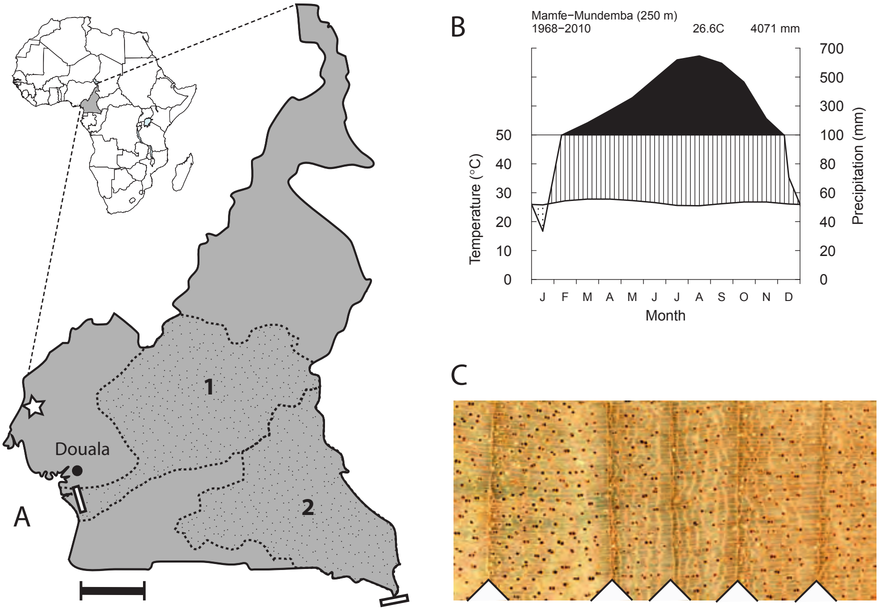


Figure 3.1 **(A)** Map of Cameroon with the location of the sampled trees (indicated by the star), the Sanaga river basin (1) and the Cameroonian half of the Sangha river basin (2). White blocks indicate where river discharge was measured, black scale bar represents a distance of 200 km. **(B)** Climate diagram for the study site. Monthly precipitation and temperature (1968-2010) of two nearby stations were averaged: Mamfé Airport weather station (40 km north of study site) and Mundemba (40 km south of the study site). Dotted area indicates the dry season (precipitation <100 mm/month), black area the rainy season (>100 mm/month). **(C)** Growth rings in the wood of *Entandrophragma utile*. Triangles indicate ring boundaries (characterized by a band of terminal parenchyma).

Sample collection and preparation

Entire stem discs at ~1 m height were collected from eight large trees with a chainsaw after selective logging in March-April 2012. No logging had taken place in the area prior to 2012. The trees were collected from an area of 1600 ha. After drying, the surfaces of the stem discs were polished with sandpaper of grids up to of 600 grains/cm^2. Part of the disc was also cut with razor blades to enhance the visibility of the rings. Ring identification took place using a LINTAB 6 measuring table (Rinntech, Germany), but ring widths were measured using high-resolution scans (1600 dpi) and WinDendro software (Regent Instruments, Canada). We checked the dating of the rings in two ways, first by visually cross-dating the ring-width series from different directions on the same disc and secondly, by cross-dating the ring-width series of different trees. Because the latter proved difficult, we checked the quality of dating by radio carbon (^{14}C) dating (Worbes et al. 2003). ^{14}C-values were determined for 9 rings from three different trees (Accelerator Mass Spectrometer, University of Groningen, the Netherlands).

For the $\delta^{18}O$ measurements, the five trees with the clearest growth rings and best internal cross-dating were selected. Wood samples were extracted from each ring of the selected trees. Wood was extracted across the entire width of the ring. A total of 665 samples were taken. Cellulose extraction was performed on the wood samples using a modification of the Jayme-Wise method (Wieloch et al. 2011). Crude cellulose samples were subsequently homogenized in a demi-water solution by a mixer mill (Retsch MM301, Germany) and oven-dried at 60 ^{0}C. The $\delta^{18}O$ of the cellulose samples was analysed at the Leicester Environmental Stable Isotope Laboratory (University of Leicester, United Kingdom), using a Sercon HT furnace coupled to a 20-20 isotope ratio mass spectrometer. The furnace was kept at 1400°C and was equipped with a quartz reactor lined with a glassy carbon tube. The glassy carbon tube was filled to a height of 150 mm with glassy carbon chips with the addition of a small amount of Nickeled carbon powder catalyst. Carbon monoxide and nitrogen were separated on a GC column, packed with molecular sieve 5A at a temperature of 40°C. The oxygen isotope composition ($\delta^{18}O$, in ‰) was calculated as:

$$\delta^{18}O_{\text{tree-ring}} = (R_{\text{sample}} / R_{\text{standard}} - 1)$$

where R_{sample} is the $^{18}O/^{16}O$ ratio of a sample and R_{standard} the $^{18}O/^{16}O$ ratio of an internationally recognized standard material (Vienna-Standard Mean Oceanic Water; VSMOW).

Climatic data

Monthly measurements of $\delta^{18}O$ in precipitation from stations in West and Central Africa were obtained from the Water Isotope System for Data Analysis, WISER (IAEA/WMO 2006). Monthly precipitation data from two local weather stations (at Mamfé and Mundemba) were obtained from these stations at site.

Regional climatic signals in tree-ring $\delta^{18}O$ values were assessed by relating $\delta^{18}O$ variability to river discharge data, regional precipitation and sea surface temperatures. Discharge data for the Sanaga and Sangha river basins, the two largest river basins in central and south Cameroon, was obtained from the Global River Discharge Database (Center for Sustainability and the Global

Environment, Gaylord Nelson Institute for Environmental Studies, University of Wisconsin-Madison). The Sanaga River drains an area of around 130.000 km^2 in central Cameroon. The Sangha river basin drains a large part of southern Cameroon as well as part of the Central African Republic (around 150.000 km^2 in total). Gridded regional precipitation at a 0.5° scale over the period 1930 to 2009 was obtained from the CRU TS 3.10.01 dataset (University of East Anglia Climate Research Unit 2012). We used sea-surface temperatures of the Gulf of Guinea from 1930-2011 from the HadISST1 1° reconstruction (HadISST1; Hadley Centre).

Data analyses

Annual ring-width series were de-trended and converted to ring-width indices (RWI) by expressing ring-width values as anomalies from a smoothing spline with a rigidity of 20 years. We took the average of the RWI and tree-ring $\delta^{18}O$ series of the five *E. utile* trees as a representation of the common signal in these trees, the 'chronology'. The Expressed Population Signal (EPS) and average inter-series correlation (r_{is}) were calculated to evaluate the strength of the RWI and $\delta^{18}O$ chronologies. The EPS quantifies how well a chronology represents the hypothetical perfect or true chronology (Wigley et al., 1984). For a chronology in which trees show a prefect synchronized signal, the EPS = 1. An EPS of 0.85 is usually used to separate between chronologies dominated by a strong common signal ($EPS \geq 0.85$) and chronologies wherein tree-level instead of stand-level signals dominate ($EPS < 0.85$; Speer (2010)). The mean inter-series correlation give the average correlation of all the series with each other.

We visualized decadal scale fluctuations in tree-ring $\delta^{18}O$ and climate data with a smoothing spline (wavelength of 5 years and a frequency response of 0.05). Pearson's correlations were used to relate tree-ring $\delta^{18}O$ values to local rainfall variability, river discharge data and the $\delta^{18}O$ of precipitation. All analyses were performed in R, version 2.12.2, (R foundation for Statistical Computing, Vienna, Austria), using the package dplR.

Pearson's correlation maps of the $\delta^{18}O$ chronology with regional precipitation and SST variability were created using the KNMI Climate Explorer (Trouet and Van Oldenborgh 2013), which uses the Grid Analysis and Display System (GrADS; Institute of Global Environment and Society, USA). All data were linearly de-trended before analyses to be certain that significant correlations are resulting from similarity in year-to-year variation, rather than concurrent long-term trends.

3.3 Results

Dating accuracy and chronologies

Dating accuracy of the growth rings was checked by radio-carbon (^{14}C) dating of nine wood samples from three individuals. Age estimations based on radiocarbon analyses exactly matched those obtained from tree-ring analyses (within 1 year margin of error; Fig. 3.2). This indicated that no or very few dating errors were made over the tested period 1950-2010 for the trees analysed. We tested three different samples: tree discs with very clear rings; with clear rings and samples with the presence of 'complex' rings: e.g. with periods of very small growth (suppressions) or strong wedging rings. We found no differences between these samples (Fig. 3.2), which shows that even the sample with 'complex' rings did not contain dating errors in the tested period.

Despite the highly accurate dating of the samples, the standardized ring-width (RWI) series of the five large *Entandrophragma* trees showed a very low common signal and poorly cross-dated with each other (r=0.038, p=0.735; EPS=0.166; Fig. 3.3). The $\delta^{18}O$ chronology, on the other hand, strongly contrasted the RWI chronology and showed a significant inter-series correlation (r_{is}=0.367; p<0.001) and high EPS (0.737; Fig 3.3) over the period 1930 - 2010. From 1900 – 2010, the inter-series correlation and EPS were slightly lower (r_{is}=0.319, p<0.001; EPS =0.694), from 1860-2010 the EPS dropped to 0.642 and r=0.310 (Supplementary Fig. 3.1). We took 1930 as the starting point for the $\delta^{18}O$ chronology because the chronology becomes gradually weaker when including years before 1930.

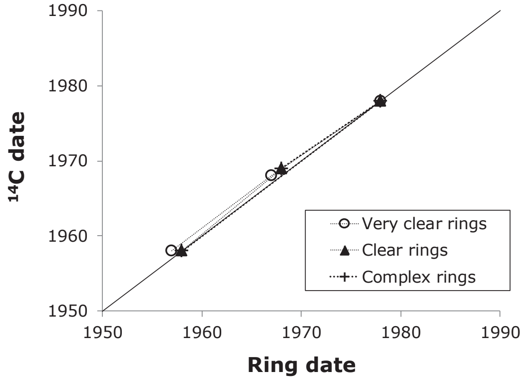


Figure 3.2 Radio carbon (^{14}C) bomb-peak dating of three *Entandrophragma utile* trees. We used three different types of samples: tree discs with very clear rings (open circles); with clear rings (closed triangles) and samples with the presence of 'complex' rings (crosses), e.g. with suppressions or strong wedging rings. Diagonal line indicates the 1:1 match between ring-based age estimations and ^{14}C-based age estimations.

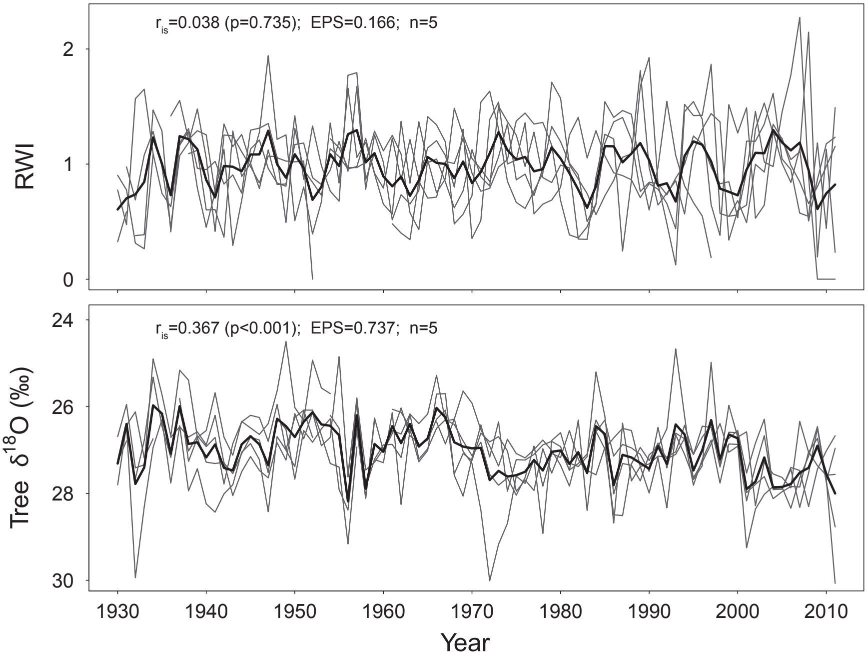


Figure 3.3 Ring-width index (RWI) and $\delta^{18}O$ series of five large *Entandrophragma utile* trees. Grey lines are the individual series; bold black line shows the average. The 'r_{is}' is the average inter-series correlation; EPS is the Expressed Population Signal; n the number of trees.

Correlations with precipitation $\delta^{18}O$

Oxygen isotopes in precipitation have been measured in just a few locations in West and Central Africa (IAEA/WMO 2006). At five locations, $\delta^{18}O$ of precipitation has been measured for more than 10 consecutive years and in five others, data were available for 5-9 years. None of these stations were located in close proximity of our study area (minimal distance was 650 km). We found no strong significant correlations between tree-ring $\delta^{18}O$ and precipitation $\delta^{18}O$. The highest correlations were found with precipitation $\delta^{18}O$ during the rainy season (May-November; Fig. 3.4). Correlation coefficients were also higher for weather with stations in West Africa (Ghana and Nigeria) than for those in Central Africa (Angola and Democratic republic of Congo), but none of these correlations were significant except for Kano, Nigeria, were precipitation $\delta^{18}O$ was measured 1961-1966 and 1971-1973 (r=0.695, p=0.037).

In Ghana, δ^{18}O has been measured in sediments of Lake Bosumtwi (Shanahan et al. 2009), about 1200 km from our study site. We found a marginally significant correlation between tree-ring δ^{18}O and δ^{18}O in sediments of Lake Bosumtwi from 1860 to 2003 (p=0.053; Supplementary Fig. 3.2).

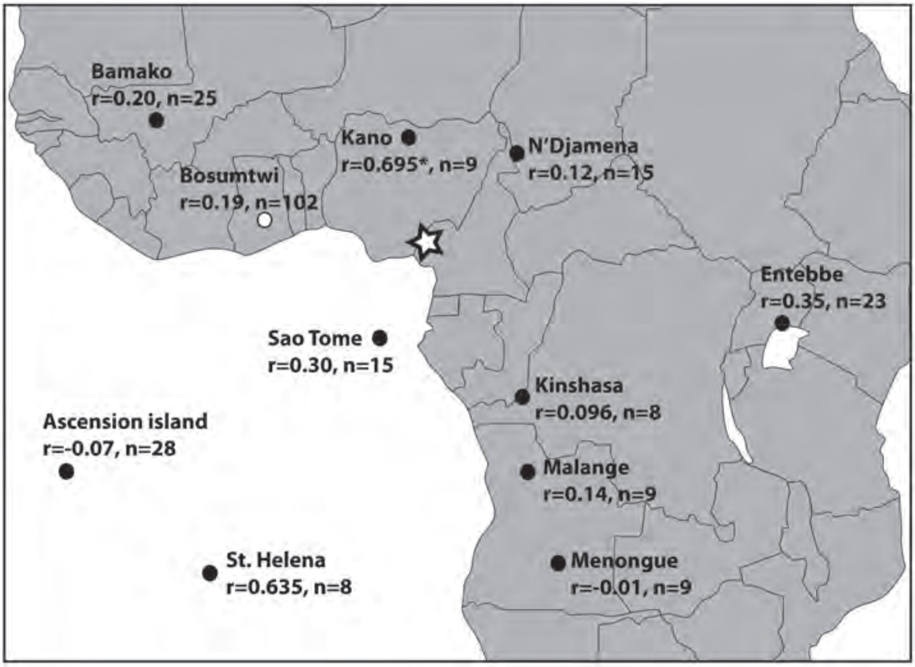


Figure 3.4 Pearson's correlations between tree-ring δ^{18}O values in *Entandrophragma utile* from Cameroon (white star) and δ^{18}O of precipitation. All stations in the region where monthly δ^{18}O in precipitation was measured for more than 5 consecutive years are shown (black dots). Best correlations were found with average δ^{18}O of precipitation during the wet season (May to November); 'r' refers to the Pearson's correlation coefficients, 'n' is the number of years used in the correlation. No significant correlations were found, except for Kano, Nigeria (p=0.037). δ^{18}O values measured on sediments of lake Bosumtwi, Ghana (open square; data from Shanahan et al. 2009) correlated with a p-value of 0.053 with tree-ring δ^{18}O (1860-2010, see Supplementary Fig. 3.2).

Tree-ring δ^{18}O and local precipitation

We found no significant correlation between variation in tree-ring δ^{18}O and variation in total annual precipitation over the period 1968 to 2010, measured at the two closest climate stations (Mamfé and Mundemba). Tree-ring δ^{18}O also did not correlate with wet season precipitation (May-November), nor when precipitation was averaged over the two climate stations. We subsequently used gridded precipitation of a 0.5° square around our study site, which is based on the interpolation of many climate stations around the study site (but without climate data from Mundemba and Mamfé). This yielded a significant negative correlation between temporal variation in the tree-ring $\delta^{18}O$ values and that in gridded total annual precipitation (1930-2009: r=-0.299, p=0.007; Fig. 3.4) and precipitation during the wet season (r=-0.301, p=0.007). The gridded precipitation correlated significantly with precipitation measured in Mamfé (1968-2009; Pearson's r=0.456, p=0.001), but not with precipitation in Mundemba.

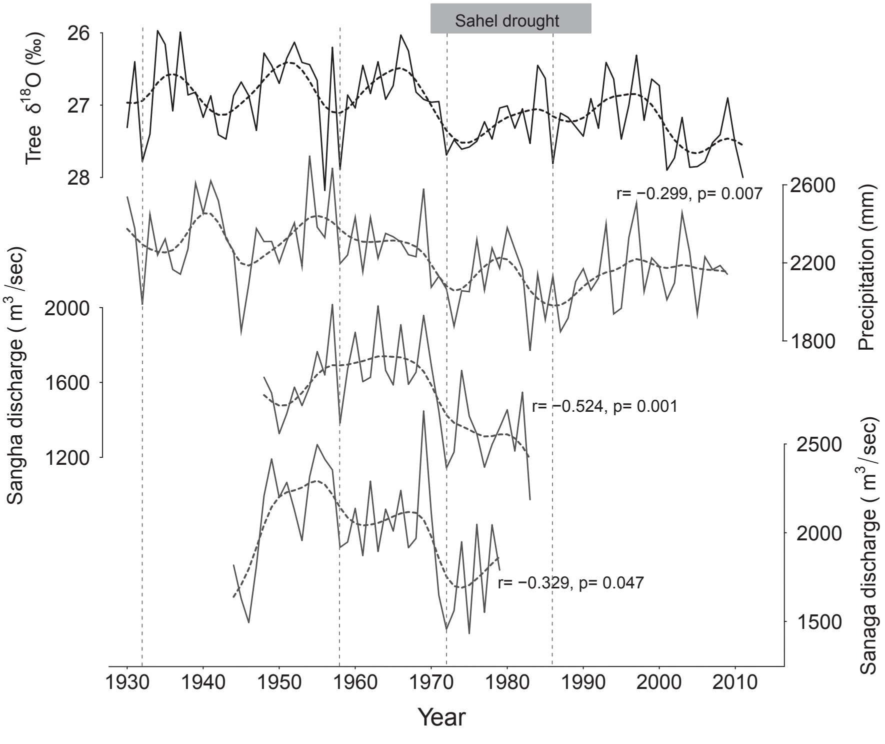


Figure 3.5 The $\delta^{18}O$ chronology based on five *Entandrophragma utile* trees (top; y-axis in reverse order) significantly correlated with interpolated total annual precipitation (square of 0.5° around the study site) over the period 1930 – 2009 (second line). The $\delta^{18}O$ chronology also significantly correlated with discharge data of the Sangha river basin (third line) and Sanaga river basin (fourth line). Grey horizontal bar indicates a period of extended drought (the Sahel drought). The grey dashed vertical lines indicate some of the peaks in the $\delta^{18}O$ chronology.

Regional climate signals in tree-ring $\delta^{18}O$

We explored the regional climatic signal in the $\delta^{18}O$ chronology in three ways: (1) by correlating $\delta^{18}O$ to river discharge data, (2) by correlating $\delta^{18}O$ to gridded precipitation over West- and Central Africa and (3) by correlating $\delta^{18}O$ to gridded sea surface temperatures.

The available discharge data of the Sanaga river (Fig. 3.1) from 1943 to 1980 correlated significantly, but weakly, to the $\delta^{18}O$ chronology ($r=-0.329$, $p=0.047$; Fig. 3.5). Discharge data of the Sangha river (Fig. 3.1) from 1948 to 1983 (measured at Ouésso, close to the border between Cameroon and the Republic of Congo) showed a significant and somewhat stronger correlation with tree-ring $\delta^{18}O$ ($r=-0.524$, $p=0.001$; Fig. 3.5). The discharges of the two river basins also correlated to each other ($r=0.600$, $p<0.001$). We found tree-ring $\delta^{18}O$ to show a significant negative correlation with precipitation (1930-2010) over large parts of West and Central Africa (Fig. 3.6). These correlations were significant for precipitation over the entire country of Equatorial Guinea, Gabon and Congo, as well as large parts of Central and South Cameroon, western part of the Democratic Republic of Congo, North-western Angola and Southern Nigeria (Fig. 3.6). Finally, we found a significant negative correlation between tree-ring $\delta^{18}O$ and gridded sea surface temperatures (SST) in the Gulf of Guinea from 1930 – 2010 (Fig. 3.6).

The correlations between tree-ring $\delta^{18}O$ and sea surface temperatures and between $\delta^{18}O$ and regional precipitation were found for all months of the wet season (March to November) and disappeared during the dry season (results not shown). This is likely because *Entandrophragma utile* is a deciduous tree species and leafless during the dry season. As a consequence growth is strongly limited, or absent, during in the dry season and no ^{18}O signal (or a very weak signal) is recorded in tree-ring cellulose during these months.

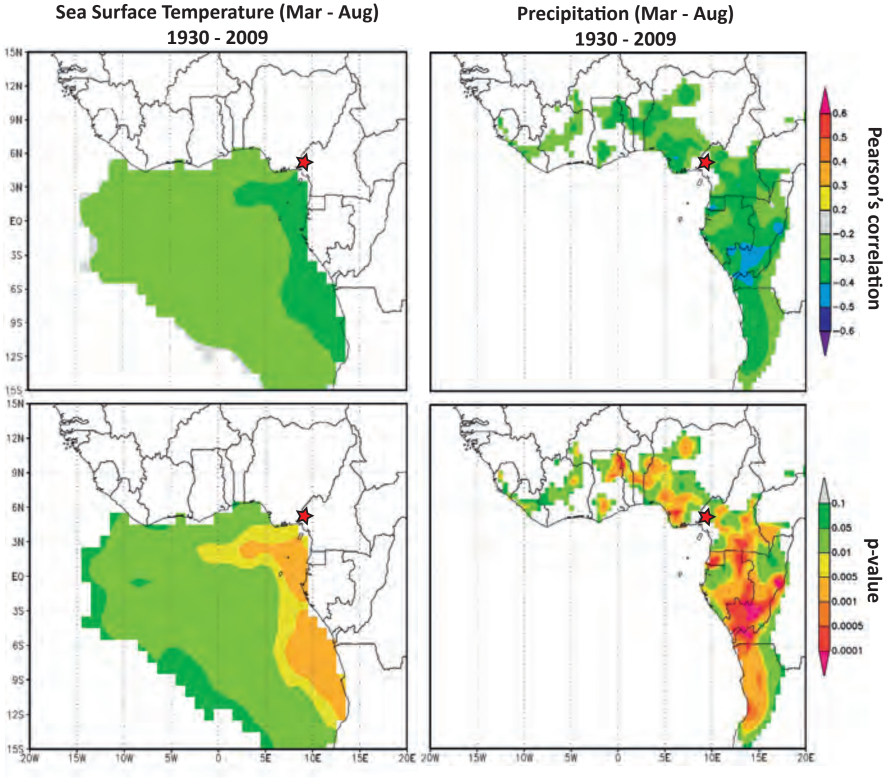


Figure 3.6 Correlations between the tree-ring $\delta^{18}O$ chronology of *Entandrophragma utile* and gridded sea surface temperature (SST) anomalies in the gulf of Guinea (left panels) and gridded precipitation (right panels) during the growth season (March to August) over the period 1930-2009. Upper panels show the Pearson correlation coefficients; lower panels the associated p-values. The red star indicates the area where trees were sampled.

3.4 Discussion

Ring-width and $\delta^{18}O$ chronologies

The average ring-width series of five large *Entandrophragma utile* trees from North-western Cameroon showed no common signal and poorly cross-dated to each other (Fig. 3.3). Radio-carbon (^{14}C) dating revealed that this result was not caused by miss-identifications and measuring errors, since tree-ring based age estimations perfectly matched ^{14}C-based age estimations (Fig. 3.2). A good match was also found in the sample with so called 'complex' rings (e.g. suppressions and strong wedging rings), indicating that false rings or locally absent rings are rarely formed in this species. The absence of a common growth signal could be caused by the very high precipitation in the study area (around 4000 mm per year) and low seasonality (Fig. 3.1b). In that case, tree growth can be controlled by local site conditions (e.g. the availability of light and nutrients) which are in general much more heterogeneous on a spatial scale than precipitation (Speer 2010). The absence of a common growth signal in tree species with clear and annual growth rings has been found in other wet tropical forests (Fichtler et al. 2003). In slightly drier areas in West and Central Africa, strong synchronized tree growth has been found and related to (wet season) precipitation (Schöngart et al. 2006, Couralet et al. 2010, Trouet et al. 2010, de Ridder et al. 2013).

Contrary to ring widths, $\delta^{18}O$ values of the *Entandrophragma* trees significantly correlated between the five trees, showing a clear common signal (the $\delta^{18}O$ chronology; Fig. 3.3). The presence of a common signal in tree-ring $\delta^{18}O$ is likely caused by the recording of the $\delta^{18}O$ signature of precipitation in tree rings, a signal that is relatively homogeneous on the spatial scale on which our trees were sampled (Bowen 2008). The strength of the common $\delta^{18}O$ signal in *E. utile* (1930 to 2010: $r=0.367$; EPS=0.737) was lower than found in chronologies of similar duration (50-100 years) in trees from Laos (Xu et al. 2011), Bolivia (Brienen et al. 2012b) and Indonesia (Schollaen et al. 2013b). In these studies however, trees were sampled in areas with a distinct dry season and an average annual precipitation of less than 2000 mm (930 - 1900 mm/year). It is possible that the high precipitation amount in our study site affects how well the $\delta^{18}O$ signature of precipitation is recorded. Possibly, a saturation of the soil causes a high run-off during extremely wet years. As a consequence, the $\delta^{18}O$ signature of recent rainwater could be less well recorded. In addition, if soils maintain a high water content during the dry season, e.g. in years when the dry season is very short, the $\delta^{18}O$ signature of rainwater of one growing season could be partially carried over to the next growing season. Heterogeneity in the drainage capacity of soils may therefore cause variability in the recording of precipitation $\delta^{18}O$ of individual trees. Unfortunately, we know little about the soil properties at the individual locations of the sampled trees or of variation in rooting depth between the sampled individuals. The significant inter-series correlation ($p<0.001$) nonetheless suggests that even that even under these very wet conditions, trees sampled in a large area (~1600 ha) recorded a highly similar $\delta^{18}O$ signal.

To test if trees indeed record the $\delta^{18}O$ variability of precipitation we correlated tree-ring $\delta^{18}O$ to precipitation $\delta^{18}O$ data (Fig. 3.4) and found a significant correlation with one of the closest stations (720 km away), Kabo in Nigeria ($r=0.695$, $p=0.037$). Precipitation $\delta^{18}O$ has been measured in very few locations in West and Central Africa and most datasets contain missing data and have large gaps between the years measured. This might at least partially explain why no other significant correlations were found with stations where precipitation $\delta^{18}O$ was measured (Fig. 3.4).

Climatic signals in the tree-ring $\delta^{18}O$ chronology

No significant correlations over the period 1968 – 2010 were found between tree-ring $\delta^{18}O$ and precipitation measured at two climate stations in the vicinity (~40 km north and south) of the study site. However, these stations also did not correlate to each other ($r=0.156$, $p=0.318$), indicating that these datasets are either of low quality or that rainfall in the area is highly variable and that precipitation data from these stations does not accurately reflect the precipitation amount at the site where our study trees were sampled. Such a high spatial variability of rainfall patterns may be caused by variable rain shadows of Mount Cameroon (4000 meter high) or lower (500-700 meters high) ridges in the vicinity of the study site._

At a slightly larger spatial scale we did found a significant negative correlation between interpolated precipitation at a 0.5° square (~55 x 55 km) around our study site and tree-ring $\delta^{18}O$ over the period 1930-2009 (Fig. 3.5). Furthermore, when we correlated tree-ring $\delta^{18}O$ of *E. utile* to gridded precipitation on a sub-continental scale we found significant correlations with (wet season) precipitation over vast areas in Cameroon, as well as other areas in Central and West Africa (Fig. 3.6). All found correlations between tree-ring $\delta^{18}O$ and precipitation were negative. Years with high precipitation coincided with low $\delta^{18}O$ values in trees, whereas dry years are represented by high $\delta^{18}O$ values. A similar negative correlation was found between tree-ring $\delta^{18}O$ and discharge of two major river systems in Central and South Cameroon (Fig. 3.5), underpinning the capacity of this species to record the precipitation amount on regional to sub-continental scales.

The stronger regional than local precipitation signal is likely caused because the effect of 'precipitation amount' on rainwater $\delta^{18}O$ works on a regional scale rather than on a local scale in the tropics (Lee and Fung 2008, Risi et al. 2008, Kurita et al. 2009). At a continental site, tree-ring $\delta^{18}O$ has been shown to reflect primarily what happens during water-vapour transport to the site: the amount of rainfall and subsequent enforcement of this signal by the recycling of rainwater by vegetation (Brienen et al. 2012b). Our study site is located only 120 km from the Atlantic Ocean and thus the distance of vapour transport may be short and recycling of rainwater limited. Instead, a direct effect of oceanic water in the Gulf of Guinea on tree-ring $\delta^{18}O$ might be expected. Both the $\delta^{18}O$ of ocean water and the water evaporating from it are affected by temperature. Water vapour forming precipitation will be depleted in heavy isotopes relative to ocean water, because water with ^{16}O needs less energy to evaporate, a process that is enforced when temperature is high (e.g.Craig and Gordon 1965, Brown et al. 2006). The negative correlation between tree-ring $\delta^{18}O$ and sea surface temperatures in the Gulf of Guinea that we found (Fig. 3.6) are consistent with this mechanism of temperature-driven ^{18}O depletion.

For *Entandrophragma* trees in North-western Cameroon, two major controls of tree-ring $\delta^{18}O$ thus seem likely. First, a direct effect of SST on the ^{18}O signal of water evaporating from the ocean, followed by the effect of the amount of precipitation during vapour transport. These two factors are however difficult to disentangle because rainfall variability in West and Central Africa is profoundly influenced by the SST of the tropical Atlantic Ocean (Chang et al. 1997). A high SST in the Gulf of Guinea is associated with high precipitation over large parts of West and Central Africa during the peak of the wet (growing) season (Balas et al. 2007). Because tree-ring $\delta^{18}O$ correlates with SST in the Gulf of Guinea, it is not surprising that it also correlates to precipitation over vast areas in West and Central Africa.

Low frequency signals and long-term trends in the $\delta^{18}O$ chronology

The $\delta^{18}O$ chronology (Fig. 3.5) is characterized by a remarkable 10-15 year cycle from 1930 to around 1970. Cycles of a similar duration have been found in a $\delta^{18}O$ chronology from sediments in lake Bosumtwi, Ghana and have been related to relative changes in SST between the hemispheres on decadal timescales, a phenomenon often called the Atlantic SST dipole (Shanahan et al. 2009). We however found no match with published Tropical Northern Atlantic Index (referring to anomalies in monthly SST in the area 5.5N to 23.5N and 15W to 57.5W) and the Tropical Southern Atlantic index (SST anomalies in the area Eq-20S and 10E-30W) or the difference between the two (Enfield et al. 1999; results not shown). We did find that the period of higher $\delta^{18}O$ values from 1970-1990, coincided with the occurrence of the Sahel drought (Fig. 3.5), a series of severe droughts in the 1970s and 1980s in the Sahel region and parts of sub-Saharan Africa (Zeng 2003). A similar pattern of relatively high $\delta^{18}O$ values during this period has been found in corals from the Island of Principe in the gulf of Guinea (Swart et al. 1998) and lake sediments in Ghana (Shanahan et al. 2009). These results suggest that tree-ring $\delta^{18}O$ in *Entandrophragma* also records decadal drought periods.

In addition to decadal-scale fluctuations, we found a significant long-term trend in the $\delta^{18}O$ chronology, amounting to a 0.5‰ increase of $\delta^{18}O$ values over the period 1860-2011 (Supplementary Fig. 3.1). Interestingly, an equal 0.5‰ increase since 1850 has been found in trees from Bolivia (Brienen et al. 2012b). Because similar trends in $\delta^{18}O$ value since 1850 were also found in Andean ice cores (Thompson et al. 2006) and Andean lake sediments (Bird et al. 2011), Brienen et al. (2012b) suggested this trend is not caused by the ontogeny (i.e. size development) of trees. In Southeast Asia, a stronger increase of tree-ring $\delta^{18}O$ values since 1950 has been reported and was related to a drying of the Asian Monsoon (Poussart and Schrag 2005, Xu et al. 2011). In our study, the Sahel drought period (1970-1990; Fig. 3.5) contributed to the observed long-term increase in $\delta^{18}O$ values, as no trend was found when excluding the last 40 years of the $\delta^{18}O$ chronology.

Conclusions and perspectives

Tree-ring $\delta^{18}O$ in *Entandrophragma utile* from North-western Cameroon correlated to precipitation at regional and sub-continental scales and to sea surface temperatures in the Gulf of Guinea. It seems likely that tree-ring $\delta^{18}O$ at this locality records a direct effect of SST on the $\delta^{18}O$ of water vapour in addition to an effect of precipitation amount during the transportation of water vapour to the study site.

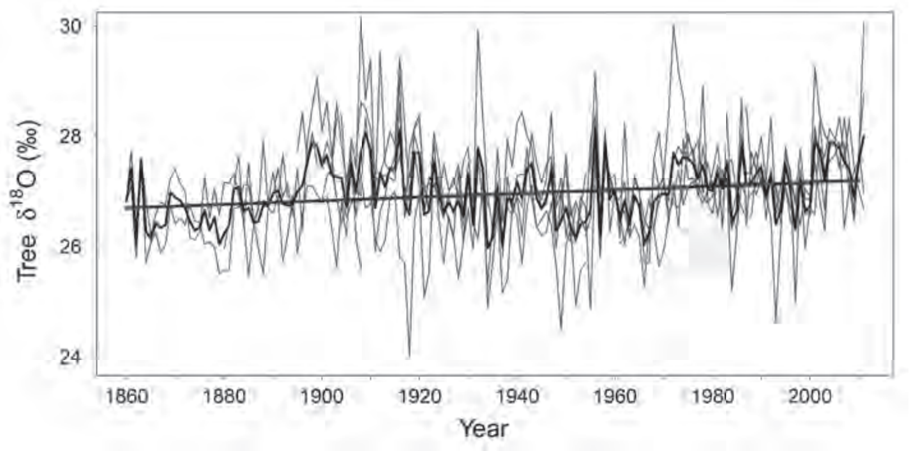
Because of the easily measurable and accurately datable growth rings in *Entandrophragma utile*, the presence of a strong common $\delta^{18}O$ signal and its relation to regional precipitation, we argue that $\delta^{18}O$ analysis of tree rings from this species represents a promising tool for a detailed reconstruction of climate variability over the last centuries in the region. An advantage of tree-ring $\delta^{18}O$ data relative to $\delta^{18}O$ series derived from lake sediments or scleractinian coral skeletons is that *Entandrophragma* trees are spatially much more widespread. In addition, *E. utile* is commercially logged throughout its range, facilitating the collection of samples (discs) from large trees. For climate reconstruction it is crucial that a set of old trees (>200 years) is collected, implying the selection of large to very large trees (roughly >150 cm dbh). The selection of such individuals for climate reconstruction should, if possible, focus on shallow rooting species in well drained soils as this gives the highest chance that tree-ring $\delta^{18}O$ records the $\delta^{18}O$ variability of recent rainfall.

A higher temporal resolution of the variability of $\delta^{18}O$ in precipitation could be obtained from intra-annual sampling of tree-ring $\delta^{18}O$ (within one annual ring) in *E. utile*. Intra-annual sampling techniques have greatly improved during the last decade and allow a semi-automatic analysis of many very small wood samples adjacent to each other (Pons and Helle 2011, Schollaen et al. 2013a). Such intra-annual sampling should be very well possible in *E. utile* because of its relatively fast growth, with average diameter growth of the studied trees being 0.72±0.4 cm/year. The data derived could provide information on the variability of $\delta^{18}O$ in precipitation with a temporal resolution of weeks. Such information can be used to shed light on the functioning of the West African Monsoon, a major but poorly understood climatological system which may be subject to change (Roehrig et al. 2013). Thus, in addition to a reconstruction of annual variation in regional precipitation and sea surface temperatures, tree-ring $\delta^{18}O$ series might further aid our understanding of important climatic drivers in West and Central Africa.

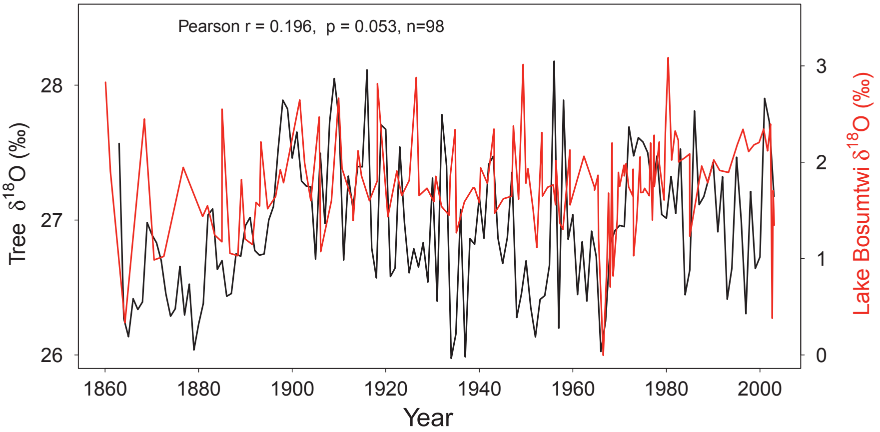
Acknowledgements

We would like to thank the personnel of logging company Transformation REEF Cameroon for their help with the collection of the samples, Arnoud Boom for performing the $\delta^{18}O$ analyses and Frans Bongers, Niels Anten, Wouter Peters and Thijs Pons for their valuable comments on a previous version of the manuscript.

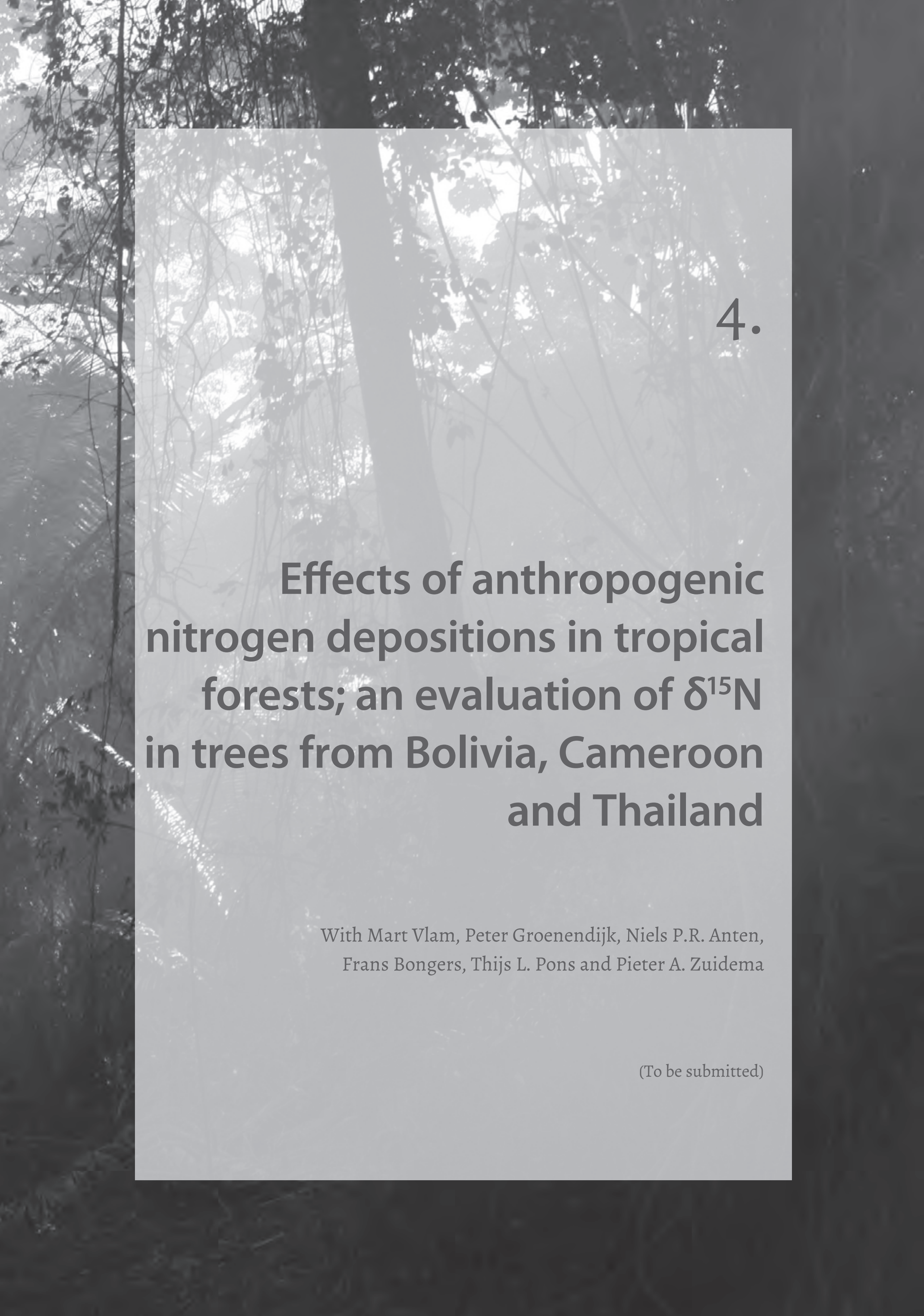
Supplementary materials



Supplementary Figure 3.1 The δ18O series of five large *Entandrophragma utile* trees from North-western Cameroon. Grey lines are the individual series; bold black line shows the average. Over the period 1860-2010 the average inter-series correlation is 0.310 ($p<0.001$), the EPS=0.642. A significant long-term increase of δ18O was found (dark grey line; $p<0.001$).



Supplementary Figure 3.2 Pearson's correlation between the δ18O chronology of *Entandrophragma utile* from Cameroon (black line) and 18O values measured on sediments of lake Bosumtwi, Ghana (red line; Shanahan et al. 2009).



4.

Effects of anthropogenic nitrogen depositions in tropical forests; an evaluation of $\delta^{15}N$ in trees from Bolivia, Cameroon and Thailand

With Mart Vlam, Peter Groenendijk, Niels P.R. Anten,
Frans Bongers, Thijs L. Pons and Pieter A. Zuidema

(To be submitted)

Abstract

Anthropogenic nitrogen depositions have likely increased in many tropical forests. Increased $\delta^{15}N$ values in the growth rings of tropical trees have been hypothesized to reflect the effect of this elevated nitrogen input, suggesting that tropical N cycles have become more 'open' and 'leaky' during the last decades. To supplement the still sparse observations of increased $\delta^{15}N$ values in tropical trees, we studied historical changes of $\delta^{15}N$ in tree rings of 400 trees from forest sites in Bolivia, Cameroon and Thailand. We used a novel sampling method that controls for potential ontogenetic (i.e. size-related) effects on tree-ring $\delta^{15}N$ values. We found a marginally significant increase of $\delta^{15}N$ values since 1950 in trees from Thailand (p=0.058), suggesting the nitrogen cycle at this location has become more open and is shifting to a nitrogen 'saturated' state. However, at the sites in Bolivia and Cameroon, no long-term change of tree-ring $\delta^{15}N$ values were found, even though estimated NH_3 and NO_x emissions strongly increased at the Cameroonian site since 1970. Possibly, the very high precipitation at that site (~4000 mm yr^{-1}) causes the local nitrogen cycle to be 'leaky', limiting the effect of additional nitrogen input on the $\delta^{15}N$ signature of soil nitrogen. For the Bolivian site, our results are in line with those expected for areas with a low anthropogenic nitrogen input. Although our results confirm previous observation of increasing $\delta^{15}N$ over time at one site, we did not find evidence for a long-term $\delta^{15}N$ change in the other forest sites. As such, our findings are not consistent with the idea that nitrogen cycles in tropical forests are generally shifting to more open and leaky systems, but rather show that this will depend on local N-deposition rates and differences in the N saturation among forests.

4.1 Introduction

During the last century, nitrogen depositions in ecosystems have increased globally as a result of the widespread use of artificial N fertilizers and the burning of fossil fuels (Gruber and Galloway 2008, Davidson 2009). In temperate regions, anthropogenic nitrogen deposition has stimulated productivity in nitrogen limited forests (Binkley and Högberg 1997, Sigurdsson et al. 2002), but excessive nitrogen input can lead to 'nitrogen saturation', a state of restricted nitrogen uptake by plants and a subsequent acidification of the soil (Vitousek et al. 1997). Higher soil acidity causes an increased leaching of nutrients and can ultimately lead to reduced tree growth and survival (Schulze 1989, Aber et al. 1998, Magill et al. 2004). In tropical forests, nitrogen depositions are still low compared to temperate ones, but they are expected to increase dramatically in the near future (Holland et al. 1999, Lamarque et al. 2005, Dentener et al. 2006). Yet, tropical forests are not as nitrogen limited as temperate ones (Hedin et al. 2009) and it is hypothesized they will much quicker reach the point of nitrogen saturation and experience the associated negative effects on tree growth and biodiversity (Matson et al. 1999). Given the important role of tropical forests in the global carbon cycle and the huge biodiversity they harbour, there is an increasing interest to assess the nitrogen status of tropical forests and to evaluate if nitrogen cycles in these forests are already changing (Phoenix et al. 2006, Galloway et al. 2008).

One relatively new and promising tool to study nitrogen cycles is the measurement of nitrogen isotopes ($\delta^{15}N$) in plant material. Of particular interest are $\delta^{15}N$ values in the growth rings of trees, as they can provide a long historical record that can be studied in any region provided that there are trees with distinct growth rings (Zuidema et al. 2013). There is general agreement that $\delta^{15}N$ variability in plant material can reflect the $\delta^{15}N$ variability of the absorbed N sources under most field conditions (Evans et al. 1996, Högberg et al. 1999). Because the $\delta^{15}N$ signature of these N sources is shaped by processes in the local N cycle, tree rings have the potential to record long-term changes in the N cycle of terrestrial ecosystems. With the exception of nitrogen-fixing trees from the family Fabaceae, which do not (always) depend on soil nitrogen, and as such will unlikely record changes in the $\delta^{15}N$ of soil nitrogen.

In temperate forests, tree-ring $\delta^{15}N$ has been shown to record the effect of elevated natural and experimental nitrogen input (Bukata and Kyser 2005, Elhani et al. 2005, McLauchlan et al. 2007, Guerrieri et al. 2011). In tropical forests, however, only two studies on tree-ring $\delta^{15}N$ have been conducted, showing an increase of $\delta^{15}N$ during the last decennia in trees from Brazil and Thailand (Hietz et al. 2010, Hietz et al. 2011). This increase has been related to higher nitrification rates following elevated N depositions. Nitrification is a process that produces ^{15}N-depleted nitrate (NO_3^-), leaving behind ^{15}N-enriched ammonium (NH_4^+). If not directly absorbed, ammonium attaches to the negatively charged surfaces of minerals and organic matter and is thus held by the soil (Certini 2005). A fraction of the nitrate, on the other hand, is leached downwards and eventually lost, causing a gradual ^{15}N enrichment of the remaining N pool (Hogberg and Johannisson 1993, Högberg 1997). Increased tree-ring $\delta^{15}N$ could thus be an indication that tropical N cycles have become more 'open' and 'leaky' during the last decades (Hietz et al. 2011) and are shifting in the direction of nitrogen saturation.

To supplement the still sparse observations of increased tree-ring $\delta^{15}N$ in tropical trees, we studied long-term $\delta^{15}N$ values in 400 trees from a forest site in Bolivia (South America), Cameroon (Africa) and Thailand (Southeast Asia). The examined sites have likely experienced very different nitrogen deposition rates over the last 40 years (as assumed from estimated nitrogen emissions; Fig. 4.1), thereby allowing a test of the effect of N depositions on N cycles. However, when interpreting temporal trends in tree-ring $\delta^{15}N$ values, two potential factors should be taken into account. The first factor is tree ontogeny (i.e. size development), which can cause changes in $\delta^{15}N$ values and thereby obscure changes caused by altered N cycles. Ontogenetic $\delta^{15}N$ changes could be the result of e.g. increased rooting depth or by shifts in the exploited nitrogen sources (Hobbie and Högberg 2012). A second confounding factor is that nitrogen has some radial mobility, meaning that it is not permanently fixed when wood is formed. There have been several reports on translocation of N across ring boundaries in ^{15}N labeling experiments (Nômmik 1966, Mead and Preston 1994, Colin-Belgrand et al. 1996, Schleppi et al. 1999, Elhani et al. 2003, Hart and Classen 2003). A simple solution of nitrogen mobility effects is to leave out the active sapwood (i.e. most recent tree rings) where most translocation takes place (Hietz et al. 2010, Hobbie and Högberg 2012).

We use a new sampling methodology that explicitly controls for potential confounding ontogenetic effects. In this method, tree-ring $\delta^{15}N$ values are compared across a fixed tree size (Rozendaal et al. 2010a; illustration in Fig. 4.3 top panel) and as such excludes ontogenetic effects on tree-ring $\delta^{15}N$ values. We also removed the most recently formed growth rings and analysed $\delta^{15}N$ values on the rest of the wood (assuming this is mostly heartwood or relatively inactive sapwood). As heartwood is dead and does not contain living cells, translocation of nitrogen is not likely. The applied correction for ontogenetic effects and the exclusion of sapwood gives an unbiased way to assess long-term trends in tree-ring $\delta^{15}N$ values and thus the potential effect of increased anthropogenic nitrogen depositions on tropical N cycles. We hypothesize that our method will confirm the previous reported change of tree-ring $\delta^{15}N$ values in Thailand and show a similar change in Cameroon (given the estimated high nitrogen emissions in the area; Fig. 4.1). For Bolivia, however, we do not expect to find any significant trend in tree-ring $\delta^{15}N$ values because nitrogen emissions are still very low in the area (Fig. 4.1).

4.2 Material and Methods

Study area

The study was carried out in a forest site in Bolivia (South America), Thailand (Southeast Asia) and Cameroon (Africa; Fig. 4.1). In Bolivia, trees were sampled in the logging concession 'La Chonta', around 300 km northeast of Santa Cruz de la Sierra (15.84 S, 62.85 W). The forest in La Chonta is a semi-deciduous moist forest and the transitional between Chiquitano dry forest and moist Amazonian forest (Peña-Claros et al. 2008). Annual precipitation in the region averages 1580 mm, with a 4 month dry season receiving <100 mm from May to September (Peña-Claros et al. 2008). Soils in the study area are mostly derived from gneiss, granitic, and metamorphic rocks and have been described as sandy-loam ultisols (Peña-Claros et al. 2012). They have a neutral pH and a high fertility due to human influences, as ca. 20 percent of the area is being covered by anthropogenic soils (Paz-Rivera and Putz 2009).

In Thailand, trees were collected in the Huai Kha Khaeng Wildlife Sanctuary (HKK), Uthai Thani province, around 250 km northwest of Bangkok (15.60 N 99.20 E; same study area as Hietz et al. 2011). The vegetation in HKK is a semi-deciduous moist forest (Bunyavejchewin et al. 2009). Mean annual rainfall averages 1473 mm, with a 4-6 months dry season from November to April (Vlam et al. 2014). Soils in HKK are variable, but most are highly weathered ultisols derived from parent material of granite porphyry (Bunyavejchewin et al. 2009). Soil texture is sandy loam at the soil surface, with increasing clay accumulation below 40 cm depth (Bunyavejchewin et al. 2009).

In Cameroon, fieldwork took place in a logging concession (Forest Management Unit 11.001) of Transformation REEF Cameroon (TRC). This area is adjacent to the northwest border of Korup National park, in Western Cameroon (5.23 N, 9.10 E). The forest consists of a semi-deciduous lowland rainforest of the Guineo-Congolian type. Annual precipitation in the region averages around 4000 mm, with a dry season from December to February (Groenendijk et al. 2014). No detailed information on soil characteristics is available for the area where trees were collected, but the soil in a 50 ha forest plot located ~50 km from the study site (CTFS Korup plot), is generally skeletal and sandy (up to 70% sand in some areas), with small but increasing clay content with increasing soil depth (Chuyong et al. 2004). Most organic matter is in the top few centimetres of the soil profile and soils are very nutrient poor as a result of the high leaching due to heavy rainfall (Chuyong et al. 2004).

Nitrogen emissions

Anthropogenic NO_x and NH_3 emission data for the study sites were obtained from the European Commission, Joint Research Centre/Netherlands Environmental Assessment Agency, EDGAR version 4.2 (http://edgar.jrc.ec.europa.eu). Per study site, emission data at a 0.1° grid cell (~11x11 km) were averaged over a 1° square (~110x110 km) centring the sampled trees. As NO_x and NH_3 can travel through the atmosphere for many kilometres before being rained out, emissions averaged over a 1° grid cell are more representative of local nitrogen depositions than at a relatively small 0.1° scale. NH_3 and NO_x emissions at a 1° grid cell were converted to km^2 scale (using the R package SDMTools, which calculates surface areas for spherical polygons based on latitude and longitude coordinates). Results per site are given in Figure 4.1.

Study species and collection

At each site, we sampled trees of two species (Table 4.1). Species were selected based on their abundance (we chose relatively common species) and the possession of clear annual growth rings. The annual nature of growth rings has been demonstrated for the Bolivian species by Lopez et al. (2012), for the species studied in Thailand by Baker et al. (2005) and Cameroon by (Groenendijk et al. 2014). At each site, trees were collected in 144-297 ha of undisturbed forest. All trees larger than 20 cm diameter at breast height (dbh) were sampled in a 50 meter radius around a randomly assigned gps point. At each site, we used ~25 random points spread over the study area and collected around 50 to 100 trees per species (ranging in size from 20 to >100 cm dbh). In Cameroon and Bolivia, a first round of selective logging took place in the study area at the time of sampling (no previous logging had taken place in any of the areas). At these sites, logging operations permitted the collection of stem discs. If no discs could be collected, a minimum of three 5-mm diameter cores per tree were collected using an increment borer (Suunto, Finland and Haglöf, Sweden). Cores were taken in at least three different directions at breast height. After drying, the surface of discs and cores were either cut or polished depending on what gave the best visibility of ring boundaries.

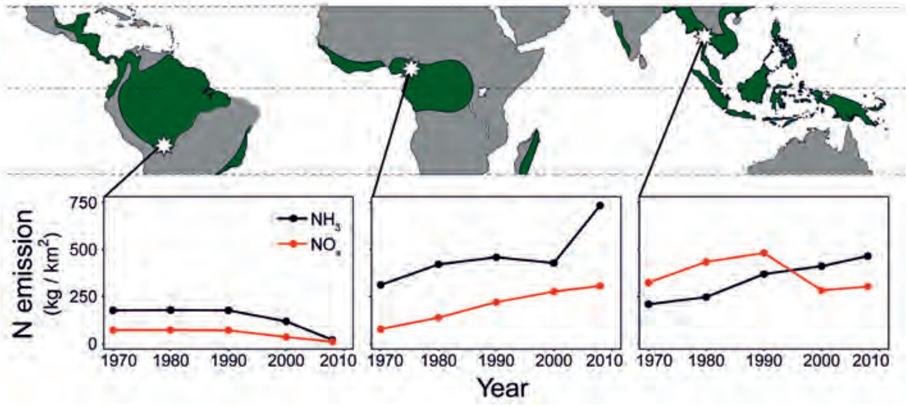


Figure 4.1 Study areas (white stars) and anthropogenic NH_3 and NO_x emissions around the study areas (averaged over a 1° grid cell centring the study sites). Wood samples were collected from three wet tropical forests (>1500mm rainfall per year; green areas). From left to right: Bolivia: La Chonta logging concession; Cameroon: a logging concession adjacent to Korup National park and Thailand: Huai Kha Khaeng Wildlife Sanctuary. Nitrogen emission data per 0.1° grid cell are from the European Commission, Joint Research Centre/Netherlands Environmental Assessment Agency, EDGAR version 4.2.

Table 4.1 Study species.

Country	Species	Family	Functional group[1]
Bolivia	*Cariniana ianeirensis* *Hura crepitans*	Lecythidaceae Euphorbiaceae	partial shade-tolerant partial shade-tolerant
Cameroon	*Daniellia ogea* *Terminalia ivorensis*	Fabaceae[2] Combretaceae	partial shade-tolerant long-lived pioneer
Thailand	*Melia azedarach* *Toona ciliata*	Meliaceae Meliaceae	long-lived pioneer long-lived pioneer

[1] Functional groups are based on the definitions in Poorter et al. (2006)

[2] Non-nodulating (Diabate et al. 2004)

Tree-ring identification and sampling strategy

Growth rings were identified using a LINTAB 6 measuring table or using high-resolution scans (1600 dpi) and WinDendro software (Regent Instruments, Canada). Rings were identified for each tree in at least three different directions following standard dendrochronological approaches (Stokes and Smiley 1996). For each tree, we visually cross-dated (i.e. matched) the ring-width series from three different directions. Matching the ring-width series within the same tree allows the detection of locally absent (missing) or false rings.

Previous studies commonly collected wood samples for $\delta^{15}N$ analysis longitudinal (i.e. from pit to bark within the same tree). This could lead to the incorporation of ontogenetic shifts in tree-ring $\delta^{15}N$ values, because sampling parallels the size development of a tree. Such ontogenetic changes in $\delta^{15}N$ could hinder the detection of $\delta^{15}N$ changes caused by increased anthropogenic nitrogen depositions. We used a new sampling method that corrects for the potential ontogenetic effects on $\delta^{15}N$. In this method, 10-year bulk wood samples were collected at a fixed diameter (see illustrated in Fig. 4.3, top panel). The diameter used was 20 cm dbh and was chosen because trees with a diameter of 20 cm are relatively large (with crowns in the sub-canopy) and likely posses well developed root systems. Much larger diameters would require the sampling of very large trees to obtain information of the distant past (Fig. 4.3, top panel). We measured the ring formed when the tree reached 20 cm (the 'central' ring), as well as the four rings formed before and the five rings formed after the central ring (yielding a bulk sample of 10 growth years). As we collected trees ranging in size from 20 to >100 cm dbh, the rings formed around the 20 cm diameter differ in age. This allowed an analysis of tree-ring $\delta^{15}N$ values over time across trees in the same ontogenetic stage, thereby avoiding that observations of $\delta^{15}N$ change are caused by tree development rather than an affect of N-cycle change.

Stable nitrogen isotopes

The 10-year bulk samples of coarse wood were ground until a very fine powder was formed using a mixer mill (Retsch MM301, Germany). Soluble N compounds were extracted from the wood samples following Saurer et al. (2004a) and Hietz et al. (2010). This extraction procedure can improve the isotope signal (Elhani et al. 2003) and is commonly used in studies on tree-ring $\delta^{15}N$. Between 20-50 mg of ground wood was placed in 2 ml vials. We subsequently added a 1 ml of toluene/ethanol (1:1) for 4 h, followed by 1 mL of ethanol for 4 h, and finally deionised water for 1 h. The entire extraction was performed at 50°C. Between extractives and after rinsing with water, the samples were centrifuged at 10000g for 5 minutes, the supernatant discarded and the wood samples oven-dried at 60°C for 48 hours.

Wood $\delta^{15}N$ values were measured on 10±1 mg of each sample at the Department of Chemical Ecology and Ecosystem Research, University of Vienna, with an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) operating in continuous-flow mode and coupled through a ConFlo III interface (Finnigan MAT, Bremen, Germany) to a gas isotope ratio mass spectrometer (DeltaPLUS, Finnigan MAT). Tree-ring $\delta^{15}N$ values are expressed relative to standard material (a mixture of proline and sucrose). The standard deviation of the repeated measurement of standard material was 0.27‰.

Statistical analyses

We compared the average $\delta^{15}N$ over the period 1950-2000 between the two species per site with a t-test. Subsequently, the average $\delta^{15}N$ between sites (from 1950-2000) was compared with a one-way ANOVA and a Bonferroni Post Hoc test.

Temporal changes in $\delta^{15}N$ per study site were analysed with a linear mixed-effect model that included 'time' as a fixed factor and 'tree species' as a random factor. Two analyses were performed: one using the entire dataset and one without the most recently formed tree rings. For the latter, we excluded all samples with a central ring (of a 10-year bulk sample) before the year 2000. In this way, the last 6 to 15 years are excluded and thus the most active part of the sapwood in which nitrogen translocation might be strong. For both the analyses with and without recent sapwood, we studied trends in tree-ring $\delta^{15}N$ since the oldest sample collected (<1900). Because nitrogen cycles have likely changed more strongly in recent decades, we also performed analyses of $\delta^{15}N$ from 1950 onwards. In summary, we assessed the presence of temporal trends in tree-ring $\delta^{15}N$ values with four different tests: with and without recent sapwood and from the oldest $\delta^{15}N$ sample measured or from 1950 onwards.

To estimate the statistical power of the employed mixed-effect models we performed a power test. To this end, we simulated data based on the observed data and forced in different temporal $\delta^{15}N$ trends. For each species a simulated dataset was created using the de-trended variance in $\delta^{15}N$ in the observed data of that species. The mean $\delta^{15}N$ value of a species was taken as the values at time = 0. For example, for *Cariniana ianeirensis* the mean $\delta^{15}N$ over the period 1900-2000 is 2.13‰, the residual standard deviation is 0.55‰. We created data closely resembling the observed data by randomly adding (following a normal distribution with mean =0 and standard deviation=1) the residual standard deviation of 0.55 to the mean of 2.13. This was done for each x-axis value in the observed data, so that the simulated data had the same sample size and same spread on the x-axis. A temporal $\delta^{15}N$ trend was inserted by adding a linear increase to the simulated data in time. This was done for each species separately. The simulated datasets of the two species per site were subsequently combined. Per site, we tested 20 trends ranging from a total increase since 1950 from 0.1 to 2‰ or a total increase of 0.1 to 4‰ since 1900. For each trend, we generated 1000 datasets and for each of these datasets we tested if a mixed-effect model identical to the one used for the observed data detected a significant effect of 'time'. The number of cases for which 'time' was significant was divided by 1000 to obtain the estimated power of the model and data to detect a given long-term change (1900-2010 or 1950-2010) in $\delta^{15}N$.

All analyses were performed in R, version 2.12.2, (R foundation for Statistical Computing, Vienna, Austria), using the package nlme.

4.3 Results

Species and site differences in $\delta^{15}N$

We found no significant difference in tree-ring $\delta^{15}N$ values averaged over the period 1950-2000 between the two species from Bolivia or between the two species from Cameroon (Fig. 4.2). In Thailand, average $\delta^{15}N$ values were significantly higher in *T. ciliata* than in *M. azedarach* ($t=2.34$, $p=0.023$). Average $\delta^{15}N$ values were significantly different between sites (combining the two species per site over the period 1950-2000; $F=104.05$, $p<0.001$). $\delta^{15}N$ was higher in Cameroon compared to Bolivia ($p<0.001$) and Thailand ($p<0.001$) and average $\delta^{15}N$ values were lower in Thailand than in Bolivia ($p<0.001$; Fig. 4.2).

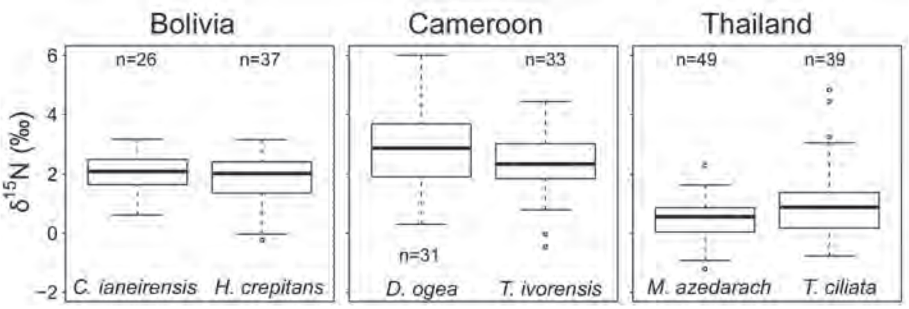


Figure 4.2 Average δ^{15}N values from 1950-2000 per species. Per site, average δ^{15}N values of the two species studied were similar, except for Thailand ($p=0.023$). When δ^{15}N values of the two species per site were averaged, δ^{15}N was higher in Cameroon compared to Bolivia ($p<0.001$) and Thailand ($p<0.001$) and average δ^{15}N values were lower in Thailand than in Bolivia ($p<0.001$).

Table 4.2 Linear mixed-effect model results on temporal changes of δ^{15}N values in trees from three sites. To assess the presence of temporal trends in tree-ring δ^{15}N values, the mixed-effect model analyses were performed on the dataset including all samples (**A**) and for the dataset excluding samples after the year 2000 (without recent sapwood samples; **B**). For each dataset, two time periods were analysed: the entire time range of data points (for all sites ≥100 years) and the period since 1950. In the mixed-effect models, 'time' was included as a fixed factor, 'tree species' as a random factor. Years refer to the 'central ring' of a 10-year bulk sample (e.g. for a sample from 2001 to 2010, this is 2005)

A (with recent sapwood)

	Period	Estimate (year)	SE	df	p-value
Bolivia	1875 - 2005	-0.0025	0.0021	111	0.2298
	1950 - 2005	-0.0014	0.0052	88	0.7859
Cameroon	1851 - 2005	0.0010	0.0027	172	0.6939
	1950 - 2005	0.0153	0.0112	72	0.1767
Thailand	1895 - 2005	0.0061	0.0052	109	0.2507
	1950 - 2005	0.0074	0.0073	105	0.3117

B (without recent sapwood)

	Period	Estimate (year)	SE	df	p-value
Bolivia	1875 - 2000	-0.0045	0.0026	83	0.0858
	1950 - 2000	-0.0092	0.0078	60	0.2452
Cameroon	1851 - 2000	0.0009	0.0030	161	0.7620
	1950 - 2000	0.0211	0.0155	61	0.1775
Thailand	1895 - 2000	0.0108	0.0062	89	0.0854
	1950 - 2000	0.0190	0.0099	85	0.0584

Temporal trends in $\delta^{15}N$

We analysed if trends in tree-ring $\delta^{15}N$ values were present over the time period considered. Per site, both species were analysed together in a linear mixed effect model (including 'species' as a random factor). For the 114 trees from Bolivia, we found no significant change of $\delta^{15}N$ over the period 1875 to 2005 (Fig. 4.3; Table 4.2a). Similarly no significant trend was found in the 175 trees from Cameroon from 1851 to 2005, or in the 112 trees from Thailand from 1895 to 2005 (Fig. 4.3; Table 4.2a). As nitrogen depositions are expected to have increased only recently in most tropical forests (Fig. 4.1), we also analysed temporal trends in tree-ring $\delta^{15}N$ values over the period 1950 to 2005 only. No significant trends were found over this period in any of the sites (Table 4.2a).

To exclude a potential confounding effect of nitrogen translocation in the sapwood, we excluded samples with a central ring after the year 2000 (thereby removing most sapwood samples) and re-ran all analyses. No significant trend in $\delta^{15}N$ was found at the study site in Bolivia and Cameroon (Table 4.2b). For the trees from Thailand, a marginally significant increase of $\delta^{15}N$ was found since 1950, amounting to a total increase of 0.95‰ ($p=0.058$; Table 4.2b). When samples with a central ring after 1990 were excluded, this increase was significant ($p=0.009$; results not shown).

Statistical power

To assess the probability that the lack of significant temporal trends in $\delta^{15}N$ values was caused by a limited sample size, we estimated the statistical power of the employed linear mixed-effect models. We tested both the power to detect changes over the period 1900-2005 and 1950-2005. An increase (or decrease) of 1‰ in $\delta^{15}N$ since 1950 or since 1900 would have been detected with a 85% and 95% probability respectively for trees from Bolivia, a 30% and 62% probability for trees from Cameroon and a 65% and 42% for the trees from Thailand (Fig. 4.4). If the temporal change of $\delta^{15}N$ would have amounted to a total increase (or decrease) of 2‰ since 1950 or 1900 the statistical power to detect this change would have been in the range of 80 to 100% at all sites (Fig. 4.4). For the datasets that exclude sapwood samples, the statistical power was comparable (Supplementary Fig. 4.1).

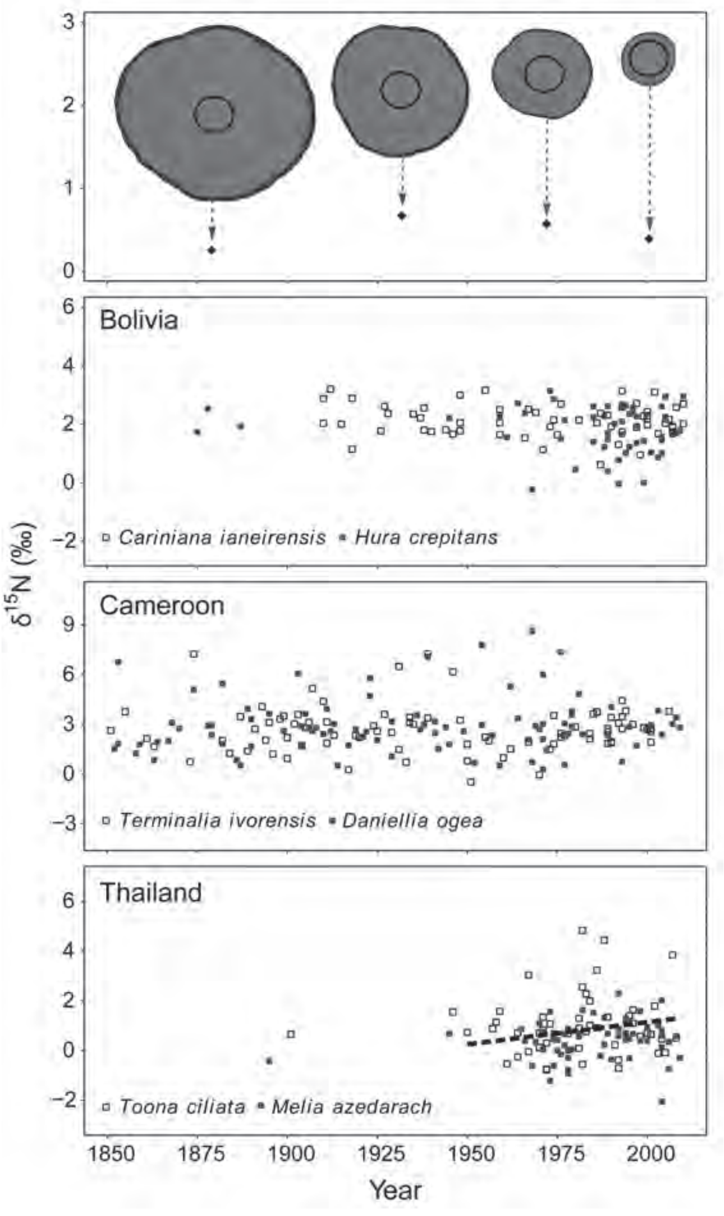


Figure 4.3 Tree-ring $\delta^{15}N$ values in time using the method illustrated in the top panel (10-year bulk wood samples around 20 cm dbh). Each point in the graph thus represents an individual tree. No significant change of tree-ring $\delta^{15}N$ values was found in Bolivia and Cameroon. In Thailand, a marginally significant increase was found since 1950 when sapwood samples were excluded (dashed black line; Table 4.2).

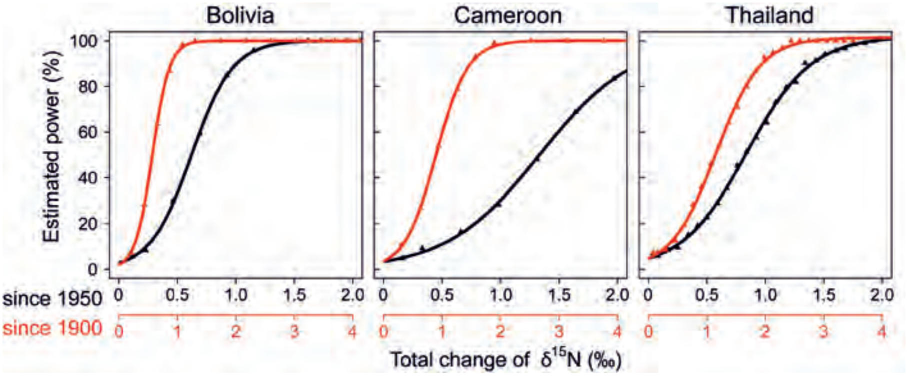


Figure 4.4 Statistical power of the linear mixed-effect models to detect long-term changes in $\delta^{15}N$. For each change in $\delta^{15}N$ (x-axis), 1000 datasets were simulated, based on the actual variance in the observed data. For each data set we tested if a mixed-effect model identical to the one used on the observed data detected a significant effect of 'time'. The number of cases for which 'time' was significant was divided by 1000 to obtain the estimated power of the model (y-axis). Two types of changes were simulated: an increase or decrease of $\delta^{15}N$ since 1950 (black line and black x-axis) and since 1900 (red line and red x-axis). Differences in statistical power are due to changes in variance and sample size (Fig. 4.2).

4.4 Discussion

A new sampling design: advantages and disadvantages

We used a new sampling methodology that compared tree-ring $\delta^{15}N$ values in time across similar sized trees (Fig. 4.3 top panel). One of the main advantages and the reason we chose to apply this method, is that it corrects for ontogenetic changes in $\delta^{15}N$. When $\delta^{15}N$ is measured within the same tree (e.g. in tree rings from pit to bark), such ontogenetic changes can potentially obscure or can be interpreted to reflect temporal changes in the nitrogen cycle. This is because during the time period studied, potential changes in the nitrogen cycle parallel the size development of the tree. Both could affect tree-ring $\delta^{15}N$ values and are therefore difficult to separate.

Although ontogenetic effects on tree-ring $\delta^{15}N$ values have not yet been studied, research over the last decades has pointed out several factors that could lead to shifts in tree-ring $\delta^{15}N$ values during tree development. We will briefly discuss three main factors. Firstly, one major control of both plant $\delta^{15}N$ values as well as of the $\delta^{15}N$ of N compounds in the soil are mycorrhizal fungi (Hobbie and Högberg 2012). In tropical rainforests, most trees have symbiotic relations with mycorrhizal fungi, which increase nutrient absorption in exchange of carbohydrates (Alexander and Högberg 1986). In general, mycorrhizal fungi retain ^{15}N-enriched N and transfer ^{15}N-depleted N to their plant hosts (Hobbie and Högberg 2012). If trees change in their dependence on mycorrhizal fungi for nitrogen uptake during development, this could affect tree-ring δ^{15}N ratios. But if such changes occur is unknown.

Secondly, plants absorb most nitrogen in the form of either ammonium (NH_4^+), or nitrate (NO_3^-), with in general a higher preference for ammonium (Högberg 1997). Ammonium and nitrate differ in δ^{15}N, with nitrate usually depleted in ^{15}N compared to ammonium (Mariotti et al. 1981, Nadelhoffer and Fry 1994, Robinson 2001). Thus any shift in the uptake of ammonium relative to nitrate during tree development will lead to a change in tree-ring δ^{15}N values. Thirdly, ontogenetic changes in δ^{15}N could be the result of increased rooting depth with size (or age). δ^{15}N in general increases with soil depth, with the highest δ^{15}N values at intermediate depth in less N-limited forests (Hobbie and Ouimette 2009). These profiles are the result of both ^{15}N depleted plant litter at the soil surface and the loss of ^{15}N depleted nitrogen during denitrification at intermediate depths (where anoxic conditions facilitate denitrification; Hobbie and Ouimette 2009). Increasing rooting depth could thus produce an increase of δ^{15}N within trees over time.

Potential ontogenetic effects on tree-ring δ^{15}N values should be taken into account when using tree-ring δ^{15}N to study effects of increased nitrogen depositions over time. Previous studies (Hietz et al. 2010, Hietz et al. 2011) have done so statistically (by including tree age or size as a factor), but this is not an ideal way to disentangle the potential effects of tree development and of nitrogen depositions on tree-ring δ^{15}N values. Reasons for this are that ontogenetic effects might be non-linear and because of possible collinearity between ontogenetic effects and changes due to N-cycle change. Although, the sampling design used in this study strongly controls for ontogeny, it has the main disadvantage that it requires many trees because each tree is sampled only once. As each sampled tree grew at a different location, this may cause a relatively large variance in δ^{15}N due to differences in soil properties and drainage characteristics between sample locations. Such a high variance further requires a large sample size for sufficient statistical power to detect long-term δ^{15}N changes. Our power test revealed that for the sample size used in this study (112 to 175 trees per site), estimated statistical power was well over 80% in most cases for changes >1‰ over the last 60-100 years (Fig. 4.4; Supplementary Fig. 4.1). However, for relatively small changes (<1‰ over the last 60 – 100 years), the power was relatively low (Fig. 4.4; Supplementary Fig. 4.1). Thus for small datasets and/or to detect small changes in $\delta^{15}N$ this methods is less suitable.

Evidence for changing nitrogen cycles

A marginally significant increase of $\delta^{15}N$ was found in trees from Thailand since 1950 when sapwood samples were excluded ($p=0.058$; Table 4.2). This result confirms previous observations of increase $\delta^{15}N$ in the same tree species at the same location (Hietz et al. 2011). Although the increase found in both studies is similar (around 1±0.5‰ since 1950), the associated p-value is much lower in this study. Possibly this is caused by a higher $\delta^{15}N$ variance due to the method used. Nonetheless, the results of this study and by Hietz et al. (2011) suggest that the nitrogen cycle is changing in the study area in Thailand, possibly turning into a more open and leaky system. Although NO_x emissions seem to have decreased since 1990, NH_3 emissions have continued to rise in the region (Fig. 4.1). A continuous high N input, might further change local N cycles. If nitrogen cycles are becoming more open en leaky, the soil is also slowly acidifying (Vitousek et al. 1997). How this will affect the forest at this location is uncertain, but it could negatively affect tree growth as a consequence of an increased nutrient leaching with higher soil acidity (Schulze 1989, Aber et al. 1998, Magill et al. 2004).

For the study site in Cameroon no long-term change in $\delta^{15}N$ values was observed, whether or not sapwood samples were excluded (Fig. 4.3; Table 4.2). This result is not consistent with the relatively high estimated NH_3 and NO_x emissions in the area (Fig. 4.1). In N-limited forests however, the cycling of nitrogen is generally very closed with minimal nitrogen losses (MacDonald et al. 2002). As such, increased anthropogenic nitrogen input will not directly lead to a ^{15}N enrichment of the soil N pool, because ^{15}N-depleted NO_3, that is susceptible for a downwards leaching, will be absorbed by the vegetation. There is very limited information on the nitrogen availability of the forest site in Cameroon, but N-poor sites with closed N cycles have been repeatedly differentiated from N-rich sites by more negative $\delta^{15}N$ values (Garten Jr 1993, Garten Jr and Van Miegroet 1994, Pardo et al. 2002, Koba et al. 2003). The relative high average $\delta^{15}N$ values in the trees from Cameroon compared to Bolivia and Thailand (Fig. 4.2), suggest the opposite case and that the nitrogen cycle at this location is not very closed. But if so, why has the high increase of N emissions since 1970 not affected N cycling and thereby tree-ring $\delta^{15}N$ values? Possibly, the high precipitation in the area (around 4000 mm yr^{-1}) causes a constant leaching of nitrate. Relatively high tree-ring $\delta^{15}N$ values are consistent with a N cycle where ^{15}N-poor nitrate is continuously lost. In that case, additional anthropogenic nitrogen input may not cause large changes in the $\delta^{15}N$ signature of soil nitrogen because the N cycle is already very open. In addition, the nitrogen input in this forest might be much lower than was assumed from the estimated NH_3 and NO_x emissions (Fig. 4.1). Obviously, emissions are not the same as depositions. Emitted NO_x and NH_3 can travel through the atmosphere for many kilometres before being rained out. The main components of the estimated regional nitrogen emissions are forest clearing and subsequent burning of biomass as well as agricultural land burning. Fires can cause large N emissions (Palacios-Orueta et al. 2005) and thereby boost estimates of average regional N emissions. However, the very low population density in a 100 km radius around the study site suggest that the occurrence of nitrogen emissions by fire are likely very localized and over a short period of time. Thus nitrogen deposition at the site where trees were sampled may be lower than expected from estimated regional NO_x and NH_3 emissions.

For the forest site in Bolivia, no temporal change of tree-ring $\delta^{15}N$ values was found (Fig. 4.3; Table 4.2), even though the statistical power was high to detect relatively small changes (Fig. 4.4; Supplementary Fig 4.1). Constant $\delta^{15}N$ values, however, confirms our expectation for this location, because anthropogenic nitrogen depositions are likely still very low in the area (Fig. 4.1).

Conclusions

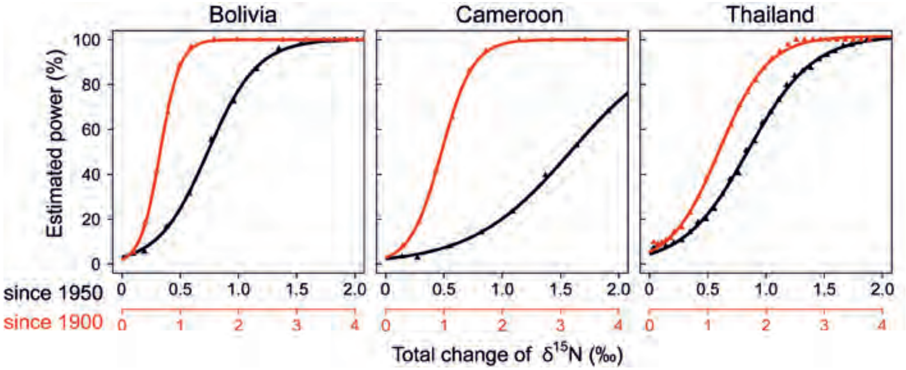
In this study we have assessed long-term changes in tree-ring $\delta^{15}N$ values using a strict control for ontogenetic effects as well as potential nitrogen translocation effects. Although our result confirms previous observations of increasing $\delta^{15}N$ over time for the study site in Thailand, we did not find evidence for a long-term $\delta^{15}N$ change in two other forest sites (Bolivia and Cameroon). As such, our findings are not consistent with the idea that nitrogen cycles in tropical forests are generally shifting to more open and leaky systems, but rather show that this will depend on local N-deposition rates and differences in the N saturation among forests.

Although the effect of anthropogenic nitrogen deposition may still be small in most tropical forests, N depositions are expected to increase dramatically in the near future in most tropical forests due to increased land-use intensification, forest fragmentation, biomass burning and fossil fuel emissions (Galloway 2004, Galloway et al. 2008). The forest site in Bolivia is particularly rich in calcium (Peña-Claros et al. 2012) and as such should have a relatively high capacity to buffer soil acidification that might occur under future nitrogen inputs. Most other tropical forests however, are hypothesized to be particularly sensitive to extra nitrogen inputs (Matson et al. 1999). Tree-ring $\delta^{15}N$ values are a very useful and relatively cheap tool to monitor the nitrogen status of tropical forests. It is probably the only technique that could provide a retrospective analysis of forest nitrogen status and as such allows the monitoring of nitrogen cycling in tropical forests.

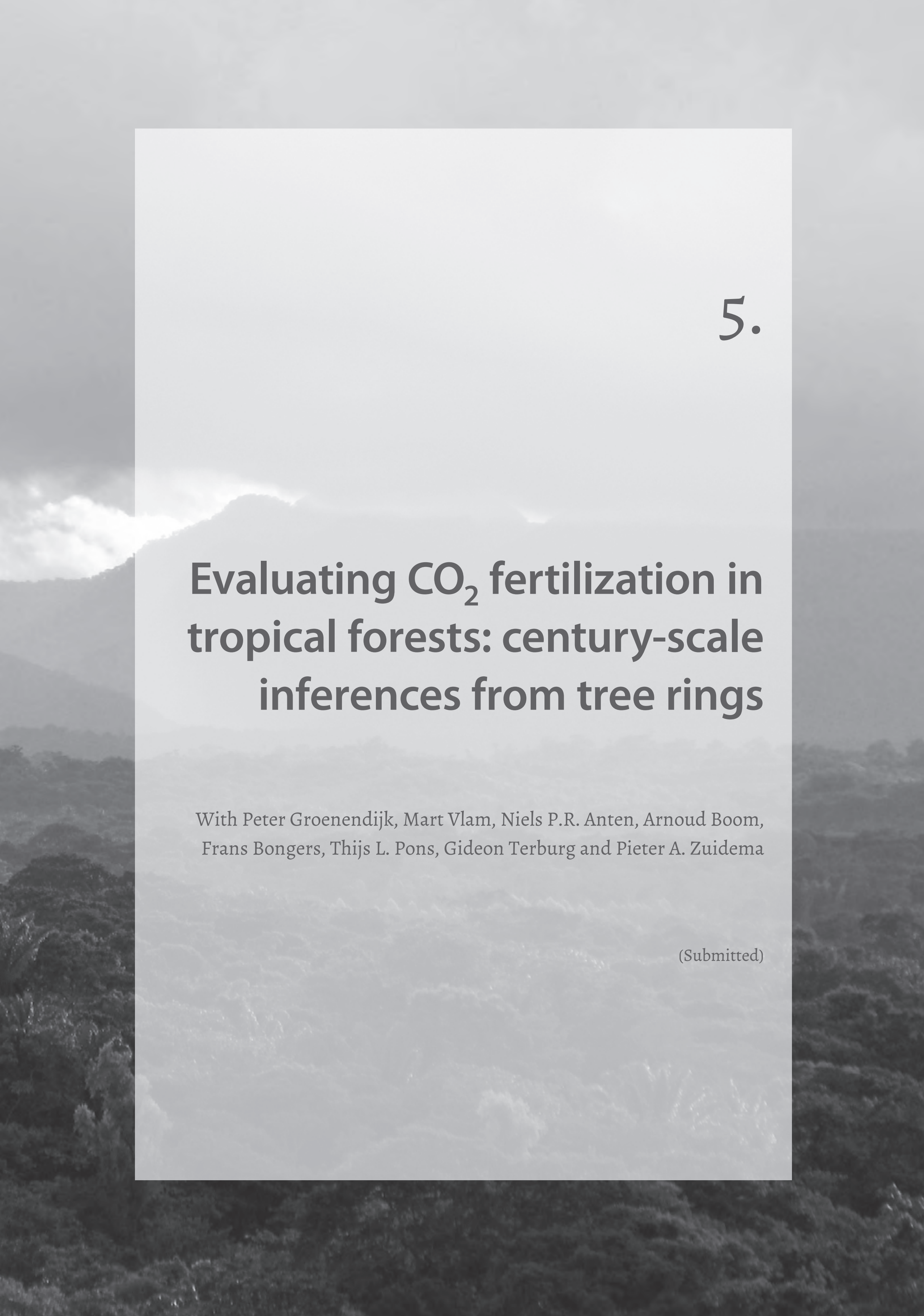
Acknowledgments

We would like to thank Margarete Watzka of the SILVER laboratory (Vienna) for her help with the $\delta^{15}N$ analyses and Niek van Rabenswaaij for his help with the preparation of wood samples.

Supplementary materials



Supplementary Figure 4.1 Statistical power of the linear mixed-effect models to detect long-term changes in δ^{15}N, excluding samples with a central ring before the year 2000 (sapwood samples). For each change in δ^{15}N, 1000 datasets were simulated, based on the actual variance in the observed data. Two types of changes were simulated: an increase or decrease of δ^{15}N since 1950 (black line and black x-axis) and since 1900 (red line and red x-axis).



5.

Evaluating CO_2 fertilization in tropical forests: century-scale inferences from tree rings

With Peter Groenendijk, Mart Vlam, Niels P.R. Anten, Arnoud Boom,
Frans Bongers, Thijs L. Pons, Gideon Terburg and Pieter A. Zuidema

(Submitted)

5.1 Abstract

Intact tropical forests have been reported to increase in biomass over time (Lewis et al. 2004, Lewis et al. 2009a), thereby absorbing a significant fraction of human-caused CO_2 emissions. It is commonly believed that the rise of atmospheric CO_2 is fertilizing tropical trees (Cox et al. 2013, Huntingford et al. 2013), allowing them to grow faster, but this hypothesis is controversial (Körner 2009). In theory, rising CO_2 levels increase plant photosynthetic rates (Gunderson and Wullschleger 1994) and enhance the ratio of photosynthesis to water loss (water-use efficiency; Holtum and Winter 2010), but evidence that these physiological responses indeed stimulate tropical tree growth is lacking. Here we tested the CO_2 fertilization hypothesis using an analysis of growth-ring data from 1100 trees at three sites distributed across the tropics. Our results show that water-use efficiency of trees consistently increased over the past 150 years. However, the hypothesized concurrent acceleration of tree growth was not found. This suggests that the widespread assumption of CO_2 fertilization of tropical trees may not be valid and could lead to serious biases in global vegetation models (Huntingford et al. 2013).

5.2 main text

Tropical forests store around 25% of the global terrestrial carbon pool and account for a third of net primary production (Bonan 2008) and are therefore a crucial component of the global carbon cycle. Repeated measurements of permanent forest plots across the tropics have shown increased tree growth over the past decades in many cases (Lewis et al. 2004, Lewis et al. 2009a), but decelerating or stable growth rates in others (Feeley et al. 2007, Clark et al. 2010). One of the main factors hypothesized to cause an increased tree growth is the rise of the atmospheric CO_2 concentration, as it can increase plant photosynthetic rates (Gunderson and Wullschleger 1994) and enhance water-use efficiency (Holtum and Winter 2010, Keenan et al. 2013). This latter response is of crucial importance in plant communities subjected to seasonal water shortage or drought periods, because it reduces water stress and extends the growing season. However, direct evidence for a CO_2 fertilization effect on growth rates of tropical trees is still missing because Free-Air CO_2 Enrichment (FACE) experiments are lacking in this biome (Norby and Zak 2011). In addition, the reported increase of tropical tree growth, that has been linked to CO_2 fertilization, was obtained on a much shorter time scale than the rise in CO_2 concentration, which started at the onset of the Industrial Revolution (Zuidema et al. 2013). Thus, it is unclear whether the rising atmospheric CO_2 concentration has resulted in a long-term stimulation of tropical tree growth.

We address this knowledge gap by analysing growth rings in the wood of long-lived tropical trees (Zuidema et al. 2013), thus obtaining a centennial record of tree growth. We simultaneously quantify the physiological responses to increased atmospheric CO_2 and changes in growth rates over the last 150 years. We analysed 1109 trees of 12 species from sites across three tropical continents (Fig. 5.1a). Our study species are of different functional groups (Supplementary table 5.1) and were locally common to very abundant. Long-term changes in gas exchange variables were obtained by measuring stable carbon isotopes ($\delta^{13}C$) in wood cellulose, from which the intercellular CO_2 concentration in leaves (C_i) and the intrinsic water-use Efficiency (iWUE) were derived (Supplementary Methods). This intrinsic WUE can represent actual water-use efficiency when the gradient in water-vapour pressure between the leaf and the atmosphere remains constant. We checked if this condition was met in our study sites over the last century and found this to be true (Supplementary Methods, Supplementary fig. 5.4). Long-term growth changes were assessed by converting tree-ring widths to the growth in the cross-sectional area of tree stems (Basal Area Increment; BAI), a good proxy for biomass growth (Baker et al. 2004). This is the first pan-tropical study to directly link physiological responses to CO_2 rise with long-term tree growth.

At each of the three sites, we sampled trees in large (144-297 ha) plots in undisturbed old-growth forest. Increment cores and tree disks were collected from trees of all sizes >5cm diameter at breast height (dbh). By including small trees and by sampling trees according to the size structure of our study species, we minimized the effect of potential sampling biases that may yield spurious positive growth trends (Brienen et al. 2012a). In our analyses of trends in growth and $\delta^{13}C$ over time, we accounted for confounding ontogenetic effects by employing the method illustrated in Figure 5.1b, which evaluates trends over time for trees of a fixed diameter. We chose two sizes for our analyses: understorey trees (dbh=8cm; ~10m tall) and canopy trees (dbh=27 cm; ~30m tall). These categories were chosen because understorey trees are expected to benefit more strongly from elevated CO_2 compared to canopy trees (Lloyd and Farquhar 2008), while canopy trees account for the bulk of forest biomass growth (Lewis et al. 2004). A total of ~100.000 rings were measured to determine tree ages and around 9000 rings were used to calculate BAI and to measure $\delta^{13}C$.

A mixed-effect model revealed a highly significant and exponential increase of C_i (Supplementary fig. 5.3) at each of the three sites, and in both understorey- and canopy trees. C_i increased by 43% and 53% over the last 150 years for understorey- and canopy trees respectively. Yet, the rate of increase in C_i was consistently lower than that of atmospheric CO_2. This 'active' response (McCarroll et al. 2009) to elevated atmospheric CO_2 resulted in a significant and large increase of iWUE. Over the last 150 years, iWUE increased by 30%-35% for understorey and canopy trees (Fig. 5.2). A highly significant increase was also found when relating iWUE to atmospheric CO_2 (Supplementary table 5.2). Evidence for an increase in tree-level iWUE was previously found in studies on some tropical tree species (Hietz et al. 2005, Nock et al. 2011, Silva and Anand 2013), but never for a set of species at a pan-tropical scale.

A long-term increase of iWUE indicates either a proportional increase of net photosynthesis and/or a decrease of stomatal conductance and thus transpiration, both of which can stimulate biomass growth if either carbon or water are limiting growth (Körner 2009). Such a stimulation of growth should become apparent as a long-term increase in tree Basal Area Increment (BAI). However, we found no overall change in BAI in a mixed-effect model analysis

(Fig. 5.2). This result was consistent across sites and for both understorey and canopy trees (Fig. 5.2; Supplementary table 5.2a). Thus, for the study species at our three sites, atmospheric CO_2 rise and associated increases in C_i and iWUE has not resulted in a detectable increase in diameter growth over the last 150 years. In order to account for the non-linear increase of atmospheric CO_2 over time, we also tested whether BAI changed with atmospheric CO_2. We found no evidence for growth stimulation (no significant effect of atmospheric CO_2, except for a negative relation for understorey trees in Thailand; Supplementary table 5.2b).

Our finding that BAI did not increase over time may have potentially been caused by the 'juvenile selection effect' (Rozendaal et al. 2010a), which implies that fast-growing small trees have a higher chance of reaching the canopy than slow-growers (Rozendaal et al. 2010a). In that case, growth of small trees realized in the distant past (i.e. in extant large trees; Fig. 5.1b) would, on average, be higher than that of small trees in the recent past (i.e. in extant small trees; Fig. 5.1b). This may lead to an apparent trend of decreasing tree growth and could thereby mask a CO_2-driven growth stimulation. Although this effect is relevant for our understorey trees (8 cm diameter), it unlikely plays a role for the canopy trees (27 cm diameter), as these have already reached the canopy and have thus passed the selection 'filter'. To ascertain that the juvenile selection effect did not mask a positive growth trend, we re-ran all analyses on the fastest growing 25% of the individuals (similar to a quantile regression). In agreement with the results obtained from the total dataset, these analyses provided no evidence for increased BAI at any of the sites, nor for all sites combined (Supplementary table 5.3).

A second potential reason for not finding an acceleration of BAI is low statistical power. We verified to what extent our dataset allowed detecting long-term growth trends, by means of a power test (Supplementary Methods). This test revealed that our analysis would detect a 1% growth change per decade with a probability of 68% for understorey trees and >99.9% for canopy trees (Supplementary fig. 5.5a). If tree growth would have increased by 2% per decade, as reported from permanent monitoring studies in the Amazon (Lewis et al. 2004), our analyses would have detected this change with >99.9% certainty for both size categories (Supplementary fig. 5.5a). The mixed-model for only the 25% fastest growers had an even higher power to detect changes (Supplementary fig. 5.5b). Thus, it is highly unlikely that our study species experienced a growth-stimulation over the last 150 years. These results contract with those obtained from permanent forest plots showing increased tree growth over time (Lewis et al. 2004, Lewis et al. 2009a). Differences in time period analysed (centennial vs. decadal), unit of analysis (species vs. stand), plot size (small vs. large) and spatial replication (few vs. many plots) do not allow a straightforward comparison of plot-based and ring-based studies and such a comparison was not our goal here. Nonetheless, our findings do clearly indicate that CO_2 rise did not stimulate tree growth of our study species on a centennial time scale.

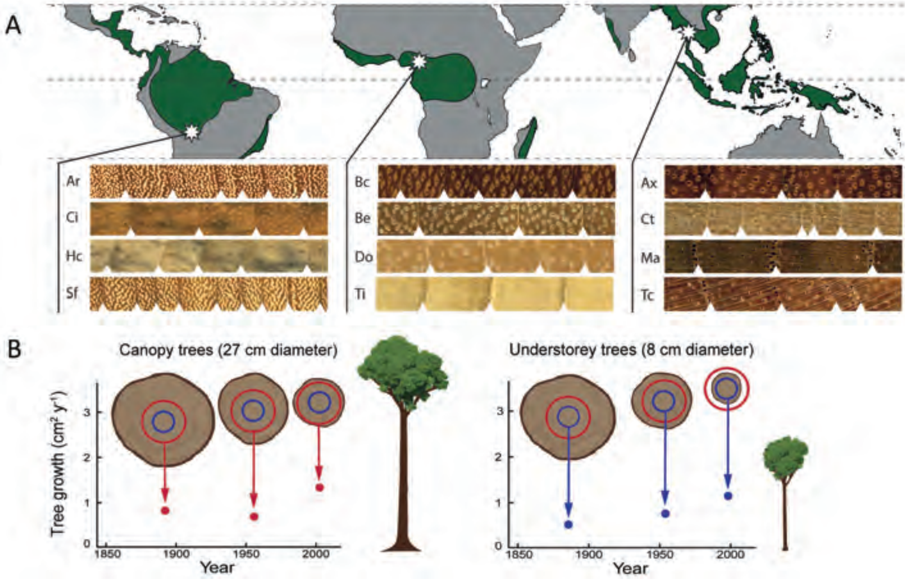


Figure 5.1 Study sites, annual growth rings of the study species and an illustration of the sampling design. **(a)** Wood samples were collected from three wet tropical forests (>1500mm rainfall per year; green areas). Bolivia: La Chonta forest concession; Cameroon: a forest concession adjacent to Korup National park and Thailand: Huai Kha Khaeng Wildlife Sanctuary. Pictures show wood samples of the study species, with white triangles indicating growth-ring boundaries. Species codes: Ar=*Ampelocera ruizii*, Ci=*Cariniana ianeirensis*, Hc=*Hura crepitans*, Sf=*Sweetia fruticosa*, Bc=*Brachystegia cynometroides*, Be=*Brachystegia eurycoma*, Do=*Daniellia ogea*, Ti=*Terminalia ivorensis*, Ax=*Afzelia xylocarpa*, Ct=*Chukrasia tabularis*, Ma=*Melia azedarach*, Tc=*Toona ciliata*. **(b)** Illustration of the sampling design. To avoid confounding effects of ontogenetic (i.e. developmental) trends in growth, we measured tree growth and intrinsic water-use Efficiency (iWUE) from five growth rings around two fixed diameters: understorey trees of 8 cm diameter (blue circles) and canopy trees of 27 cm diameter (red circles). We collected trees of different sizes, allowing the comparison of recently formed peripheral rings from young trees to more central rings in large trees, which were formed a long time ago. This methodology thus permits a comparison of tree growth and iWUE in time at a constant ontogenetic stage.

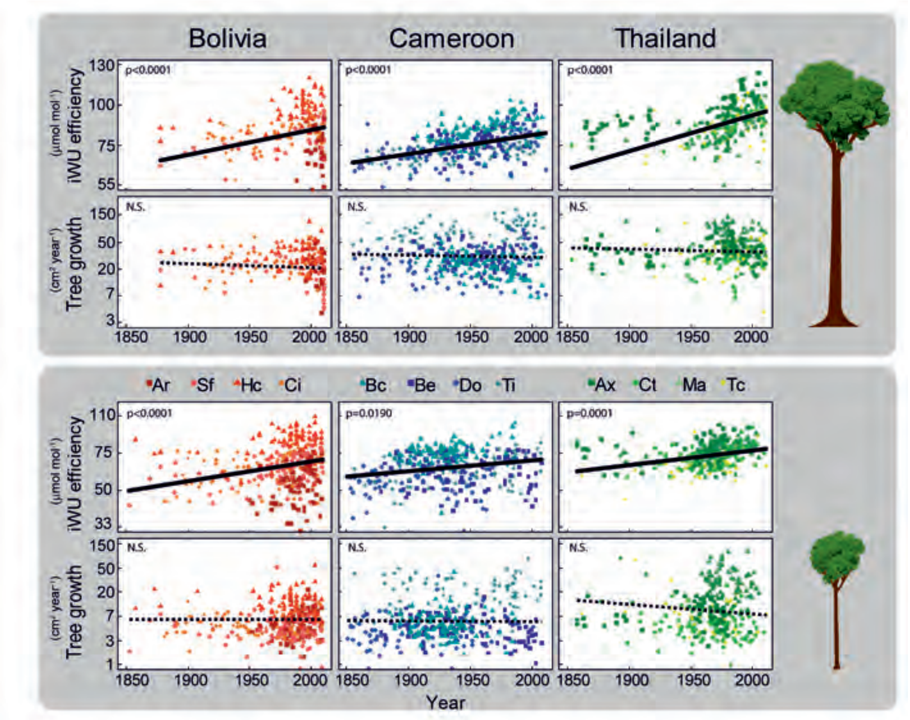


Figure 5.2 Long-term increase of intrinsic Water-Use Efficiency (iWU efficiency) in tropical trees did not result in enhanced tree growth (Basal Area Increment; BAI). Upper six panels present results for canopy trees (dbh=27cm); lower panels for understorey trees (dbh=8cm). Y-axes are on a log scale. Results of the linear mixed-effect models for ln-transformed values of 5-year average iWUE and BAI at two diameters: iWUE increased significantly for all sites combined (canopy trees t=5.74, p<0.0001; understorey trees t=4.96, p<0.0001) and for sites separately (p-values in panels, see Supplementary table 5.2). BAI did not change for all sites combined (canopy trees t=-0.90, p=0.367; understorey trees t=-0.57, p=0.571) nor for individual sites (Supplementary table 5.2). Black lines represent per-site trends from the linear mixed-effect model (dotted are non-significant trends); symbols represent individual trees; species codes in caption of Fig. 5.1.

Why has increased C_i and iWUE not resulted in increased growth rates? We discuss three possible explanations. First, a CO_2-induced stimulation of photosynthesis may not have resulted in increased growth due to an external climate-related stressor, such as increased temperature or decreased precipitation. There is no evidence for decreased precipitation at our study sites, but mean daily temperatures did increase by 0.1°C per decade since 1950 (Supplementary Methods). At the leaf level, however, temperatures may have increased more if evaporative cooling of the leaf decreased with elevated CO_2 levels (Cernusak et al. 2013). On the short-term, higher temperature increases respiration rates, but plants are able to acclimate relatively quickly to such changes (Atkin et al.

2005). It is not clear though, how the potential negative effects of higher (leaf) temperatures interact with the positive effects of atmospheric CO_2 rise over the longer term. A second explanation is that the additional assimilates generated due to CO_2 enrichment have not been invested in stem growth and could thus not be detected in tree rings (or in tree diameter measurements). Such allocation shifts might include increased fruit production (Wright and Calderón 2006) and investment in root biomass (Iversen 2010). The third and – in our view – most likely factor explaining the lack of tree growth stimulation is the so called CO_2-acclimation of photosynthesis (Gunderson and Wullschleger 1994, Rogers and Humphries 2000). Increased atmospheric CO_2 can increase photosynthesis, but the sink capacity of trees for these extra assimilates may be limited by nutrients (e.g. phosphates), thereby hampering a biomass growth stimulation and causing a down-regulation of the photosynthetic capacity. CO_2 acclimation has become apparent in FACE experiments in temperate forests, which have shown that elevated CO_2 does not lead to a sustained long-term increase of tree biomass growth under most field conditions (Körner 2009, Norby and Zak 2011, Bader et al. 2013). These observations are consistent with the results presented here. We anticipate that the planned FACE experiment in the Amazon will shed light on the strength of CO_2 acclimation in tropical trees in a mature forest (Tollefson 2013).

If the absence of a long-term growth stimulation in our study species was caused by CO_2-acclimation, then the observed strong increase in iWUE was mainly driven by decreased stomatal conductance over time and hence a reduced evaporative water loss. Subsequently, if our results are representative of the general long-term response of tropical trees to rising CO_2, then this may importantly change hydrological cycles. Decreased transpiration by plants can lead to lower humidity, higher air temperatures and a reduction of the recycling of precipitation (Betts et al. 2007). It is not clear to what extent hydrological cycles have already changed due to reduced transpiration because these alterations are occurring concomitantly with other changes such as deforestation (Piao et al. 2007).

As tropical forests are a crucial component of the global carbon cycle, it is important to predict their responses to atmospheric change. Such predictions are made using dynamic global vegetation models (DGVMs). Currently, most – if not all – DGVMs predict increases in tropical forest biomass as a result of CO_2 fertilization (Huntingford et al. 2013). These simulation results thereby suggest that tropical forests will have the capacity to act as CO_2 sinks for the coming century, thus reducing global warming. However, if a CO_2 fertilization effect on tree growth is absent – as our findings suggest – DGVMs overestimate tropical forest biomass growth and sink capacity (Huntingford et al. 2013).

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5.3 Supplementary materials

Materials and Methods

Study area

The study was carried out across three continents (Fig. 5.1a): Bolivia in South America, Thailand in Southeast Asia and Cameroon in Africa. In Bolivia, trees were collected in the logging concession ‘La Chonta’, around 300 km northeast of Santa Cruz de la Sierra (15.84 S, 62.85 W). The forest in La Chonta is a semi-deciduous moist forest and the transitional between Chiquitano dry forest and moist Amazonian forests (Peña-Claros et al. 2008). Annual precipitation in the region averages 1580 mm, with a 4 month dry season receiving <100 mm from May to September (supplementary fig. 5.1). In Thailand, trees were collected in the Huai Kha Khaeng Wildlife Sanctuary (HKK), Uthai Thani province, around 250 km northwest of Bangkok (15.60 N 99.20 E). The vegetation in HKK is a semi-deciduous moist forest (Bunyavejchewin et al. 2009). Mean annual rainfall averages 1473 mm, with a 4-6 months dry season from November to April (supplementary fig. 5.1). In Cameroon, field work took place in Forest Management Unit 11.001 of Transformation REEF Cameroon (TRC). This area is adjacent to the northwest border of Korup National park, in South-western Cameroon (5.23N, 9.10E). The forest consists of a semi-deciduous lowland rainforest of the Guineo-Congolian type. Annual precipitation in the region averages around 4000 mm, with a dry season from December to February (supplementary fig. 5.1). No evidence for long-term changes in precipitation have been found for the study sites (Nock et al. 2011, Asefi-Najafabady and Saatchi 2013, Seiler et al. 2013), but temperature at all sites has increased by 0.1°C per decade since 1950 (Grace et al. 1995, Nock et al. 2011, Seiler et al. 2013)

Study species and collection

At each site, we sampled trees of four species (supplementary table 5.1). Species where selected based on their abundance (we chose relatively common species) and the possession of clear annual growth rings. The annual nature of growth rings has been demonstrated for the Bolivian species by Lopez et al. (2012), for the species studied in Thailand by Baker et al. (2005) and Cameroon by Detienne et al. (1998) and by radio carbon (^{14}C) dating for the species on which no previous studies have been conducted (supplementary fig. 5.2). At each site, trees were collected in 144-297 ha of undisturbed forest, located in a larger area of undisturbed forest (Thailand) or a mixture of selectively logged and pristine forests (Cameroon and Bolivia). Thus, no strong edge effects of disturbances outside the study areas were expected. All trees larger than 5 cm diameter at breast height (dbh) were sampled in a 50 meter radius around a randomly assigned gps point. At each site, we used ~25 random points spread over the study area and collected around 100 trees per species (ranging in size from 5 to >100 cm dbh). This spatial sampling approach ensured that samples from all species were obtained from the entire study area. If the target of 100 samples per species was not reached within the circular plots, we sampled in the entire study area or chose an adjacent area (for two species in Cameroon). In Cameroon and Bolivia, a first round of selective logging took place in the study area at the time of sampling (no previous logging had taken place in any of the areas). At these sites, logging operations permitted the collection of stem discs for ~30% of the sampled trees. If no discs could be collected, 0.5-cm diameter cores were collected using an increment borer (Suunto, Finland and Haglöf, Sweden). Cores were taken in at least three different directions at breast height. After drying, the surface of discs and cores were either cut or polished depending on what gave the best visibility of ring boundaries.

Tree growth

Ring width was measured using a LINTAB6 measuring table and TSAPWin software (Rinntech, Germany) or using high-resolution scans (1600 dpi) and WinDendro software (Regent Instruments, Canada). Ring widths were measured for each tree in at least three different directions following standard dendrochronological approaches (Stokes and Smiley 1996). We checked the quality of the dating of rings in two ways. (1) For each tree, we visually cross-dated (i.e. matched) the ring-width series across the three directions. Matching the ring-width series in different directions allows the detection of locally absent (missing) or false rings in many cases. (2) If possible, we constructed an overall (master) chronology for the study species (Stokes and Smiley 1996) and subsequently checked whether annual variation in ring-width of individual trees matched with the chronology. A tree-ring chronology describes the common annual variation in ring width of a group of trees and are commonly used to verify the dating of tree-ring series (Stokes and Smiley 1996). We note that our study was not aimed at, or designed to, establish strong chronologies, because we included many small (juvenile) trees which often poorly cross-date (but have nonetheless annual rings; Soliz-

Gamboa et al. 2012), included a site with high precipitation and low seasonality (Cameroon) and did not select species based on their responsiveness to climate variation. In all, we were not able to establish a master chronology for 6 of the 12 species. In species for which no chronology could be established, we verified ring identification using marker years, e.g. relatively narrow rings formed during dry years (e.g. el Niño years). Tree ring widths were converted to growth in cross-sectional area of the tree (Basal Area Increment, BAI), as this gives a good estimate of biomass growth (Baker et al. 2004).

Because diameter growth of trees changes with tree size (and age), it is important to separate ontogenetic growth changes from potential growth changes over time. We applied the method proposed and implemented by Rozendaal et al. (2010a) and compared growth rates in the years around two fixed diameters: understorey trees (8 cm dbh) and canopy trees (27 cm dbh). Thus, to assess changes in growth over time for canopy trees, we selected the ring that was formed when a tree reached the target size of 27 cm dbh. We did this for all trees that were sufficiently large (>27cm dbh). We measured this 'central' ring as well as the two rings formed before and after the central ring, and averaged the widths of these five rings. In this way, the obtained estimate of tree growth was only minimally affected by year-to-year variance in growth. As we collected trees ranging in size from 5 to >100 cm dbh, the rings formed around the 27 cm diameter differ in age and allow comparisons of growth rates over long time spans for trees of the same size (Fig. 5.1b). The same procedure was executed for understorey trees, i.e. at a dbh of 8 cm. A total of ~100.000 rings were measured to derive the age of the rings around the 8 and 27 cm diameter sections in the 1109 sampled trees. Around 9000 rings were used to calculate BAI.

Stable carbon Isotopes

The analysis of trends in intrinsic water-use efficiency (iWUE) was done in a similar way as those for BAI, again using canopy and understorey trees. By using the same selected tree rings from which we obtained BAI values, we could directly compare physiological responses to elevated CO_2 levels (iWUE) with growth responses over the same period. We cut wood from the selected tree rings (around 8 and 27 cm dbh) and obtained 5-year bulk samples from which cellulose was extracted using a modification of the Jayme-Wise method (Wieloch et al. 2011). Crude cellulose samples were subsequently homogenized in a demi-water solution by a mixer mill (Retsch MM301, Germany) and oven-dried at 60 ^{0}C.

All cellulose samples were analysed in a continuous flow mode with an element analyser coupled to a mass spectrometer (Sercon Hydra 20-20) at Leicester Environmental Stable Isotope Laboratory, University of Leicester, United Kingdom. The carbon isotope composition ($\delta^{13}C$, in ‰) was then calculated as:

$$\delta^{13}C_{\text{tree-ring}} = (R_{\text{sample}} / R_{\text{standard}} - 1)*1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of a sample and R_{standard} the $^{13}C/^{12}C$ ratio of an internationally recognized standard material (V-PDB). Discrimination against the heavier ^{13}C ($\Delta^{13}C$) was calculated as:

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_{\text{tree-ring}}) / (1+ \delta^{13}C_{\text{tree-ring}}) \quad (2)$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ of atmospheric CO_2 (currently about -8.1‰). $\delta^{13}C_a$ decreased by 1.7‰ since the onset of the industrial revolution due to the burning of fossil fuels, which are depleted in ^{13}C. We used published data for $\delta^{13}C_a$ (McCarroll and Loader 2004, Keeling et al. 2010) to correct for this change. We then used Farquhar's model (Farquhar et al. 1982) for the discrimination of ^{13}C in plants to estimate CO_2 concentration in the intercellular spaces of the leaves (C_i) from the discrimination values determined in equation 2:

$$\Delta^{13}C \approx a + (b - a)\, C_i/C_a \quad (3)$$

where a (4.4‰) refers to the slower diffusion of $^{13}CO_2$ compared to $^{12}CO_2$ through the stomata, and b (27‰) to the discrimination by the CO_2 fixing enzyme Rubisco. C_a is the CO_2 concentration of the atmosphere and was obtained from direct measurements of atmospheric CO_2 concentrations (Dlugokencky and Tans 2013). Intrinsic water-use efficiency (iWUE), the rate of CO_2 assimilation (A) divided by the stomatal conductance for water vapour (gs; Ehleringer et al. 1993), was estimated from C_i, as:

$$\text{iWUE} = A/gs = (C_a - C_i)/1.6 \quad (4)$$

Note that $\delta^{13}C$ at the level of wood cellulose is not the same as at the level of primary assimilates because of downstream discrimination during transport and cellulose synthesis, which cause $\delta^{13}C$ values of cellulose in growth rings to be lower than the $\delta^{13}C$ of leaf sugars (about 1-2‰; Gleixner et al. 1993, Badeck et al. 2005). $\Delta^{13}C$ does thus not represent real C_i and iWUE values, but can nonetheless be used to determine relative changes in C_i and hence iWUE.

Vapour Pressure Deficit

iWUE can represent actual water-use efficiency (the ratio of assimilation to transpiration; Ehleringer et al. 1993) if the vapour pressure (VP) difference between the air and inside the leaves remains constant. This VP difference can increase as a result of global warming and/or decreased precipitation and lead to increased transpiration over time. To assess long-term changes of the VP difference between the air and inside the leaves, we checked if the vapour pressure deficit (VPD), the difference between actual VP and the saturated VP (VP_{sat}), has increased over the investigated period. We calculated VP_{sat} from maximum monthly air temperatures according to Allen et al. (1998):

$$VP_{sat} = 0.6108 \, \mathrm{Exp}\,(17.27\, T_{air}) / (T_{air} + 237.3) \qquad (5)$$

Maximum monthly air temperature (T_{air}) over the period 1901 to 2009 for each study site were obtained from the CRUTS3.0 gridded dataset (University of East Anglia Climate Research Unit 2009). We used gritted data as long-term climate data are not available for two of our sites. To estimate VPD, we subtracted VP_{sat} from the actual VP over the period 1901-2009 for each study site (also from the gridded CRUTS3.0 dataset). We assumed that the VPD is representative for the VP difference between leaf and air, which is valid when the difference between air and leaf temperature does not change and the increase in temperature over the experimental period is small. However, this is not necessarily true when transpiration changes as a result of decreasing transpiration with increasing CO_2 (Cernusak et al. 2013). We calculated the average VPD during the growth season (October to May in Bolivia; March to December in Cameroon and May to December in Thailand) and used a regression analysis to detect changes in VPD over time.

Statistical analyses

We analysed long-term changes in intrinsic water-use Efficiency (iWUE) and Basal Area Increment (BAI) with a linear mixed-effect (LME) model and included 'time' as a fixed factor and 'tree species' as a random factor in the analysis of trees per country and both 'tree species' and 'country' as random factors in the analysis of an overall pan-tropical trend (i.e. combining all trees measured). The natural logarithm was taken of C_i and iWUE to obtain linear relationships and for BAI to stabilize the variance. We tested two models: one with only random intercepts and one with both random intercepts and slopes. In all analyses, the model with both random intercepts and slopes was the most parsimonious, yielding the lowest Akaike's Information Criteria (AIC). We performed the analyses for the 8 and 27 cm diameter trees separately.

We estimated the statistical power of the linear mixed-effect models to detect long-term changes in BAI. For each species a simulated dataset was created by randomly applying its de-trended variance in BAI to a certain positive slope (growth trend). Species were subsequently re-combined to generate a total dataset of 1109 virtual trees. We tested 20 growth trends ranging from an increase of 0.1 to 2% per decade from 1900-2010. For each trend, we generated 1000 datasets and for each of these datasets we tested if an LME model identical to the one used for the observed data detected a significant effect of 'time'. The number of case for which 'time' was significant was divided by 1000 to obtain the estimated power of model and data to detect a given growth trend.

To exclude the possibility that the detection of a long-term change in growth rates was affected by a sampling error known as the 'juvenile selection effect' (Rozendaal et al. 2010a), we studied temporal changes in iWUE and BAI on the fastest growing 25% of the trees (canopy and understory trees separately). The juvenile selection effect can arise if fast-growing small trees have a higher chance to reach the canopy than slow-growing small trees. In that case, the large canopy trees of today were relatively fast growers as small trees. Comparing the growth of the 8 cm diameter in the centre of a large trees (Fig. 5.1b) will then on average be higher than the average growth of extant small trees (Fig. 5.1b), as these still include slow growing individuals that will never make it to the canopy. By comparing the fastest growing 25% of the trees over time, this bias can be avoided. We therefore selected the fastest growing 25% of the individuals per species and per decade. All species were combined and on this reduced dataset we performed a mixed-effect model to evaluate temporal trends in iWUE and BAI, using ‘time’ as a fixed factor and ‘tree species’ and ‘country’ as random factors. This approach is equivalent to a quantile regression for the 75th percentile.

All analyses were performed in R, version 2.12.2, (R foundation for Statistical Computing, Vienna, Austria), using the package NLME.

Supplementary table 5.1 The tree species studied

Country	Species	Family	Functional group[1]	Phenology[2]	Ring boundary[3]	Annual rings
Bolivia	*Ampelocera ruizii*	Ulmaceae	ST	Evergreen	Marginal parenchyma	Lopez et al. (2012)
	Cariniana ianeirensis	Lecythidaceae	PST	Deciduous	Compressed fibres	Lopez et al. (2012)
	Hura crepitans	Euphorbiaceae	PST	Deciduous	Compressed fibres	Lopez et al. (2012)
	Sweetia fruticosa	Fabacea	LLP	Brevi-deciduous	Marginal parenchyma	Brienen and Zuidema (2003)
Cameroon	*Brachystegia cynometroides*	Fabaceae	PST	Brevi-deciduous	Marginal parenchyma	^{14}C (supp. fig. 5.2)
	Brachystegia eurycoma	Fabaceae	PST	Brevi-deciduous	Marginal parenchyma	^{14}C (supp. fig. 5.2)
	Daniellia ogea	Fabaceae	PST	Brevi-deciduous	Marginal parenchyma	^{14}C (supp. fig. 5.2)
	Terminalia ivorensis	Combretaceae	LLP	Deciduous	Variation in wood density	Detienne et al. (1998)
Thailand	*Afzelia xylocarpa*	Fabaceae	LLP	Deciduous	Marginal parenchyma	Baker et al. (2005), Vlam et al. (2014)
	Chukrasia tabularis	Meliaceae	PST	Evergreen/ Brevi-deciduous	Marginal parenchyma	Baker et al. (2005), Vlam et al. (2014)
	Melia azedarach	Meliaceae	LLP	Decidious	Ring porous	Baker et al. (2005), Vlam et al. (2014)
	Toona ciliata	Meliaceae	LLP	Deciduous	Ring porous	Baker et al. (2005), Vlam et al. (2014)

[1] Functional groups are based on the definitions in Poorter et al. (2006): ST=shade-tolerant, PST=partial shade-tolerant, LLP=long-lived pioneer.
[2] Data on leaf phenology are from Mostacedo et al. (2003) for the Bolivian species; Williams et al. (2008) for the Thai species; Lemmens et al. (2012) and Poorter et al. (2004) for the Cameroonian species.
[3] Ring boundary categories after Worbes (1995), classification based on personal observations.

Supplementary table 5.2 Linear mixed-effect model results for canopy trees (27cm dbh) and understorey trees (8 cm dbh). We analysed long-term changes in intrinsic water-use efficiency (iWUE) and Basal Area Increment (BAI), using 'time' as a fixed factor (**A**) or 'atmospheric CO_2 concentration (C_a)' as a fixed factor (**B**). In both analyses, 'tree species' was inserted as a random factor when trees per country were studied or 'tree species' and 'country' were random factors in the study of an overall pan-tropical trend (i.e. combining all trees measured). The natural logarithm was taken of iWUE and BAI to obtain linear relationships and to stabilize the variance. We tested two models: one with only random intercepts and one with both random intercepts and slopes. In all analyses, the model with random intercepts and slopes was the most parsimonious, yielding the lowest Akaike's Information Criteria (AIC).

A

iWUE		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	0.7206	0.6125	798	1.1766	0.2397	1.0792	0.6424	979	1.6800	0.0933
	Time	0.0019	0.0003	798	5.7371	>0.0001	0.0016	0.0003	979	4.9554	>0.0001
Bolivia	*Intercept*	0.6624	0.6060	183	1.0931	0.2758	-0.0103	1.0492	339	-0.0098	0.9922
	Time	0.0019	0.0003	183	6.1569	>0.0001	0.0021	0.0005	339	4.2875	>0.0001
Cameroon	*Intercept*	1.5335	0.4383	357	3.4986	0.0005	1.7932	0.9638	359	1.8605	0.0636
	Time	0.0014	0.0002	357	5.9745	>0.0001	0.0012	0.0005	359	2.3570	0.0190
Thailand	*Intercept*	-0.8987	0.7945	256	-1.1312	0.2590	1.0886	0.8200	279	1.3276	0.1854
	Time	0.0027	0.0004	256	7.0548	>0.0001	0.0016	0.0004	279	4.0234	0.0001

BAI		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	5.7648	2.5289	798	2.2796	0.0229	3.7063	3.1653	979	1.1709	0.2419
	Time	-0.0012	0.0013	798	-0.9022	0.3672	-0.0009	0.0016	979	-0.5668	0.5710
Bolivia	*Intercept*	6.0552	2.5897	183	2.3382	0.0205	2.0601	1.9440	339	1.0597	0.2900
	Time	-0.0015	0.0013	183	-1.1209	0.2638	-0.0001	0.0010	339	-0.1468	0.8834
Cameroon	*Intercept*	5.0374	4.4369	357	1.1353	0.2570	2.1715	3.7071	359	0.5858	0.5584
	Time	-0.0008	0.0023	357	-0.3328	0.7395	-0.0002	0.0020	359	-0.0805	0.9359
Thailand	*Intercept*	5.6418	4.9566	256	1.1383	0.2561	10.0827	5.1494	279	1.9580	0.0512
	Time	-0.0010	0.0024	256	-0.4105	0.6818	-0.0040	0.0025	279	-1.6035	0.1100

B

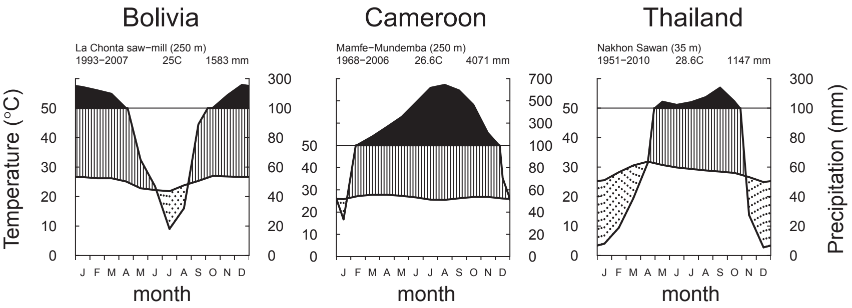
iWUE		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	3.6210	0.0719	798	50.368	>0.0001	3.4256	0.1238	979	27.667	>0.0001
	C_a	0.0023	0.0003	798	7.6472	>0.0001	0.0023	0.0004	979	6.6039	>0.0001
Bolivia	*Intercept*	3.7035	0.1318	183	28.107	>0.0001	3.2987	0.2374	339	13.893	>0.0001
	C_a	0.0019	0.0004	183	4.3797	>0.0001	0.0025	0.0005	339	5.2344	>0.0001
Cameroon	*Intercept*	3.6813	0.0647	357	56.936	>0.0001	3.3100	0.3554	359	9.3416	>0.0001
	C_a	0.0019	0.0002	357	8.6644	>0.0001	0.0026	0.0012	359	2.1102	0.0355
Thailand	*Intercept*	3.4731	0.1015	256	34.229	>0.0001	3.5006	0.1312	279	26.689	>0.0001
	C_a	0.0029	0.0003	256	10.641	>0.0001	0.0023	0.0003	279	6.7243	>0.0001

BAI		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	3.8792	0.5288	798	7.3363	>0.0001	2.3882	0.7963	979	2.9991	0.0028
	C_a	-0.0012	0.0016	798	-0.7507	0.4531	-0.0013	0.0021	979	-0.6178	0.5368
Bolivia	*Intercept*	3.7593	1.1113	183	3.3827	0.0009	1.3204	1.0549	339	1.2516	0.2116
	C_a	-0.0016	0.0035	183	-0.4662	0.6416	0.0012	0.0029	339	0.4263	0.6701
Cameroon	*Intercept*	3.3398	0.8435	357	3.9564	0.0001	2.0514	1.0466	359	1.9599	0.0508
	C_a	0.0005	0.0030	357	0.1645	0.8694	-0.0006	0.0037	359	-0.1683	0.8664
Thailand	*Intercept*	4.1651	0.9804	256	4.2485	>0.0001	3.9019	1.0055	279	3.8806	0.0001
	C_a	-0.0014	0.0024	256	-0.6174	0.5375	-0.0049	0.0021	279	-2.3135	0.0214

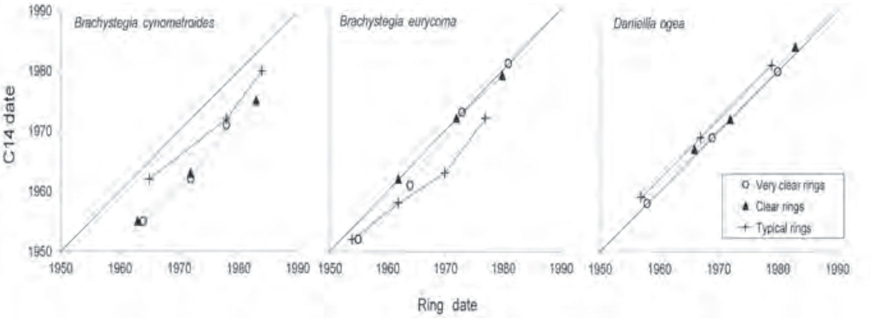
Supplementary table 5.3 Linear mixed-effect model results for the 25% fastest growing individuals of both canopy trees (27cm dbh) and understorey trees (8 cm dbh). Per size class (canopy and understorey) and per species, the fastest growing 25% of the individuals were selected per decade. The selected individuals per species were combined with the selected individuals of other species. This was done separately for canopy and understorey trees. On these two datasets, temporal trends in intrinsic water-use efficiency (iWUE) and Basal Area Increment (BAI), were analysed with a mixed-effect model, using 'time' as a fixed factor and 'tree species' as a random factor in the analysis of trees per country and both 'tree species' and 'continent' as random factors in the analysis of an overall pan-tropical trend (i.e. combining all trees measured). The natural logarithm was taken of iWUE and BAI to obtain linear relationships and to stabilize the variance.

iWUE		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	0.7822	0.5792	198	1.3506	0.1784	1.4337	1.2172	245	1.1779	0.2400
	Time	0.0018	0.0003	198	6.1095	>0.0001	0.0014	0.0006	245	2.2914	0.0228
Bolivia	*Intercept*	0.1864	1.1379	46	0.1638	0.8706	-1.2116	1.3761	85	-0.8804	0.3811
	Time	0.0021	0.0006	46	3.6401	0.0007	0.0028	0.0007	85	4.1075	0.0001
Cameroon	*Intercept*	1.3634	0.6951	88	1.9613	0.0530	3.2560	0.9917	92	3.2832	0.0015
	Time	0.0015	0.0004	88	4.1876	0.0001	0.0005	0.0005	92	0.9007	0.3701
Thailand	*Intercept*	-1.1572	1.0683	62	-1.0833	0.2829	-0.2471	1.4154	66	-0.1746	0.8619
	Time	0.0028	0.0005	62	5.4396	>0.0001	0.0023	0.0007	66	3.2811	0.0017

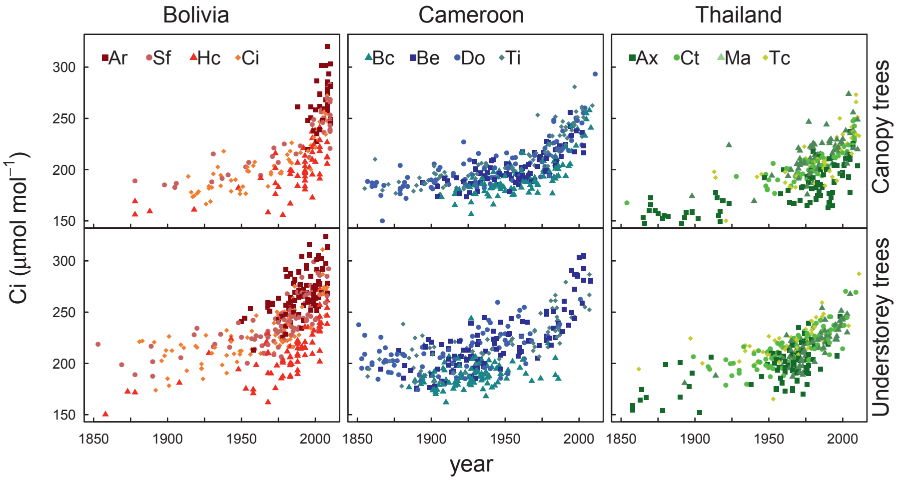
BAI		**Canopy trees**					**Understorey trees**				
		Estimate	**SE**	**df**	**t-value**	**p-value**	**Estimate**	**SE**	**df**	**t-value**	**p-value**
All sites	*Intercept*	2.6707	2.2428	198	1.2025	0.2306	0.8440	2.4392	245	0.3460	0.7296
	Time	0.0006	0.0012	198	0.5355	0.5929	0.0010	0.0012	245	0.7086	0.4792
Bolivia	*Intercept*	2.3058	6.3075	46	0.3656	0.7164	-3.7685	3.3273	85	-1.1326	0.2606
	Time	0.0007	0.0033	46	0.2094	0.8351	0.0031	0.0017	85	1.8175	0.0727
Cameroon	*Intercept*	1.4986	3.3206	88	0.4513	0.6529	3.7840	3.0027	92	1.2602	0.2108
	Time	0.0013	0.0018	88	0.7146	0.4767	-0.0007	0.0016	92	-0.4294	0.6687
Thailand	*Intercept*	4.3858	4.0261	62	1.0893	0.2802	1.6843	3.6302	66	0.4640	0.6442
	Time	-0.0001	0.0020	62	-0.0692	0.9450	0.0006	0.0018	66	0.3286	0.7435



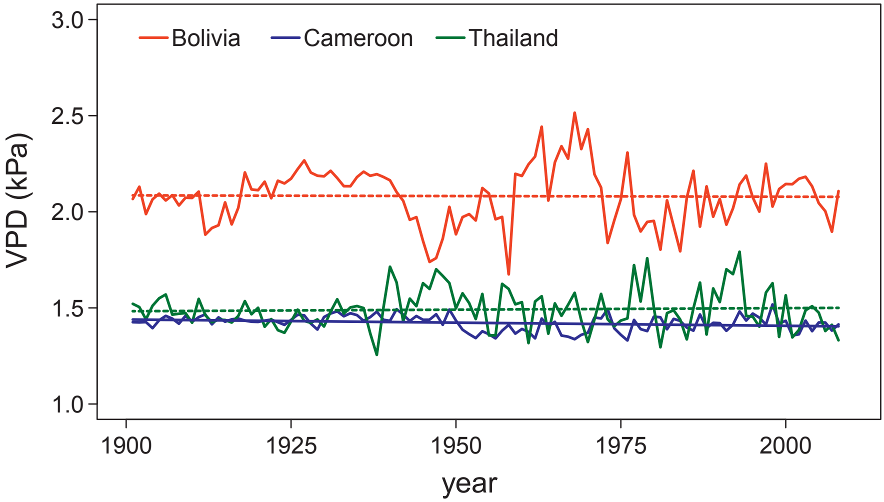
Supplementary figure 5.1 Climate diagrams for the study sites. For Bolivia, precipitation data are from the La Chonta saw-mill, located 30 km north of the study site. Temperature data are from Ascención de Guarayos (1987-2006), 60 km west of the study site. In Cameroon, monthly precipitation and temperature of two nearby stations were averaged: Mamfé Airport weather station (40 km north of study site) and Bulu meteorological station (40 km south of the study site). In Thailand precipitation and temperature data are for Nakhon Sawan, the closest station, 100 km east of the study site. The total annual precipitation at the Thai study site (HKK) is however higher than in Nakhon Sawan (around 1500 mm) and temperature lower (23.5 °C; Bunyavejchewin et al. 2009), but no long-term climate data are available for HKK. Dotted area indicates the dry season (precipitation <100 mm/month), black area the rainy season (>100 mm/month).



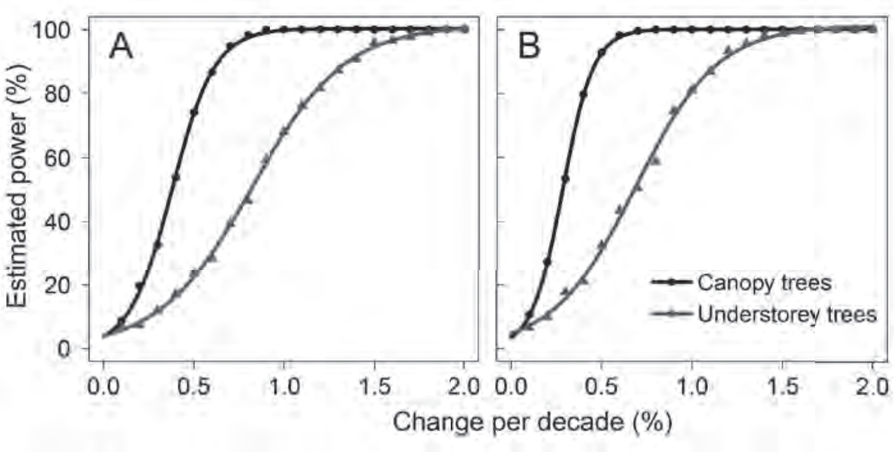
Supplementary figure 5.2 ^{14}C bomb-peak dating confirms annual ring formation in three species from Cameroon, but also points to dating errors in two species. We tested whether ring based age estimations matched well (within 1 year margin of error) with ^{14}C based age estimations. We used three different types of samples: tree discs with very clear rings (open circles); increment cores with clear rings (closed triangles) and cores with 'typical' rings (crosses), e.g. with suppressions or strong wedging rings. For the tested *Daniellia ogea* trees, this match was very good, showing the annual nature of ring formation as well as a good quality of dating based on rings. For *Brachystegia cynometroides* and some of the *Brachystegia eurycoma* samples, the ring based age estimation was lower than the results of the ^{14}C dating, indicating that rings were missed during measurements (points below the diagonal line). The maximum measurement errors were -7 years for *B. eurycoma* and -10 for *B. cynometroides*. Dividing the number of misidentified rings by the period under analysis provides a probability of ring misidentification (expressed in percentage, i.e. errors per 100 years). For *B. eurycoma*, this percentage of misidentified rings was -3.1%, i.e. there is a chance of missing a ring every ~32 years, and for *B. cynometroides* -12.3%. As the ring identification error in *B. cynometroides* was rather large, we checked to what extent the results of our mixed model analyses (Supplementary table 5.2) were altered when we removed this species. Mixed-effect models without *B. cynometroides* yielded qualitatively the same results, i.e. a significant increase in iWUE over time and no significant increase in tree growth over time.



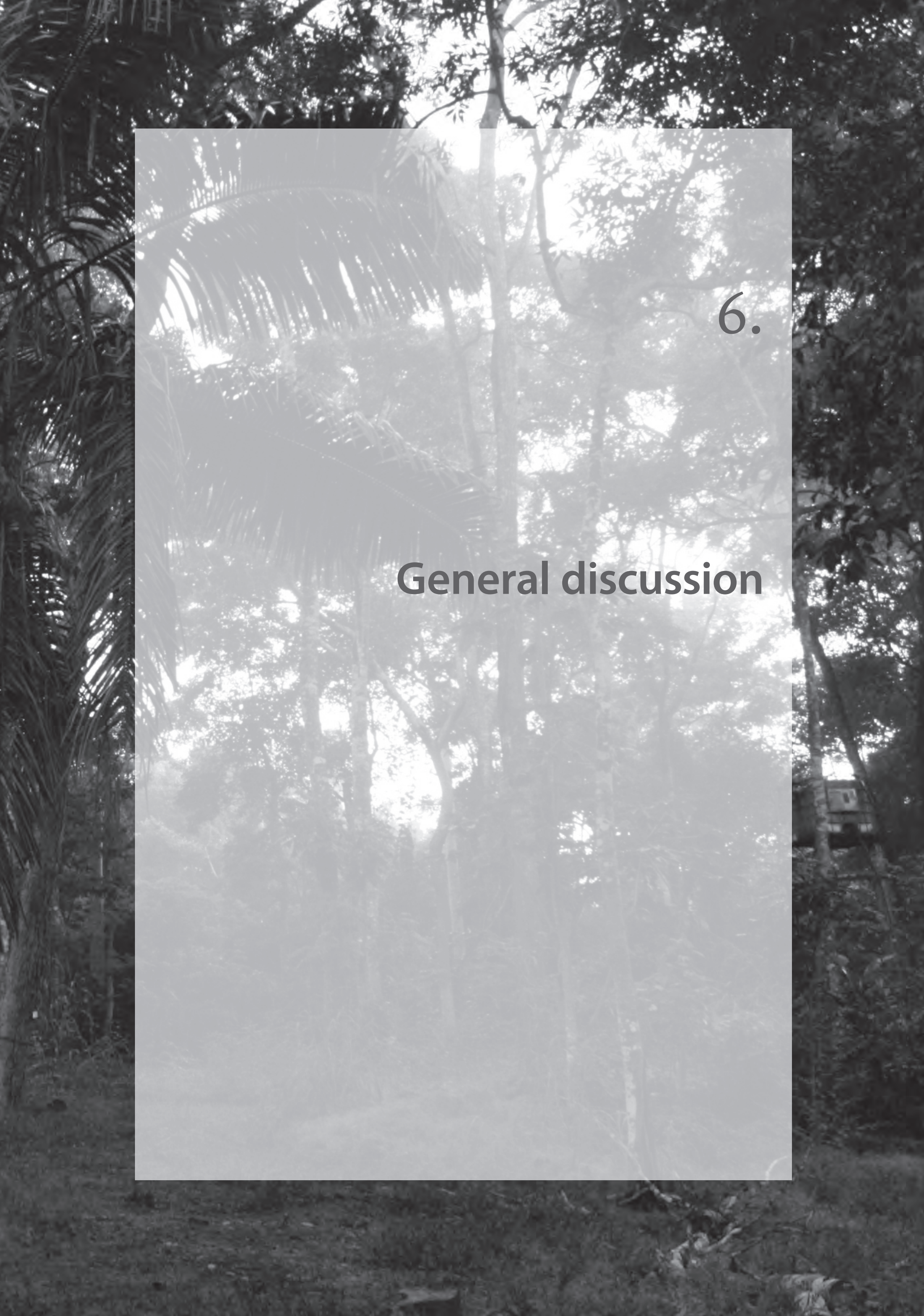
Supplementary figure 5.3 Intercellular CO_2 concentration in leaves (C_i) increased exponentially in all trees analysed. An estimate for C_i was derived from tree-ring cellulose $\delta^{13}C$ using equations 1-3 in methods section. We used linear mixed-effect models to analyse the temporal change in C_i (log-transformed C_i was used to obtain linear relationships) and found a highly significant increase across the three continents (for the canopy trees (27 cm dbh) in Bolivia: t=9.62, p<0.0001, Cameroon: t=13.68, p<0.0001, Thailand: t=9.58, p<0.0001 and for the understorey trees (8 cm dbh) in Bolivia: t=16.13, p<0.0001; Cameroon: t=6.67, p=0.0003, Thailand: t=6.92, p<0.0001).



Supplementary figure 5.4 Vapour pressure deficit (VPD) did not increase from 1901 to 2009 at the studied sites. Temporal trends in VPD were examined with a regression analyses. No trend in VPD was found in Bolivia and Thailand (dashed lines: p>0.05), but a significant negative trend was found in Cameroon (t=-2.769, p=0.007; solid line). We assumed that the VPD is representative for the VP difference between leaf and air, which is valid when the difference between air and leaf temperature does not change and the increase in temperature over the experimental period is small. These results indicate that the observed positive trends in iWUE (Fig. 5.2) represent positive trends in actual WUE, or underestimate such a trend for the Cameroon site.



Supplementary figure 5.5 The statistical power of the linear mixed-effect models to detect long-term changes in Basal Area Increment (BAI) when all trees are combined (A) and for the fastest growing 25% of the trees (B). We performed a power test with the goal to check the ability of the mixed-effect model to detect varying growth trends (0.1 to 2% per decade from 1900 to 2010). We used simulated datasets based on the actual sample size and same variance structure as our observed data (see Materials and Methods). Although, the dataset analysed in panel A (pertaining to mixed-effect models presented in Supplementary table 5.2) is much larger than in panel B (pertaining to mixed-effect models presented in Supplementary table 5.3), the power to detect growth trends in the fastest growing 25% of the trees is slightly higher due to a substantially lower variance, compared to the total dataset used in panel A.



6.

General discussion

Tropical forests are a major component of the global carbon cycle, harbour an incredible biodiversity and provide many ecosystem services (Myers 1992, Bonan 2008). Changes in forest structure and forest cover can therefore have significant consequences. Besides the ongoing destruction of tropical forests due to logging and the conversion to agricultural land, there is evidence that intact and undisturbed tropical forests are changing as well. Most notably there seems to have been an acceleration of tree growth and increased forest biomass over the last decades (e.g. Lewis et al. 2009a). The drivers of these changes are however still unknown.

In this thesis, we investigated three factors that may have caused a long-term alteration of tree growth: the rise of atmospheric CO_2, increased nitrogen depositions and changes in precipitation. We also studied the changes in growth that can occur on a short temporal scale due to forest dynamics. We measured tree-ring widths to obtain (long-term) information on tree growth and stable isotopes in the wood of tree rings to provide information on the environmental and physiological drivers of tree growth. This first part of this chapter gives a review of the use of stable isotopes as proxies for environmental changes (part 6.1), followed by a summary of the strengths and limitations of stable-isotope research (part 6.2). Finally, an overview is given of what stable-isotope research has taught us about environmental changes in tropical forests (part 6.3). The results obtained in this thesis are integrated with those from other studies.

6.1 Stable isotopes in tropical tree rings: a short review

Currently stable isotopes of three different elements have been measured on tropical trees, namely those of carbon, oxygen and nitrogen. These isotopes have been used to study a wide range of topics. In the field of environmental sciences, stable isotope research covers four main themes: (1) the study of environmental changes on a short temporal and small spatial scale (e.g. due to gap dynamics and logging), (2) the study of the effect of elevated atmospheric CO_2 levels on plant physiology, (3) the study of climate and (4) to assess the effects of increased anthropogenic nitrogen depositions.

Environmental changes due to gap dynamics

Logging or the death of a tree by natural causes, can results in considerable environmental changes in the surrounding area, e.g. in light, nutrient and water availability. This can strongly affect the growth rates of remaining trees. Stable isotopes in tree rings have been used to understand the environmental drivers of such growth changes as well as of tree mortality after logging (e.g. logging and stand thinning; Powers et al. 2008, Powers et al. 2009b, Powers et al. 2010). Of particular interest is $\delta^{13}C$, from which an estimate of the CO_2 concentration in the intercellular spaces within leaves (C_i) can be derived. C_i is an important parameter that has long been studied to assess tree physiological responses. The interpretation of C_i is however complex, because it is the outcome of both CO_2 supply through the stomata (gs) and the demand for CO_2 by photosynthesis (A). All environmental conditions that affect either A or gs influence C_i and, when persistent, will be reflected in $\delta^{13}C$ of leaf sugars. When $\delta^{13}C$ in the wood of an annual tree ring is analysed, the calculated C_i represents the average annual (or growth-season) C_i and can be seen as the sum of all environmental factors that have influenced photosynthesis and/or stomatal conductance during the year. This makes a simple interpretation of a change in $\delta^{13}C$ often difficult.

To determine if changes in C_i are predominantly caused by changes in A or in gs, previous studies have used $\delta^{18}O$ as a proxy for gs by assuming that changes of leaf $\delta^{18}O$ are due to changes in relative humidity (Scheidegger et al. 2000, Cullen et al. 2008, Powers et al. 2008, Powers et al. 2010). Because relative humidity strongly controls stomatal conductance, the most-likely behaviour of the stomata can be derived given a certain change of $\delta^{18}O$ values in plants. Although this method may work well on temperate grasses and on trees when the $\delta^{18}O$ signature of source water is known, it is difficult to apply to tropical trees because of (1) the strong influence of the $\delta^{18}O$ signature of absorbed water (e.g. precipitation) instead of relative humidity in mature trees (Brienen et al. 2012b) and (2) the lack of data on the $\delta^{18}O$ signature of precipitation in most areas in the tropics (IAEA/WMO 2006).

In CHAPTER 2 we analysed tree-ring $\delta^{13}C$ values to understand the variable growth response after gap formation of a common tree species in North-eastern Bolivia. To disentangle the different environmental factors that affect C_i on a short temporal scale, we assumed that variation in growth proportionally reflects variation in photosynthesis. This assumption has previously been applied to separate site conditions from climatic influences (Saurer et al. 1997). In years with wide growth rings, photosynthetic rates must have been relatively high to support this growth. High photosynthetic rates, in combination with a high C_i, can only occur when stomatal conductance was large, i.e. not water limited. Similarly, low photosynthetic rates and low C_i point to an effect of water stress. These simple interpretations are depicted in the conceptual model of Figure 6.1, in which the most likely behaviour of gs and A (given a certain change in growth and C_i) are used to reconstruct the underlying changes in environmental conditions (light/nutrient vs. water availability). We used this model to understand growth responses after gap formation by logging. Of particular interest was the lack of a growth stimulation in most juvenile trees. This is a surprising result because small trees are expected to benefit strongly from increased light availability after gap formation, given their shaded position in the forest understory. The lack of a growth increase in juvenile trees was hypothesized to be caused by an increased drought stress. However, the measurement of $\delta^{13}C$ showed that increased water stress is an unlikely cause for the absence of increased growth, but rather suggested that light conditions had not improved after the formation of a single tree-fall gap. Even though a proper testing of this model was beyond the scope of the study, we showed that combining growth rates with changes in C_i is a valuable tool to better understand the causes of temporal variation in tree growth.

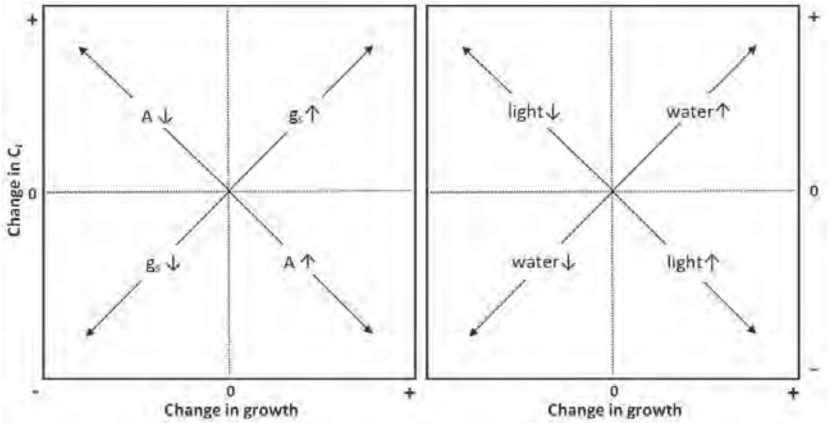


Figure 6.1 A conceptual model to deduce the environmental conditions underlying observed changes in the intercellular CO_2 concentration in leaves (C_i) and diameter growth rates. The left panel shows the most likely physiological causes for each combination of change in C_i and growth: a change in assimilation rate (A) or stomatal conductance (gs). The right panel shows the suggested changes in light/nutrients and water availability causing changes in physiology.

Atmospheric CO_2; a global rise of C_i and iWUE

An estimate of plant water-use efficiency (i.e. the amount of carbon gained through photosynthesis divided by the amount of water lost through transpiration) can be derived if one knows the mean C_i of the plant (intrinsic water-use efficiency, see Box 1 in General Introduction). Current research has mainly focussed on intrinsic water-use efficiency (iWUE) in the context of rising atmospheric CO_2 levels and to assess how this has affected the physiology of plants (*A* and *gs*). Studies on long-term iWUE values in trees from temperate and boreal forests have shown an increase of iWUE during the last century (Saurer et al. 2004b, e.g. Peñuelas et al. 2011, Silva and Anand 2013). For tropical trees, measurements of $\delta^{13}C$ also revealed a long-term improvement of iWUE (Table 6.1). In CHAPTER 5 we analysed more than 1100 trees from three sites across the tropics. In line with other studies, we found a significant increase of both C_i and iWUE over the last 150 years. This result was consistent across the three sites and in both small understorey trees and larger canopy trees. But what is the significance of a long-term increase of iWUE?

Perhaps the most valuable information that can be derived from iWUE is whether plants are actively or passively responding to the rise of atmospheric CO_2. If plants are completely passive, not changing stomatal conductance and/or photosynthetic rates, any increase of atmospheric CO_2 will lead to an equal absolute increase of C_i (McCarroll et al. 2009). In that case, iWUE will remain constant (Fig. 6.2). The increase of iWUE however indicates that plants are not passive, but actively responding to elevated atmospheric CO_2 (Fig. 6.2). This active response is either a reduction of stomatal conductance, an increase of photosynthetic rates or a combination of both. Changes in *A* and/or *gs* can have important consequences if they are occurring in trees on a global scale. Increased *A* could result in increased tree growth, whereas decreased *gs* could decrease evaporative water loss.

However, increased iWUE does not always mean that real water-use efficiency (the ratio assimilation to transpiration) improved. To understand why, it is important to realise that an increased *A* or decreased *gs* affects the flow of CO_2 and H_2O in and out of the leaf. Carbon molecules flow into the leaf due to a much lower partial pressure of CO_2 inside the leaf relative to ambient CO_2 levels. Water molecules, on the other hand, do the opposite, because of the near saturated water vapour pressure inside the leaves, and, in most cases, a much lower ambient vapour pressure. When stomata close, both the flow of water and carbon molecules are affected, but not equally (Marshall et al. 2007). C_i will drop due to an ongoing photosynthesis and as C_i drops the gradient between CO_2 concentrations in and outside the leaves increases, stimulating the diffusion of carbon molecules into the leaves and making the CO_2 flow more efficient. For water, the gradient (v) remains more stable, because the vapour pressure in the leaves will remain saturated independent of stomatal aperture. This will increase the ratio of carbon gain to water loss. Likewise, elevated photosynthetic rates will increase the difference between C_i and C_a, and thereby speed up the diffusion of CO_2, but it does not affect v and thereby does not affect the flow of water molecules. This again increases the ratio of carbon gain to water loss. However, these interpretations only work when v remained constant (i.e. when the atmospheric vapour pressure remained constant). On a longer time scale, the assumption of a constant v is unlikely correct, because the vapour gradient between leaf and atmosphere can be strongly affected by climate change (e.g. increased temperature and decreased precipitation). Thus, a temporal increase of iWUE will not represent a real increase of water-use efficiency (e.g. instantaneous WUE; see Box 1 in General introduction) in most cases. This should be taken into account when interpreting iWUE as a measure of real WUE. In CHAPTER 5 we checked the assumption of constant v by calculating the Vapour Pressure Deficit (VPD; a rough estimate for v) over the last 100 years based on ambient vapour pressure and temperature data. We found a near constant VPD at two of the study sites, suggesting that the increased iWUE has likely resulted in an increase of real WUE.

So, the apparent global rise of iWUE in trees during the last century suggests an active response, which entails changes in *gs* and/or *A*. These changes could have led to an improvement of actual water-use efficiency over time and an increased photosynthetic carbon gain.

Table 6.1 Overview of studies on long-term (>30 years) C_i and iWUE trends in tropical trees.

	Tree species	Country	Prec. (mm)	Period	C_i	iWUE	Reference
South America	*Cedrela odorata*	Brasil	3000	1850 – 1990	↑	↑	Hietz et al. (2005)
	Swietenia macrophylla	Brasil	3000	1850 – 1990	↑	↑	Hietz et al. (2005)
	Dipteryx micrantha	Peru	1740	1820 – 2006	↑	↑	Jenkins (2009)
	Mimosa acantholoba	Mexico	930	1968 – 2005	≈	↑	Brienen et al. (2011)
	Pseudolmedia laevis	Bolivia	1580	1900 – 2010	↑	↑	Nijmeijer (2012)
	Ampelocera ruizii	Bolivia	1580	1950 – 2010	↑	↑	This thesis
	Cariniana ianeirensis	Bolivia	1580	1880 – 2010	↑	↑	This thesis
	Hura crepitans	Bolivia	1580	1860 – 2010	↑	↑	This thesis
	Sweetia fruticosa	Bolivia	1580	1850 – 2010	↑	↑	This thesis
South-east Asia	*Chukrasia tabularis*	Thailand	1473	1910 – 2006	↑	↑	Nock et al. (2011)
	Chukrasia tabularis	Thailand	1473	1900 – 2010	↑	↑	This thesis
	Chukrasia tabularis	Bangladesh	2250	1920 – 2010	↑	↑	Rahman (2013)
	Melia azedarach	Thailand	1473	1910 – 2006	↑	↑	Hietz et al. (2005)
	Melia azedarach	Thailand	1473	1950 – 2010	↑	↑	This thesis
	Toona ciliata	Thailand	1473	1910 – 2006	↑	↑	Hietz et al. (2005)
	Toona ciliate	Thailand	1473	1900 – 2010	↑	↑	This thesis
	Afzelia xylocarpa	Thailand	1473	1850 – 2010	↑	↑	This thesis
	Eusideroxylon zwageri	Malaysia	>3000	1850 – 2009	↑	↑	Loader et al. (2011)
	Shorea johorensis	Malaysia	2873	1850 – 2009	↑	↑	Loader et al. (2011)
	Shorea superba	Malaysia	2873	1850 – 2009	↑	↑	Loader et al. (2011)
Africa	*Brachystegia cynometroides*	Cameroon	4000	1850 – 2010	↑	↑	This thesis
	Brachystegia eurycoma	Cameroon	4000	1850 – 2010	↑	↑	This thesis
	Daniellia ogea	Cameroon	4000	1850 – 2010	↑	↑	This thesis
	Terminalia ivorensis	Cameroon	4000	1850 – 2010	↑	↑	This thesis

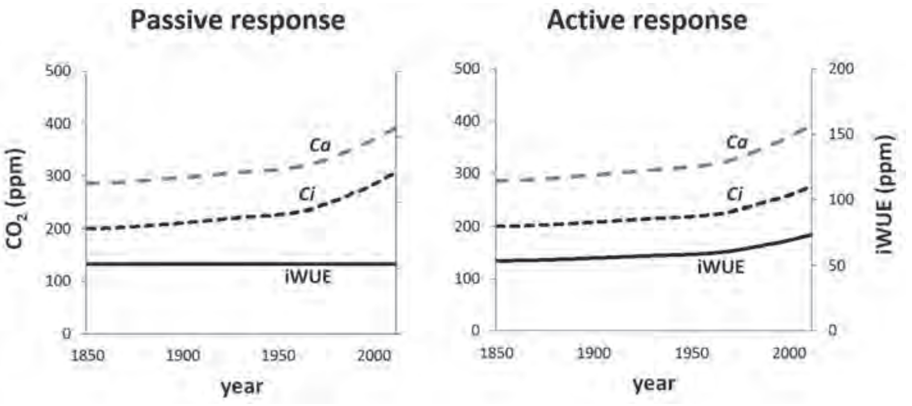


Figure 6.2 The active and passive response to rising atmospheric CO_2 (sensu McCarroll et al. 2009). If plants are totally passive, not changing either photosynthetic rates (*A*) or stomatal conductance (*gs*), any increase of atmospheric CO_2 (C_a) will result in an equal absolute increase of the CO_2 concentration in the intercellular space of leaves (C_i). In that case, no change of the intrinsic water-use efficiency (iWUE) will occur. If plants respond to increased C_a by decreasing stomatal conductance and/or by increasing photosynthesis, C_i will increase at a slower pace than C_a and iWUE will increase.

Climate reconstructions

The measurement of $\delta^{13}C$ values in tree-rings has also been used for climate reconstructions. Dry years, when water stress prevails and stomatal conductance is generally restricted, will lead to a lowered C_i and relatively high tree-ring $\delta^{13}C$ values. Conversely, wet years will result in relatively low $\delta^{13}C$ values in tree rings. In the tropics, tree-ring $\delta^{13}C$ values have been significantly correlated to rainfall in South Africa (Hall et al. 2009, Hobbie et al. 2009) and Ethiopia (Gebrekirstos et al. 2009). Although the inter-annual variability of climate can be studied with tree-ring $\delta^{13}C$ values, long-term trends in $\delta^{13}C$ are difficult to link to climate change, due to the above mentioned effects of rising atmospheric CO_2 levels on $\delta^{13}C$ values.

A more recent development is the study of stable oxygen isotopes in tree rings. Oxygen isotopes in the growth rings of tropical trees seem to have a high potential to record rainfall amount on a regional scale and will most likely become a crucial tool in dendroclimatology in the near future. The underlying mechanism is that the $\delta^{18}O$ ratio of newly formed wood is strongly determined by the isotopic signature of the water absorbed by the roots and to a lesser degree by the evaporation of water from the leaves (McCarroll and Loader 2004, Barbour 2007 and see information in General introduction). When trees are rooting superficially, the water absorbed is rainwater that recently fell. In the sub-tropics and tropics the $\delta^{18}O$ signature of rainwater is strongly determined by condensation processes in clouds (Dansgaard 1964). Water molecules with a heavy ^{18}O condense more easily, making the isotopic content of the remaining vapour increasingly depleted in ^{18}O. As clouds rain out and move inland, the remaining water vapour becomes increasingly lighter, a process that is more dominant in years with intense rainfall (Risi et al. 2008). This causes a close relation between precipitation amount and the isotopic composition of rain (Villacís et al. 2008, Kurita et al. 2009). Because of the capacity of trees to record the $\delta^{18}O$ signature of rain, tree-ring $\delta^{18}O$ can be used to study paleo-climatic variability on large (regional) scales. Tree-ring $\delta^{18}O$ has been correlated to basin-wide precipitation in the Amazon (Ballantyne et al. 2011, Brienen et al. 2012b), regional precipitation in Thailand (Poussart and Schrag 2005) and Indonesia (Schollaen et al. 2013b) and to El Niño Southern Oscillation (ENSO) variability in Laos (Xu et al. 2011). When the $\delta^{18}O$ of the absorbed soil water is known, tree-ring $\delta^{18}O$ can further be used to determine the evaporative enrichment in the leaves (Barbour 2007). Because this enrichment is strongly affected by the gradient of vapour pressure inside the leaves and the ambient vapour pressure, $\delta^{18}O$ has been used as a proxy for relative humidity (Cullen and Grierson 2007, Kahmen et al. 2011).

In CHAPTER 3 we explored the climate signals in the $\delta^{18}O$ values of five large *Entandrophragma utile* trees from Cameroon and found a significant negative correlation between tree-ring $\delta^{18}O$ and precipitation over large parts of West and Western central Africa (1930-2009). In addition, we found a negative correlation between tree-ring $\delta^{18}O$ and sea surface temperature (SST) in the Gulf of Guinea. These two results are related because rainfall variability in West and Central Africa is profoundly influenced by the SST of the tropical Atlantic Ocean (Chang et al. 1997). Thus a high SST in the Gulf of Guinea is associated with high precipitation over large parts of West and Central Africa (Balas et al. 2007) and recorded in tree rings by a relatively low $\delta^{18}O$ value. On the other hand, dry years, when SST is low are recorded by relatively high tree-ring $\delta^{18}O$ values.

In line with previous studies, we show that the analysis of tree-ring $\delta^{18}O$ in tropical tree species is a powerful tool for reconstructing the variability of precipitation on regional scales. However, not all tree species seem suitable for such usage. We have measured annual $\delta^{18}O$ series in six large *Toona ciliata* trees from Thailand and found that the inter-annual variation in $\delta^{18}O$ did not correlate between trees (no common signal; Fig. 6.3), whereas the ring-width series of the same trees significantly correlated with each other (from 1950-2010: average correlation between series=0.33, p=0.01) and fitted in the master chronology made for this species (Vlam et al. 2014). This indicates that the lack of a common $\delta^{18}O$ signal in the analysed *T. ciliata* trees was unlikely caused by dating errors. We also did not find any common signal in the $\delta^{18}O$ series of five large *Cariniana ianeirensis* trees from Bolivia (Fig. 6.3). Although we do not fully understand why some species do and others do not show common climatic signal in tree-ring $\delta^{18}O$ values, the most likely reason for this variance is the difference between species in rooting depth. Deep rooting species likely exploit deeper ground water, which may have a $\delta^{18}O$ signature that is formed over multiple years. Shallow rooting trees, on the other hand, absorb rainwater that recently fell and can thus record the $\delta^{18}O$ variability of rainwater. We therefore argue that shallow rooting tree species on well drained soils have the highest probability to record the $\delta^{18}O$ variability of precipitation and thus have the highest potential as tools for climate reconstructions.

Interestingly, a long-term increase of $\delta^{18}O$ values has been encountered in most studies conducted on tropical tree species. Brienen et al. (2012b) found an increase of 0.5‰ since 1850 in eight *Cedrela ordorata* trees from Northern Bolivia. We also found a significant increase in several *Cariniana ianeirensis* trees from Bolivia (about 600 km south of the study location of Brienen et al. 2012b; Fig. 6.3). In Southeast Asia, a strong increase of tree-ring $\delta^{18}O$ values since 1950 has been reported (Poussart and Schrag 2005, Xu et al. 2011). For *Toona ciliata* trees from Thailand we found increased $\delta^{18}O$ values in several trees since 1940-50, but no significant trends were found in others (Fig. 6.3). The average $\delta^{18}O$ values of five large *Entandrophragma utile* trees also showed an increase of 0.5‰ since 1850 (Fig. 6.3).

An explanation for these observations could be an effect of tree ontogeny (i.e. size development). An example of such an ontogenetic trend would be an increase of transpiration with tree size. Increased transpiration will cause a higher ^{18}O enrichment of leaf water and result in a higher tree-ring $\delta^{18}O$ value in larger trees compared to smaller trees. However, a similar increase of $\delta^{18}O$ values as recorded by trees from Bolivia has also been found in Andean ice cores (Thompson et al. 2006) and Andean lake sediments (Bird et al. 2011). Therefore, Brienen et al. (2012b) suggested that these trends are unlikely caused by the ontogeny of trees. Although the factors causing such a pan-tropical trend in tree-ring $\delta^{18}O$ values (or rainwater $\delta^{18}O$ values) remain unknown, this increase might be an effect of climate change, e.g. increased temperature and decreased precipitation.

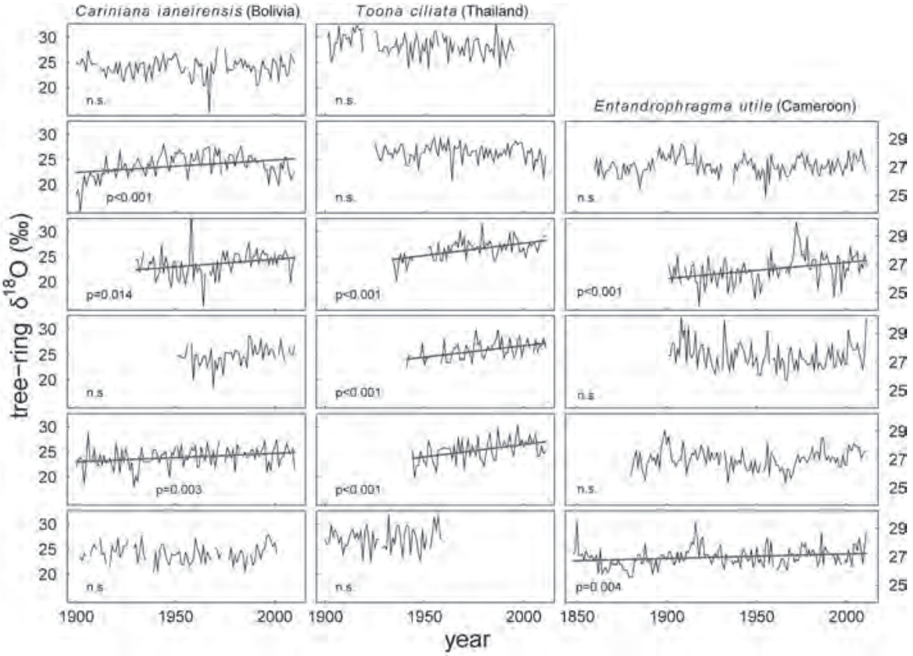


Figure 6.3 Tree-ring $\delta^{18}O$ series of six trees from Bolivia (*Cariniana ianeirensis*; unpublished results), six trees from Thailand (*Toona ciliata*; unpublished results) and five trees from Cameroon (*Entandrophragma utile*; CHAPTER 3). All significant long-term trends were positive (given p-values are of a linear regression), 'n.s.' indicates no significant trend was found. Axis for the $\delta^{18}O$ values of *Cariniana ianeirensis* and *Toona ciliata* on the left side, for *Entandrophragma utile* on the right side.

Anthropogenic nitrogen depositions

In temperate and boreal forests, nitrogen isotopes in tree rings have been studied on various tree species and have been shown to record the effect of elevated natural and experimental nitrogen input (Bukata and Kyser 2005, Elhani et al. 2005, McLauchlan et al. 2007, Guerrieri et al. 2011). In tropical forests, only two studies on tree-ring $\delta^{15}N$ values have been conducted, showing an increase of $\delta^{15}N$ during the last decennia in tree species from Brazil and Thailand as well as in leaf samples from Panama (Hietz et al. 2010, Hietz et al. 2011). This increased $\delta^{15}N$ has been interpreted as a signal that tropical nitrogen cycles have become more 'open' and 'leaky' during the last decades in response to increased anthropogenic nitrogen depositions (Hietz et al. 2011; Fig. 1.4 in general introduction for mechanism).

In CHAPTER 4 we analysed long-term $\delta^{15}N$ values of 400 trees from the study site in Bolivia, Cameroon and Thailand. For the trees from Bolivia and Cameroon, we found that $\delta^{15}N$ values have remained constant over the last decades. This indicates that anthropogenic nitrogen depositions are likely still small for these sites and have not yet affected N cycling. However, for the study site in Thailand, we found a marginally significant increase of tree-ring $\delta^{15}N$ values since 1950. This confirms the previously reported changes for the study site in Thailand (Hietz et al. 2011) and suggests a more open and leaky N cycle. But if this is the case, the soil is also slowly acidifying (Vitousek et al. 1997). How this will affect the forest at this location is uncertain, but it could negatively affect tree growth as a consequence of an increased nutrient leaching with higher soil acidity (Schulze 1989, Aber et al. 1998, Magill et al. 2004).

When studying the potential effects of increased nitrogen depositions, one confounding factor should be taken into account: the translocation of nitrogen across ring boundaries. Nitrogen in tree rings has some radial mobility, meaning that it is not permanently fixed when wood is formed (Nômmik 1966, Mead and Preston 1994, Colin-Belgrand et al. 1996, Schleppi et al. 1999, Elhani et al. 2003, Hart and Classen 2003). A simple solution for potential translocation effects would be to focus on the $\delta^{15}N$ of heartwood (Hobbie and Högberg 2012). Because the heartwood of trees is composed of dead cells, the translocation of nitrogen is unlikely. However, the analysis of $\delta^{15}N$ in heartwood has two disadvantages. Firstly, it is often very difficult to separate heartwood from living sapwood and secondly, sapwood is located on the outer and youngest part of the stem, which is also the period during which most of the effects of increased N deposition are believed to have happened (Hietz et al. 2011).

The measurement of $\delta^{15}N$ in the wood of tropical trees is still a new field and several uncertainties have to be overcome to allow a more accurate interpretation of tree-ring $\delta^{15}N$ values. However, the measurement of tree-ring $\delta^{15}N$ is probably the only technique that could provide a detailed retrospective analysis of forest nitrogen status and thereby allow the monitoring of nitrogen cycling in tropical forests. Such a tool is strongly needed given the hypothesised sensitivity of tropical forests for extra nitrogen input (Matson et al. 1999) and the predicted increase of anthropogenic nitrogen deposition in tropical forests in the near future (Holland et al. 1999, Lamarque et al. 2005, Dentener et al. 2006).

6.2 Strength and limitations of stable isotope analyses

The most important strength of the analysis of stable isotopes in tree-rings is the long temporal scale at which physiological responses and environmental change can be studied. This strongly contrasts with permanent monitoring plots (with typically less than 30 years of data) and experimental studies, e.g. Free Air CO_2 Enrichment (FACE) experiments (less than a decade). However, isotope research also has several limitations. The most prominent ones are: (1) many factors affect stable isotope values in tree-rings, of which some are not yet completely understood, (2) the occurrence of sampling biases, (3) the dependence on ring-forming tree species and (4) the relatively high labour and analytical costs. These limitations will be briefly discussed as well as the options how to overcome them.

As for the first, in this thesis several words have been repeatedly used: 'estimate', 'likely' and 'might', illustrating the difficulty of interpreting stable isotopes and to derive rigid conclusions from them. This is mostly because of the many factors affecting them. Among these factors is the change in stable isotope ratios that can occur during tree ontogeny (size development). In CHAPTER 4 and 5 we employed a method that corrects for ontogeny (Rozendaal et al. 2010a), but with the disadvantage that it requires the sampling of many trees for sufficient statistical power. However, even when ontogenetic effects are ruled out, a multitude of factors might be at the basis of stable isotope variability. The factors are often difficult to disentangle. Furthermore, some of these factors are not yet completely understood, such as the effect of nitrogen translocation on tree-ring $\delta^{15}N$ values.

In addition to this complexity, several potential sampling biases can arise in tree-ring research. In the diameter-section method employed in CHAPTER 5, a sampling bias known as the 'juvenile selection effect' might occur. This bias has been hypothesized to cause false growth trends and is based on the possibility that fast-growing small trees have a higher chance of reaching the canopy than slow-growers (Rozendaal et al. 2010a). In that case, growth of small trees realized in the distant past (i.e. in extant large trees) would, on average, be higher than that of small trees in the recent past (i.e. in extant small trees). This is because the population of extant small trees still contains many slow-growing individuals that will never make it to the canopy. The likely reason for this slow growth is that they recruited in a location with unfavourable conditions (e.g. in deep shade). This lowers the average growth rate of the pool of extant small trees compared to the extant large trees, which contains the successful, fast-growing, proportion of the pool of small trees in the distant past. Thus comparing juvenile growth of extant small trees with juvenile growth realised in the distant past by extant large trees, may lead to an apparent trend of decreasing tree growth. For the analysis of temporal trends in $\delta^{13}C$ the same bias might occur. For example, if extant juvenile trees are on average more light limited than the juvenile growth of extant large trees, they likely have higher $\delta^{13}C$ values. One simple way to overcome juvenile selection effects is to restrict the analysis to canopy trees (e.g. the 27 cm diameter section used in CHAPTER 5), because these have all passed the juvenile selection filter. If the focus is however also on juvenile trees, a possible solution to exclude juvenile selection effects is to compare only the fastest growing proportion of the sampled trees in time. In CHAPTER 5, we re-ran all analyses on the fastest growing 25% of the trees, similar to what is done with a quantile regression. While this analysis excludes a possible 'juvenile selection effect', it also excludes the use of 75% of the collected samples, which is a substantial waste of energy.

The application of tree-ring research is limited to tree species with clear annual growth rings. This could lead to a bias when ring-forming species respond fundamentally different to global change than none ring-forming species. The tree species studied in this thesis are from various plant families, as well as functional groups, which make this bias likely irrelevant. However it is important to note, that the tree species currently used in tree-ring research represent only a very tiny fraction of all tropical tree species present.

Finally, a more practical limitation of tree-ring research is that it is labour intensive. This restricts the number of trees that can be processed in a given amount of time. In addition, the costs of stable isotope analysis are relatively high. For a single wood sample, the measurement of $\delta^{13}C$ costs around €3-4 in most commercial laboratories and around €7-8 for either $\delta^{18}O$ or $\delta^{15}N$. This limits the applicability of isotope research to well-funded research groups, but even for these groups, a high number of samples will quickly add up to souring analytical costs. On the other hand, these costs are probably lower than the costs involved in the establishment of FACE experiments and the repeated measurement of a large number of forest monitoring plots.

6.3 Current debates and outlook

Environmental change in tropical forests

We found a consistent increase of C_i and iWUE in the examined tree species. Previous studies have also reported increased iWUE in tropical trees (Hietz et al. 2005, Nock et al. 2011, Silva and Anand 2013), but never at a pan-tropical scale. The results from tropical forest are in line with studies on temperate and boreal forest (Saurer et al. 2004b, e.g. Peñuelas et al. 2011, Silva and Anand 2013) and suggest that trees across the globe have increased C_i and iWUE.

Increased iWUE indicates elevated photosynthetic rates and/or reduced stomatal conductance. Atmospheric CO_2 increased by ~40% over the last 150 years and could have increased photosynthetic rates (Gunderson and Wullschleger 1994) and reduced stomatal conductance (Holtum and Winter 2010), but as pointed out above, many environmental conditions can affect C_i and thereby influence iWUE. These factors could enforce or partially compensate CO_2 effects. Two environmental conditions that are potentially changing in tropical forests and that can have a strong effect on C_i are climate change (e.g. temperature and precipitation amount) and altered nutrient cycles. In CHAPTER 4 we have shown that anthropogenic nitrogen depositions and the effects thereof on nitrogen cycles are likely still small in two of the areas studied in this thesis. However, for the study site in Thailand, a marginally significant increase of tree-ring $\delta^{15}N$ was found. This result confirms the reported increase of tree-ring $\delta^{15}N$ at this location (Hietz et al. 2011) and suggests that anthropogenic nitrogen deposition increased. A higher nitrogen input could have stimulated photosynthetic rates provided that nitrogen was a limiting factor.

Climate change entails an increase of temperature for the study area in Thailand and Bolivia (around 0.1°C per decade since 1950-60; Nock et al. 2011, Seiler et al. 2013). Total annual precipitation did not change over the last 60 years for the study site in Thailand (Nock et al. 2011), but possibly a slight drying trend occurred since 1980 at the study site in Bolivia (Seiler et al. 2013). For the study area in Cameroon, we found a significant increase of tree-ring $\delta^{18}O$ values over time, which was related to the occurrence of a drier period from 1970 to around mid-1990 (known as the Sahel drought; CHAPTER 3). Increased temperature and/or decreased precipitation can increase water stress, lowering *gs* and C_i. However, the overall rise of C_i (Table 6.1) suggests that even if a climate-induced lowering of C_i occurred, the CO_2 increase had a dominant effect on C_i.

Increased tree growth and forest biomass

Increased iWUE, caused by either a reduced stomatal conductance and/or increased photosynthetic rates, should lead to increased growth if either water or carbon is limiting tree growth. In CHAPTER 5 we assessed if increased C_i and iWUE has led to a stimulation of tree growth. We found this did not occur and that growth rates of the examined tree species have remained constant over the last 150 years. A similar combination of increased iWUE but no apparent increase of growth was found by Nock et al. (2011) and Peñuelas et al. (2011). On a shorter temporal scale, these findings are also in line with the results from Free Air CO_2 Enrichment (FACE) experiments in temperate forests, which have shown that elevated CO_2 does not always lead to a sustained long-term increase of tree growth under field conditions (Körner 2009, Norby and Zak 2011, Bader et al. 2013).

However, the absence of a growth stimulation contrasts reports of increasing tree growth and forest biomass in permanent forest plots studied over shorter periods of time (Phillips et al. 1998, Lewis et al. 2004, Chave et al. 2008, Phillips et al. 2008, Lewis et al. 2009b). While inventories of permanent forest plots are very powerful tools, there is an ongoing debate if changes in permanent forest plots give an accurate estimate of changes over large spatial scales (i.e. tropical forests in total). This is because forest biomass of a small patch of forest (e.g. 1 ha) is unlikely ever in a steady state (Fisher et al. 2008). Trees always grow and maintain their old biomass in the form of dead heartwood. Biomass will thus increase until some large scale disturbance, like a hurricane or a forest fire, causes a collapse of the forest community and resets the standing biomass. After such a disturbance, vegetation starts to develop again and biomass accumulates. As such, forest biomass development in a small forest patch can be seen as a continuous cycle of build-up and loss of biomass (Chambers et al. 2013). Because large scale disturbances are rare and have a low return frequency, inventory data based on small forest plots and short temporal scales likely overestimate net gains of forest biomass in the tropics, because the collapsing phase is consistently under sampled (Fisher et al. 2008, Körner 2009). A recent study in the central Amazon basin showed that forest biomass over long temporal and large spatial scales is likely to be in a steady state (Chambers et al. 2013).

In our study we did not measure forest biomass but focussed on tree growth. Increased tree growth and increased forest biomass are not per definition related. An increase of forest biomass could occur without changes in tree growth, e.g. due to changes in tree density. On the other hand does increased tree growth not have to result in an increase of forest biomass. An acceleration of tree growth might lead to a faster life cycle of trees and as such speed up forest dynamics. As long as recruitment and mortality rates change in conjunction with tree growth, an acceleration of tree growth will not lead to increased forest biomass. But, in addition to increased forest biomass, permanent forest plots have also provided evidence for increased tree growth (e.g. Lewis et al. 2004). However, for the estimated change of tree growth from permanent forest plots, the same potential bias as for forest biomass might be relevant. A recent study of ca. 600.000 trees from 403 tree species has shown that mass growth of most tree species continuously increases with tree size (Stephenson et al. 2014). Thus, if the building phase of forests is systematically over-represented in permanent forest plots, than this might lead to an overestimation of tree growth because the main forest stage contains predominantly trees that are increasing in size and growth rates.

Here we provide data that are clearly inconsistent with the generally assumed CO_2 driven acceleration of tree growth. This raises the question why elevated atmospheric CO_2 does not lead to a stimulation of tree growth, given that CO_2 can both stimulate photosynthesis and improve WUE. In CHAPTER 5 we discussed three of the most likely explanations: (1) an investment of growth in other plant parts than diameter (as measured by tree-rings), (2) compensation by a negative effect of increased temperature and (3) a CO_2 acclimation of photosynthesis caused by nutrient limitation (e.g. of phosphate).

The puzzling discrepancy between increased iWUE (and C_i) and constant growth rates is an important finding that could have major implications and that should be further investigated. Given the high labour and analytical costs, future tree-ring research could focus on the dominant tree species. In the Amazon basin, a recent study has shown that 1.4% of the tree species account for half of all tree individuals present (Ter Steege et al. 2013). This means that the response of the Amazon forest to global change will be dominated by relatively few species. The fourth most dominant tree species in the Amazon is *Pseudolmedia laevis*, with an estimated population of 4.3 billion individuals (Ter Steege et al. 2013). *Pseudolmedia laevis* forms annual growth rings that have been studied previously (Rozendaal et al. 2010a). There are likely many more dominant ring-forming species, whereas other dominant tree species with less distinct growth rings can be studied using a high resolution inter-annual sampling of stable isotopes (cf. Loader et al. 2011, Pons and Helle 2011, Schollaen et al. 2013a). A focus on dominant species will increase the power to extrapolate results to basin-wide responses.

Tree-ring research and permanent plot based research are currently two separate fields that both study tree growth, forest dynamics and the effect of global change. They obviously each have their own limitations and strengths, but the two approaches can certainly supplement each other. In permanent plots, tree-ring research can improve the understanding of the disturbance history of the plot (Vlam 2014) as well as the physiology of trees (based on stable isotopes). Combing tree-ring and permanent-plot research seems the most promising way to increase our understanding of the effects of human-induced environmental changes in tropical forests.

Changing hydrological cycles

Increased C_i, but no apparent stimulation of growth, suggests that the increased iWUE is mainly driven by a reduced stomatal conductance, with no, or a very low stimulation of photosynthesis. A lowered stomatal conductance leads to a reduced evaporative water loss. If plants across the tropics are decreasing their transpiration, this has important hydrological implications. A lowered transpiration will lead to a lower air humidity, higher temperatures and a reduction of the recycling of precipitation (Betts et al. 2007). In the long run, this may severely affect regions such as the western Amazon that strongly depend on precipitation generated by transpired water. It is not clear to what extent hydrological cycles have already changed due to reduced transpiration by plants because these alterations are occurring concomitantly with other changes such as deforestation (Piao et al. 2007).

The observed long-term increase of tree-ring $\delta^{18}O$ values in virtually all studies conducted thus far on tropical trees (Fig. 6.3 for trees analysed in this thesis) might reflect a reduced recycling of precipitation by vegetation and a lowering of air humidity. A reduced recycling of precipitation will lower precipitation amount on regional scales (Lettau et al. 1979, Eltahir and Bras 1994) and increase the $\delta^{18}O$ value of rain at more continental sites. Lowered air humidity will increase the enrichment of ^{18}O in leaves (Dongmann et al. 1974, Scheidegger et al. 2000). Both processes can potentially lead to increased $\delta^{18}O$ values in tree-rings, but no hard evidence supports this hypothesis yet.

A CO_2-driven climate change and a possible plant-driven climate change can be studied by measuring stable isotopes in tree rings. A spatial sampling of a tree species like *Entandrophragma utile* throughout central Africa and of *Cedrela odorata* throughout the Amazon basin could provide a detailed reconstruction of climatic variability over the last two centuries on a sub-continental scale. Both *E. utile* and *C. odorata* have a wide-spread distribution (in tropical Africa and the Amazon respectively) and are commercially logged. Collaboration with logging companies could facilitate the sampling of large trees. Climate reconstructions based on stable isotopes in tree rings could help to fill the huge lacuna of instrumental data that limits the understanding of climate change in most tropical regions.

Carbon sink vs. carbon stock

In this thesis several potential environmental changes in tropical forests were studied, as well as the effect of these changes on tree growth. Changes in tree growth could lead to changes in carbon fluxes. However, carbon fluxes should not be confused with carbon stocks. The direction of carbon fluxes in intact tropical forests is the subject of an ongoing debate, but that tropical forests store a massive amount of carbon is beyond dispute. The total carbon stock in tropical forests is estimated to be around 240 gigaton (Saatchi et al. 2011, Baccini et al. 2012), which is ca. 25% of the total terrestrial carbon pool (Bonan 2008). The ongoing degradation and destruction of tropical forests is causing massive releases of carbon into the atmosphere. Estimated net emission due to deforestation in the tropics is around 1 gigaton (i.e. 10^{12} kg) of carbon for each single year over the period 2000 - 2010 (Baccini et al. 2012, Harris et al. 2012). Compared to global annual emissions due to the burning of fossil fuels over the same period (around 8 gigaton/year; Boden et al. 2010), this is around 13%; a substantial amount. Clearly, the destruction of tropical forests will speed up atmospheric change and global warming, independent of the changes in intact tropical forests.

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Summary

Forests in the wet tropics harbour an incredible biodiversity, provide many ecosystem services and regulate climatic conditions on regional scales. Tropical forests are also a major component of the global carbon cycle, storing 25% of the total terrestrial carbon and accounting for a third of net primary production. This means that changes in forest structure and forest cover in the wet tropics will not only affect biodiversity and ecosystem services, but also have implications for the global carbon cycle and – as a result – may speed up or slow down global warming. Deforestation rates are still high in the tropics and have profoundly affected the extent of forests in many countries. Additionally, there are indications that undisturbed and pristine tropical forests are changing. The most notable changes found by the monitoring of permanent forest plots are an increase of tree growth and forest biomass per unit of surface area over the last decades. If this is indeed the case, it would entail that the world's tropical forests are potentially absorbing a significant fraction of human caused CO_2 emissions and as such are mitigating global warming. However, increased tree growth and forest biomass have not been found in all studies. Furthermore, it is unknown whether the observed changes in intact forests are part of a long-term change, or merely reflect decadal scale fluctuations. These uncertainties lead to an ongoing debate on whether tree growth and forest biomass have increased in tropical forests and – if so – to what extent. In addition, there is also a scientific discussion on the factor(s) that could underlie the potential changes in tree growth and forest biomass. Possibly, they are caused by an internal driver, like the lasting effect of large scale disturbances in the past, or by external drivers. Possible external factors affecting tropical forest dynamics are (1) climate change (temperature and precipitation), (2) increased nutrient depositions and (3) increased atmospheric CO_2 concentration.

In this thesis, I investigated the environmental changes that could have formed the basis for changes in tropical tree growth. I used two relatively new tools in tropical forest ecology: tree-ring measurements and stable isotope analyses. Tree-ring widths were measured to obtain long-term information on tree growth. Stable isotopes in the wood of tree rings were analysed to provide information on the environmental and physiological drivers of tree growth changes. This thesis is part of a larger project on the long-term changes in intact forests in the wet

tropics (the TroFoClim project, led by Pieter Zuidema) and also includes the PhD theses of Mart Vlam and Peter Groenendijk. In this project, ~1400 trees of 15 species were examined that were collected in three forest sites distributed across the tropics (in Bolivia, Cameroon and Thailand).

For the assessment of long-term changes in growth and stable isotopes, it is important to understand shorter term fluctuations due to forest dynamics (i.e. gap formation), because these interfere with changes on a longer temporal scale. The formation of a gap in a closed canopy forest, after the death of a tree, can cause considerable environmental changes in the surrounding area, e.g. in light, nutrient and water availability. This can strongly affect the growth rates of the remaining trees. However, in most studies the environmental drivers of changes in tree growth after gap formation are not considered. In CHAPTER 2 I measured carbon isotope discrimination ($\Delta^{13}C$) in annual growth rings of *Peltogyne* cf. *heterophylla*, from a moist forest in North-eastern Bolivia, and evaluated the environmental drivers of growth responses after gap formation. Growth and $\Delta^{13}C$ was compared between the seven years before and after gap formation. Forty-two trees of different sizes were studied, half of which grew close (<10m) to single tree-fall gaps; the other half grew more than 40 m away from gaps (control trees). I found that increased growth was mainly associated with decreased $\Delta^{13}C$ suggesting that this response was driven by increased light availability and not by improved water availability. Interestingly, most small trees did not show a growth stimulation after gap formation. This result was hypothesized to be caused by an increased drought stress. However, the measurement of $\Delta^{13}C$ showed that increased water stress is unlikely the cause for the absence of increased growth, but rather suggested that light conditions had not improved after gap formation. These results show that combining growth rates with changes in $\Delta^{13}C$ is a valuable tool to better understand the causes of temporal variation in tree growth.

An important potential driver of long-term changes in tree growth is climate change, e.g. global warming and altered annual precipitation. To understand the effect of climate change on tree growth, the availability of reliable data on historical climate is off course crucial. For the study areas in Bolivia and Thailand, previous studies have investigated the occurrence of temporal trends in temperature and precipitation. For the study area in Cameroon however, as well as for West and Central Africa in general, the availability of instrumental climate data is very restricted. This limits the possibility to relate past climatic variation to changes in tree growth and calls for proxies that allow reconstruction of past climatic conditions. In CHAPTER 3 I assessed the potential use of stable isotopes of oxygen ($\delta^{18}O$) in tree rings as a tool for the reconstruction of precipitation in tropical Africa. I measured $\delta^{18}O$ in tree rings of five large *Entandrophragma utile* trees from North-western Cameroon. A significant negative correlation was found between annual tree-ring $\delta^{18}O$ values (averaged over the five individuals) and annual precipitation amount during 1930-2009 in large areas of West and Central Africa. I also found tree-ring $\delta^{18}O$ to track sea surface temperatures (SST) in the Gulf of Guinea (1930-2009). These two results are related because rainfall variability in West and Central Africa is profoundly influenced by the SST of the tropical Atlantic Ocean. Thus a high SST in the Gulf of Guinea is associated with high precipitation over large parts of West and Central Africa and recorded in tree rings by a relatively low $\delta^{18}O$ value. On the other hand, dry years when SST is low, are recorded by relatively high tree-ring $\delta^{18}O$ values. I also found a significant long-term increase of tree-ring $\delta^{18}O$ values. This trend seems to be caused by lowered precipitation from 1970 to 1990 (the Sahel drought period). From 1860 to 1970, no significant long-term trend was observed in tree-ring $\delta^{18}O$ values, suggesting no substantial change in precipitation amount occurred over this period.

Another potential driver of altered tree growth and biomass in intact tropical forests is the increase of anthropogenic nutrient depositions, especially nitrogen. The deposition of nitrogen has likely risen due to an increased industrialization and use of artificial N fertilizers in most tropical countries. Nitrogen can stimulate plant growth, as is well known from the positive effect of N fertilizer application on crop yields. Previous studies have shown that the stable isotope ratio of nitrogen ($\delta^{15}N$) increased during the last decennia in the wood of trees from Brazil and Thailand as well as in tree leaves from Panama. This increased $\delta^{15}N$ has been interpreted as a signal that tropical nitrogen cycles have become more ‘open’ and ‘leaky’ during the last decades in response to increased anthropogenic nitrogen depositions. The underlying mechanism is that high rates of nitrogen deposition and high ambient nitrogen availability lead to an increased nitrification. This process can cause a gradual ^{15}N-enrichment of soil nitrogen. In CHAPTER 4 I analysed changes in tree-ring $\delta^{15}N$ values of 400 trees of six species from the three study sites. In the trees from Cameroon no long-term change of tree-ring

$\delta^{15}N$ values was found (1850-2005), even though NH_3 and NO_x emissions seem to have increased strongly around the study area since 1970. Possibly, the very high precipitation at that site causes the local nitrogen cycle to be already very 'leaky', limiting the effect of additional nitrogen input on the $\delta^{15}N$ signature of soil nitrogen. Alternatively, nitrogen input in this forest might be much lower than reconstructed NH_3 and NO_x emissions suggest. For the study site in Bolivia, no significant change of tree-ring $\delta^{15}N$ values was found (1875-2005), which is in line with the expected result for areas with a low anthropogenic nitrogen input. I found a marginally significant increased of $\delta^{15}N$ values since 1950 in trees from Thailand, which confirms previous observations. This points to an effect of increased anthropogenic nitrogen deposition, which could have stimulated photosynthetic rates, if indeed nitrogen was limiting tree growth.

The most often hypothesized factor to cause a long-term increase of tree growth is the rise of atmospheric CO_2. Since the onset of the industrial revolution (~1850) global atmospheric CO_2 concentration has increased by 40%. Elevated CO_2 can directly affect plants by increasing the activity, as well as the efficiency, of the CO_2 fixing enzyme rubisco and thereby increase photosynthetic rates. Potentially more important in plant communities subjected to periods of limited water availability (like a dry season) is that elevated CO_2 can cause a reduction of stomatal conductance, which lowers evapo-transpiration and hence reduces water losses. This increases water-use efficiency (i.e. the amount of carbon gained through photosynthesis divided by the amount of water loss through transpiration) and might allow plants to extend their growth season and/or increase their photosynthetic activity during the hottest hours of the day when water-stress might be severe. Elevated atmospheric CO_2 is thus a very likely candidate to have stimulated tropical tree growth (also referred to as CO_2 fertilization), provided at least that plant growth was either carbon or water limited. In CHAPTER 5 I tested the CO_2 fertilization hypothesis by analysing growth-ring data of 1100 trees from the three study sites. The measurement of tree-ring widths allowed an assessment of historical growth rates, whereas stable carbon isotopes ($\delta^{13}C$) in the wood of the trees were used to obtain an estimate of the CO_2 concentration in the intercellular spaces in leaves (C_i) and of water-use efficiency (intrinsic water-use efficiency; iWUE). I used a sampling method that controls for ontogenetic (i.e. size developmental) changes in growth and $\delta^{13}C$. With this method, trees were compared across a fixed diameter (i.e. same ontogenetic stage). I chose two diameters: 8 cm (referring to small understorey trees) and 27 cm (referring to larger canopy trees). A mixed-effect model revealed a highly significant and exponential increase of C_i at each of the three sites, and in both understorey and canopy trees. Over the last 150 years C_i increased by 43% and 53% for understorey and canopy trees respectively. Yet, the rate of increase in C_i was consistently lower than that of atmospheric CO_2. This 'active' response to elevated atmospheric CO_2 resulted in a significant and large increase of iWUE. Over the last 150 years, iWUE increased by 30% and 35% for understorey and canopy trees. A long-term increase of iWUE indicates either a proportional increase of net photosynthesis and/or a decrease of stomatal conductance and thus transpiration, both of which could have stimulated biomass growth. However, I found no increase of tree growth over the last 150 years in any of the sites. Although there are several possible explanations for these findings, I argue that it is most likely that tropical tree growth is generally not limited by water and carbon, but by a persistent nutrient limitation (e.g. of phosphates) and that this has prevented tropical trees to use the extra CO_2 for an acceleration of growth.

In this thesis I have studied environmental and physiological drivers of tree growth changes. I found evidence of decreased precipitation over the last decades at the study site in Cameroon (CHAPTER 3), a changed nitrogen cycle at the study site in Thailand (CHAPTER 4) and an overall change in the physiology of all tree species studied (increased iWUE; CHAPTER 5). One of the main findings of this thesis is however, that these changes have not led to a net change of tree growth over the last 150 years (CHAPTER 5). This is an important finding that could have two major implications. Firstly, the absence of a long-term growth stimulation suggests that the increase of iWUE is mainly driven by a reduced stomatal conductance, which likely leads to a reduced evaporative water loss. If trees across the tropics are reducing evapo-transpiration, this will change affect hydrological cycles, e.g. leading to a lower humidity, higher air temperatures and a reduced precipitation. Secondly, the absence of a growth stimulation over the last 150 years suggests that the carbon sink capacity of tropical forests is currently overestimated, e.g. by Dynamic Global Vegetation Models, which assume strong CO_2 fertilization effects and as such a high capacity of tropical forests to mitigate global warming. I anticipate that the planned Free Air Concentration Enrichment (FACE) experiments in the tropics will shed light on the reasons why increased CO_2 does not stimulate the growth rates of tropical trees. Furthermore, I argue that combining tree-ring measurements and stable isotope analyses together with permanent plot research is the most promising way to increase our understanding of the changes in tropical forests.

Samenvatting

Bossen in de natte tropen (>1500 mm neerslag/jaar) bevatten en enorme biodiversiteit en verzorgen vele ecosysteemdiensten waaronder de regulatie van lokale klimaatcondities. Tropische bossen zijn ook een belangrijk component van de mondiale koolstofcyclus omdat ze circa 25% van de totale terrestrische koolstof bevatten en omdat ze één derde van de totale netto primaire productie voor hun rekening nemen. Dit betekent dat veranderingen in de structuur en omvang van tropische bossen niet alleen effect hebben op biodiversiteit en ecosysteemdiensten, maar daarnaast ook implicaties hebben voor de mondiale koolstofcyclus en als gevolg daarvan voor de opwarming van de aarde. De grootste bedreiging voor het voortbestaan van tropische bossen in veel gebieden is de omzetting naar landbouwgrond. Het oppervlak aan tropisch bos dat hierdoor jaarlijks verdwijnt neemt nog steeds toe. Daarnaast zijn er ook aanwijzingen dat nog ongerepte tropische bossen aan het veranderen zijn. Die aanwijzingen komen uit bospercelen die periodiek worden gemeten. De meest opvallende verandering die in deze bospercelen wordt gevonden, is een toename van de groei van bomen en een toename van de biomassa van het bos gedurende de laatste 30 jaar. Als tropische bossen inderdaad toenemen in biomassa per oppervlakte eenheid, dan zou dit betekenen dat ze een behoorlijke fractie opnemen van de jaarlijkse CO_2 uitstoot door de verbranding van fossiele brandstoffen. In dat geval remmen ze mogelijk de opwarming van de aarde.

De toename in biomassa en boomgroei wordt echter niet waargenomen in alle bospercelen. Ook is het nog onduidelijk of de veranderingen in tropische bossen horen bij een lange termijn verandering of toch voornamelijk fluctuaties zijn die op een tijdschaal van tientallen jaren plaatsvinden. Deze onduidelijkheden zorgen voor een voortdurende wetenschappelijke discussie over de vraag òf intacte tropische bossen daadwerkelijk aan het veranderen zijn. Daarnaast is er veel discussie gaande over de factoren die veranderingen in tropische bossen kunnen veroorzaken. Dat kunnen interne factoren zijn, bijvoorbeeld grootschalige verstoringen die in een ver verleden plaatsvonden, of externe factoren. Drie mogelijke externe factoren zijn: (1) klimaatverandering (bv. toegenomen temperatuur en veranderde neerslag), (2) toegenomen nutriënt deposities en (3) toegenomen atmosferische CO_2 concentratie. In deze dissertatie zijn deze drie externe factoren bestudeerd, evenals hun effect of de lange termijn groei van tropische bomen. Hiervoor heb ik gebruik gemaakt van twee relatief nieuwe technieken in de tropische bosecologie: jaarringmetingen en de analyse van stabiele isotopen. Jaarringen werden gemeten om informatie te verkrijgen over de groei van bomen op een lange tijdschaal. Stabiele isotopen in het hout van deze jaarringen werden gebruikt om informatie te geven over factoren die mogelijk hebben geleid tot veranderingen in boomgroei. Dit promotieonderzoek is onderdeel van een groter project naar veranderingen in intacte en onverstoorde tropische bossen (het TroFoClim project van Pieter Zuidema) en bevat ook het promotieonderzoek van Mart Vlam en Peter Groenendijk. In het TroFoClim project werden in totaal ~1500 bomen gemeten van 15 soorten, uit drie bosgebieden verspreid over de tropen (in Bolivia, Kameroen en Thailand).

Voor de analyse van lange termijn veranderingen in groei en stabiele isotopen, is het ook van belang om veranderingen op korte termijn te begrijpen (bv. door bosdynamiek), omdat dit met elkaar interfereert. Een voorbeeld van een factor die veranderingen in groei en stabiele isotopen op de korte termijn kan veroorzaken is de plotselinge opening in het bladerdak door het omvallen van een boom. De vorming van zo'n opening kan een aanzienlijke verandering teweeg brengen in de omgeving (bv. in de beschikbaarheid van licht, water en nutriënten) en zo een sterk effect hebben op de groei van de overgebleven bomen. In de meeste onderzoeken worden deze effecten echter niet nader bestudeerd. In HOOFDSTUK 2 heb ik de discriminatie tegen een isotoop van koolstof ($\Delta^{13}C$) gemeten in jaarringen van de boomsoort *Peltogyne* cf. *heterophylla* uit het Noordoosten van Bolivia en onderzocht hoe veranderingen in omgevingsfactoren (na de vorming van een opening in het kronendak) de groei van overgebleven bomen beïnvloedt. De gemiddelde boomgroei en $\Delta^{13}C$ werd hiervoor vergeleken in de zeven jaar voor en na de vorming van een opening in het kronendak. Tweeënveertig bomen van verschillende groottes werden bestudeerd, waarvan de ene helft op minder dan 10 m afstand van een opening stond en de andere helft op meer dan 40 meter (controle bomen). Een toename in de groei van bomen dichtbij een omgevallen boom werd voornamelijk geassocieerd is met een afname in $\Delta^{13}C$, hetgeen wijst op een reactie veroorzaakt door toegenomen lichtbeschikbaarheid en niet door een toegenomen waterbeschikbaarheid. Interessant is dat de meeste kleine bomen geen toename in groei lieten zien na het omvallen van een naburige grote boom. Gedacht werd dat dit kwam door een toegenomen droogtestress, maar de $\Delta^{13}C$ waardes in het hout van deze bomen laten zien dat droogtestress erg onwaarschijnlijk is. Het is aannemelijker dat de lichtbeschikbaarheid nauwelijks toenam voor deze kleine bomen. Deze resultaten laten vooral zien dat het combineren van veranderingen in groei met veranderingen in $\Delta^{13}C$ een waardevolle techniek is om temporale variatie in boomgroei te begrijpen.

Een belangrijke factor die veranderingen in ongerepte tropische bossen kan hebben veroorzaakt is klimaatverandering (bv. via toegenomen temperatuur of via veranderingen in neerslag). Voor de studie naar de effecten van klimaatverandering is de beschikbaarheid van goede en betrouwbare klimaatdata uiteraard cruciaal. Voor de studiegebieden in Bolivia en Thailand heeft onderzoek naar klimaatverandering plaatsgevonden en is bekend hoe temperatuur en neerslag over de laatste tientallen jaren veranderd zijn. Voor het studiegebied in Kameroen, en voor West en Centraal Afrika in het algemeen, is klimaatdata slechts heel beperkt beschikbaar. Dit limiteert de mogelijkheid om klimaatverandering te relateren aan veranderingen in boomgroei en vraagt om proxy's die een reconstructie van de variabiliteit van het klimaat in het verleden mogelijk maken. In HOOFDSTUK 3 heb ik het gebruik van zuurstof isotopen ($\delta^{18}O$) als proxy's voor de reconstructie van neerslag in tropisch Afrika onderzocht. Ik heb hiervoor $\delta^{18}O$ gemeten in de jaarringen van vijf grote *Entandrophragma utile* bomen uit Noordwest Kameroen. Een significante negatieve correlatie is gevonden tussen jaarlijkse $\delta^{18}O$ (gemiddelde waarde van de vijf bomen) en jaarlijkse neerslag in grote delen van West en Centraal Afrika voor de periode 1930-2009. Daarnaast heb ik ook gevonden dat $\delta^{18}O$ in jaarringen correleert met de oppervlakte temperatuur van zeewater in de golf van Guinea (1930-2009). Deze twee resultaten zijn gerelateerd aan elkaar omdat de hoeveelheid regen in West- en Centraal Afrika sterk beïnvloed wordt door de temperatuur van zeewater in de golf van Guinea. In jaren met relatief warm water in de golf van Guinea is de neerslag hoog in grote delen van West en Centraal Afrika en worden in de jaarringen van *E. utile* relatief lage $\delta^{18}O$ waardes vastgelegd. Omgekeerd, zorgt een koude zee voor drogere jaren en zijn de $\delta^{18}O$ waardes in jaarringen relatief hoog. Ik vond ook een significante toename van $\delta^{18}O$ op de lange termijn (1860-2010). Deze trend lijkt te zijn veroorzaakt door een verminderde neerslag in de periode van 1970 tot 1990 (de Sahel droogte). Er werd geen trend in $\delta^{18}O$ waardes gevonden van 1860 tot 1970, dit wijst er op dat er geen substantiële verandering in de totale neerslag was gedurende deze periode.

Een andere factor die een verandering in de groei van bomen in ongerepte tropische bossen kan hebben veroorzaakt is de toename van antropogene nutriëntdeposities (vooral van stikstof). De depositie van stikstof is waarschijnlijk in de meeste tropische landen gestegen door een toegenomen industrialisatie en gebruik van kunstmest. Stikstofdeposities kunnen plantengroei stimuleren, net zoals kunstmest dat doet bij gewassen. Onderzoek heeft laten zien dat gedurende de laatste decennia de stabiele isotopen ratio van stikstof ($\delta^{15}N$) is toegenomen in het hout van enkele boomsoorten uit Brazilië en Thailand, evenals in bladeren van bomen uit Panama. Deze toename van $\delta^{15}N$ wordt geïnterpreteerd als een teken dat de stikstofcyclus in tropische bossen aan het veranderen is en dat deze transformeert naar een meer 'open' systeem. Het onderliggende mechanisme is dat hoge stikstofdeposities en relatief hoge stikstofbeschikbaarheid leiden tot een toename van nitrificatie. Dit proces kan zorgen voor een geleidelijke verrijking van ^{15}N in de bodem. In HOOFDSTUK 4 heb ik onderzocht of $\delta^{15}N$ waardes in 400 bomen van zes soorten uit de drie studiegebieden zijn veranderd in de loop van de tijd. In de bomen uit Kameroen werd geen verandering gevonden in $\delta^{15}N$ over de periode 1850-2005, ondanks dat NH_3 en NO_x emissies waarschijnlijk zijn toegenomen rondom het onderzoeksgebied sinds 1970. Mogelijk zorgt de zeer hoge neerslag in dit gebied ervoor dat de lokale stikstofcyclus al erg open is en dat daardoor het effect van antropogene stikstofdeposities op de $\delta^{15}N$ van stikstof in de bodem (en zo uiteindelijk in het hout van bomen) beperkt blijft. Een alternatieve verklaring is dat stikstofdeposities in dit gebied veel lager zijn dan de gereconstrueerde NH_3 en NO_x emissies doen vermoeden. In Bolivia werd geen verandering in $\delta^{15}N$ in het hout van bomen gevonden in de periode 1875-2005. Dit is in overeenstemming met verwachtingen omdat NH_3 en NO_x emissies nog erg laag zijn rondom het gebied. Een marginaal significante toename van $\delta^{15}N$ sinds 1950 werd gevonden in de bomen uit Thailand. Dit bevestigt eerder onderzoek in het gebied en wijst op een verandering van de stikstofcyclus als gevolg van toegenomen deposities. Een dergelijke verandering kan de groei van bomen hebben gestimuleerd, mits deze voorheen stikstof gelimiteerd was.

De meest aangedragen factor die mogelijk een lange termijn stimulatie in tropische boomgroei kan hebben veroorzaakt is de toename van atmosferische CO_2. Sinds het begin van de industriële revolutie (rond 1850) is de CO_2 concentratie in de atmosfeer gestegen met 40%. Toegenomen CO_2 kan een direct effect hebben op planten via de stimulatie van de activiteit en efficiëntie van het enzym rubisco, dat zorgt voor de fixatie van CO_2. Zodoende kan een toename van CO_2 de fotosynthese verhogen. Mogelijk nog belangrijker voor planten die regelmatig te maken hebben met watergebrek, is dat CO_2 een vermindering van de stomataire geleidbaarheid veroorzaakt, dat op zijn beurt weer een vermindering van de verdamping veroorzaakt en zo ook van het verlies van water uit bladeren. Hierdoor neemt de efficiëntie van watergebruik (koolstofopname via fotosynthese t.o.v. waterverlies via verdamping) toe, hetgeen planten in staat kan stellen langer door te groeien (o.a. in de droge tijd of tijdens de hete momenten van de dag wanneer waterstress groot is). Toegenomen atmosferische CO_2 is dus een heel waarschijnlijke factor die kan hebben geleid tot een toename van groei (ook wel CO_2 fertilizatie genoemd), mist de groei van bomen gelimiteerd wordt door koolstof of water. In HOOFDSTUK 5 heb ik de CO_2 fertilizatie hypothese getest door jaarringdata te analyseren in 1100 bomen uit de drie studiegebieden. De meting van jaarringen gaf informatie over histroische boomgroei. Daarnaast maakte de meting van stabiele koolstof isotopen ($\delta^{13}C$) in het hout een schatting mogelijk van de CO_2 concentratie in de intercellulaire ruimte in bladeren (C_i) en van de efficiëntie van het gebruik van water ('intrinsic water-use efficiency'; iWUE). Ik heb een bemonsteringsmethode gebruikt waarbij rekening werd gehouden met ontogenetische veranderingen in groei en $\delta^{13}C$ (i.e. veranderingen tijdens de ontwikkeling van een boom). In deze methode worden bomen van dezelfde grootte met elkaar vegeleken (dus in hetzelfde ontwikkelingsstadium). Twee diameters werden gebruikt in deze methode: 8 cm (relatief kleine bomen) en 27 cm (grotere bomen met kruinen in het kronendak). Een significante en exponentiële toename van de C_i werd gevonden met een 'mixed-effect model' in elk van de drie studiegebieden en voor zowel de kleine als grote bomen. Over de afgelopen 150 jaar nam C_i respectivelijk toe met 43% en 53% voor de kleine en grote bomen. De toename van C_i was echter minder dan de toename van atmosferische CO_2, hetgeen resulteerde in een significante toename van iWUE. Gedurende de laatste 150 jaar nam iWUE toe met 30% in kleine bomen en met 35% in de grotere bomen. Een toename van iWUE op de lange termijn suggereert dat de netto fotosynthese toenam of dat de stomataire geleidbaarheid (en dus transpiratie) afnam. Theoretisch kunnen beide veranderingen leiden tot een toename van groei, maar geen enkel bewijs werd gevonden dat de groei van de onderzochte bomen is toegenomen over de afgelopen 150 jaar in de drie gebieden. Hoewel er vele mogelijke verklaringen zijn voor deze uitkomst, lijkt de meest waarschijnlijke dat de groei van tropsiche bomen over het algemeen niet gelimiteerd wordt door koolstof en water, maar door een persistente nutriëntlimitatie (bv. van fosfaten).

Een limitatie van nutriënten kan er voor hebben gezorgt dat toegenomen CO_2 niet resulteerde in een toename van groei.

In deze thesis heb ik physiologsiche en omgevingsfactoren onderzocht die de groei van bomen kan hebben beinvloed. Bewijs is gevonden dat het droger was gedurende de laatste decenia in het studiegebied in Kameroen (HOOFDSTUK 3), dat de stikstofcyclus is veranderd in het studiegebied in Thailand (HOOFDSTUK 4) en dat de physiologie van alle onderzochte boomsoorten is veranderd (toegenomen iWUE; HOOFDSTUK 5). Een van de belangrijkste resultaten van dit onderzoek is echter dat deze veranderingen niet hebben geleid tot een netto verandering in boomgroei over de laatste 150 jaar (HOOFDSTUK 5). Dit is een interessante vondst die twee belangrijke implicaties kan hebben. Ten eerste, de afwezigheid van groeistimulatie op de lange termijn suggereert dat de toename van iWUE vooral het gevolg is van een afname van de stomateire geleidbaarheid, hetgeen leidt tot een afgenomen verdamping. Als bomen in tropsiche bossen gezamelijk minder verdampen beinvloedt dit de hydrologische cyclus en kan o.a. resulteren in een lagere luchtvochtigheid, een hogere lucht temperatuur en een verminderde neerslag. Ten tweede, de afwezigheid van een groeistimulatie laat zien dat de opname van koolstof in tropische bossen waarschijnlijk veel lager is dan wordt aangenomen in 'Dynamic Global Vegetation Models'. Deze modellen worden gebruikt om veranderingen van vegetatie in de toekomst te simuleren en gaan uit van een sterke CO_2 fertilizatie. Daardoor voorspellen deze modellen dat tropsiche bossen een sterke capaciteit hebben om de opwarming van de aarde te remmen. Ik verwacht dat de reeds geplande 'Free Air Concentration Enrichment' (FACE) experimenten meer duidelijkheid zullen geven over waarom de toename van atmospherische CO_2 niet heeft geleid tot een toename van groei van tropische bomen. Daarnaast denk ik dat de combinatie van jaarringonderzoek met het onderzoek in permanente bospercelen van grote waarde kan zijn om veranderingen in de dynamiek van tropische bossen beter te begrijpen.

Resumen

Los bosques húmedos tropicales albergan una increíble biodiversidad, proveen servicios ecosistémicos y regulan las condiciones climáticas a escalas regionales. Así mismo, estos bosques son un componente importante del ciclo global del carbono, almacenando 25% del carbono total terrestre y representando un tercio de la productividad primaria neta. Estó último significa que los cambios en la estructura y la cobertura del bosque húmedo tropical no solo afectarán la biodiversidad y la provisión de ecosistémicos, sino que también tendrán implicaciones para el ciclo global de carbono, y como resultado, la aceleración o el detenimiento del calentamiento global. Las tasas de deforestación todovia están aumentando en los trópicos y forman una gran amenza para la existencia de bosques tropicales en la mayoría de los países. Adicionalmente, se ha indicado que los bosques tropicales no perturbados se encuentran en proceso de cambio. Los cambios más notables corresponden a un incremento en la tasa de crecimiento de los árboles y la biomasa del bosque en las últimas décadas. Estos cambios son reportados través del monitoreo de parcelas permanentes de bosque. Si esta tendencia realmente confirmada, implicaría que los bosques tropicales del mundo, potencialmente están absorbiendo una fracción significante de las emisiones de CO_2 causadas por los humanos y por lo tanto, están mitigando el calentamiento global. Sin embargo, este no ha sido consistentemente encontrado en todos los estudios. Además, se desconoce si los cambios observados en bosques no pertubados son parte de un cambio a largo plazo o solo el reflejo de fluctuaciones a escala de décadas. Estas incertidumbres llevan a un debate permanente sobre si realmente hay un aumento en el crecimiento de los árboles y la biomasa en bosques tropicales, y si así fuese, cual es el alcance de esta situación.

Los factores que podrían explicar los cambios potenciales en el crecimiento de los árboles y la biomasa del bosque aun se desconocen. Posiblemente, son causados por controles internos (ej. un efecto duradero de perturbaciones a gran escala en el pasado) o controles externos. Los factores externos que posiblemente estén afectando la dinámica de los bosques tropicales son (1) cambio climático (temperatura y precipitación), (2) incremento en la deposición de nutrientes y (3) el incremento en las concentraciones CO_2 atmosférico. En esta tesis, he investigado los cambios en estos factores externos, así como sus efectos en el crecimiento de los árboles. He usado dos herramientas relativamente novedosas

para bosques tropicales: medidas de anillos de crecimiento, y análisis de isótopos estables. Los anillos de crecimiento fueron medidos para obtener información a largo plazo sobre el crecimiento de los árboles. Así mismo, los isótopos estables en los anillos de crecimiento fueron analizados para proveer información sobre los controles ambientales y fisiológicos de los cambios en el crecimiento de los árboles. Esta tesis es parte de un proyecto sobre los cambios a largo plazo en bosques tropicales no pertubados (el proyecto TroFoClim, liderado por Pieter Zuidema) e incluye también las tesis doctorales de Mart Vlam y Peter Groenendiijk. En este proyecto, ca. 1400 árboles de 15 especies arbóreas fueron examinados y colectados en tres bosques distribuidos alrededor de la franja tropical (Bolivia, Camerún y Tailandia).

Para la estimación de cambios a largo plazo en el crecimiento y en los isótopos estables, es importante entender las fluctuaciones a corto plazo que son consecuencia de la dinámica del bosque (ej. la formación de claros), ya que estos procesos interfieren con los cambios a una escala temporal mayor. La formación de un claro en un dosel cerrado del bosque, como consecuencia de la muerte de un árbol, puede causar considerables cambios ambientales en el área que le rodea, ej. cambios en la disponibilidad de luz, nutrientes y agua. Este hecho puede afectar fuertemente las tasas de crecimiento de los árboles remanentes. Sin embargo, los factores ambientales que dirigen los cambios en el crecimiento de los árboles, tras la formación de los claros, no son considerados en la mayoría de los estudios. En el CAPITULO 2 he medido la discriminación contra un isotopo del carbono ($\Delta^{13}C$) en anillos de crecimiento anual de la especie *Peltogyne* cf. *heterophylla*, proveniente de un bosque húmedo en el noreste de Bolivia, y he evaluado los factores ambientales que controlan las respuestas de crecimiento tras la formación de los claros. El crecimiento y los valores del isótopo $\Delta^{13}C$ fueron comparados entre los siete años antes y después de la formación del claro. Cuarenta y dos árboles de diferente tamaño fueron estudiados, la mitad de estos fueron individuos que crecieron cerca (<10m) a un claro ocasionado por la caída de un árbol; y la otra mitad fueron individuos que crecieron a mas de 40 m de distancia del claro (árboles control). He encontrado que el incremento en crecimiento estuvo asociado con la disminución de $\Delta^{13}C$ sugiriendo que esta respuesta fue conducida por el incremento en la cantidad de luz disponible y no por una mayor disponibilidad de agua. Es interesante anotar que la mayoría de los árboles pequeños no mostraron un incremento del crecimiento después de la formación del claro, lo que sugiere un posible aumento en el stress por seqúia. Sin embargo, las mediciones de $\Delta^{13}C$ mostraron que un mayor stress por sequía es una causa improbable de la ausencia de cambios en el crecimiento, lo que apunta que las condiciones de luz no han mejorado después de la formación del claro. Estos resultados muestran que la combinación de tasas de crecimiento con cambios en $\Delta^{13}C$ es una herramienta invaluable para lograr un mejor entendimiento de las causas en la variación temporal del crecimiento de los árboles.

Un importante factor potencial que controla los cambios a largo plazo en el crecimiento de árboles es el cambio climático, por ejemplo el calentamiento global y los patrones de precipitación anual alterados. Para entender el efecto del cambio climático en el crecimiento de árboles, la disponibilidad de datos confiables sobre la historia del clima es crucial. Para los sitios del estudio en Bolivia y Tailandia, existían estudios previos sobre la ocurrencia de tendencias temporales en temperatura y precipitación. Sin embargo, para el sitio de estudio en Camerún, así como para África Central y Occidental, la disponibilidad de datos climáticos se encuentra restringida. Esto limita la posibilidad de relacionar la variación climática con los cambios en el crecimiento de los árboles y motiva el uso de sustitutos que permitan la reconstrucción de condiciones climáticas pasadas. En el CAPITULO 3 he evaluado el uso potencial de isótopos estables de oxigeno ($\delta^{18}O$) en anillos de crecimiento de árboles como una herramienta para la reconstrucción de la precipitación en África tropical. Medí los valores de $\delta^{18}O$ en anillos de crecimiento de cinco árboles grandes de *Entandrophragma utile* del noroeste de Camerún. Encontré una correlación significativamente negativa entre los valores $\delta^{18}O$ de los anillos anuales (promediados sobre cinco individuos) y la cantidad de precipitación anual durante 1930-2009 en áreas extensas de África Occidental y Central. También encontré que el isótopo $\delta^{18}O$ en los anillos de crecimiento puede ser usado para evaluar la temperatura superficial del mar (SST) en el Golfo de Guinea (1930-2009). Estos dos resultados están relacionados ya que la variabilidad de la lluvia en África Occidental y Central está profundamente influenciada por la temperatura superficial del mar (SST) del Océano Atlántico tropical. Así, un alto SST en el Golfo de Guinea se asocia con alta precipitación sobre grandes extensiones de África Occidental y Central y se registra en los anillos de los árboles por un bajo $\delta^{18}O$ relativo. Por el contrario, en años secos cuando la SST es baja, el registro del $\delta^{18}O$ en los anillos de los árboles presenta valores relativamente altos. También encontré un incremento significativo a largo plazo de los valores de $\delta^{18}O$ en los anillos de los árboles. Es posible que esta tendencia sea causada por una precipitación baja desde 1970 a 1990 (el periodo de sequía de Sahel). De 1860 a 1970, no se observo una tendencia significativa a largo plazo en los valores del $\delta^{18}O$ de los anillos de los árboles, sugiriendo que no ha ocurrido un cambio substancial en la cantidad de precipitación en este periodo.

Otra potencial causa del crecimiento alterado de los árboles y la biomasa en bosques tropicales no pertubados es el incremento de la deposición de nutrientes de origen humano, especialmente nitrógeno. La deposición del nitrógeno probablemente ha incrementado debido a la industrialización y al uso de fertilizantes de nitrógeno en la mayoría de los países tropicales. El nitrógeno puede estimular el crecimiento de las plantas; ya que es bien conocido el efecto positivo de la aplicación de fertilizantes de nitrógeno sobre la producción de cultivos. Estudios previos han demostrado que los valores de los isótopos estables de nitrógeno ($\delta^{15}N$) han incrementado durante los últimos decenios en la madera de algunas especies de árboles de Brasil y Tailandia así como en hojas de árboles en Panamá. Este incremento en $\delta^{15}N$ ha sido interpretado como una señal de que el ciclo del nitrógeno en los trópicos está siendo más “abierto”. El mecanismo subyacente es que altas tasas de deposición de nitrógeno y la alta disponibilidad del nitrógeno ambiental lleva a un incremento en la nitrificación. Este proceso puede causar un enriquecimiento gradual de ^{15}N en el suelo. En el CAPITULO 4 he analizado los cambios en los niveles de $\delta^{15}N$ en los anillos de crecimiento de 400 árboles de seis especies de los tres sitios de estudio. En los árboles de Camerún no se encontraron cambios a largo plazo (1850-2005) en los niveles de $\delta^{15}N$ en los anillos de crecimiento, aun cuando las emisiones de NH_3 y NO_x han incrementado considerablemente alrededor del área de estudio desde 1970. Posiblemente, la alta precipitación en este sitio causa que el ciclo local del nitrógeno esta “abierto”, limitando el efecto de nitrógeno adicional en la señal del $\delta^{15}N$ del nitrógeno del suelo. Alternativamente, la entrada de nitrógeno en este bosque podría ser mucho más baja de lo que sugieren las reconstrucciones de emisiones de NH_3 y NO_x. En el sitio de estudio en Bolivia, no hubo cambio significativo en los valores de $\delta^{15}N$ en los anillos de los árboles (1875-2005), lo cual concuerda con el resultado esperado para áreas con un bajo ingreso de nitrógeno por causas humanas. Encontré un incremento marginalmente significativo de valores de $\delta^{15}N$ desde 1950 en árboles de Tailandia, lo cual confirma observaciones previas para este sitio. Estos resultados señalan que hay un efecto en el incremento de la deposición del nitrógeno, lo cual podría haber estimulado las tasas de fotosíntesis, si de hecho el nitrógeno ha sido un factor limitante del crecimiento de los árboles en este sitio.

El factor que más a menudo se ha empleado para generar hipótesis sobre la causa del incremento a largo plazo del crecimiento de los árboles es el aumento del CO_2 atmosférico. Desde el comienzo de la revolución industrial (~1850), la concentración del CO_2 atmosférico total ha aumentado en un 40%. Este hecho puede directamente afectar las plantas al aumentar la actividad así como la eficiencia de la enzima rubisco que fija el CO_2, resultando en un incremento de las tasas fotosintéticas. Lo que es potencialmente más importante en comunidades de plantas, sujetas a periodos de limitada disponibilidad de agua (tal como sitios con estaciones secas), es que el incremento de CO_2 puede causar una reducción en la conductancia de los estomas, lo cual disminuye la evapotranspiración y por lo tanto reduce las pérdidas de agua. Este incremento en la eficiencia del uso del agua (ej. la cantidad de carbón ganada a través de la fotosíntesis dividida por la cantidad de agua perdida a través de la transpiración) podría permitir a las plantas extender su periodo de crecimiento y/o incrementar su actividad fotosintética durante las horas de mayor temperatura en el día cuando el stress por agua podría ser severo. El CO_2 atmosférico es así un candidato probable como factor estimulante del crecimiento de árboles tropicales (también referido como fertilización por CO_2), siempre y cuando el crecimiento de las plantas esté limitado por carbono o agua. En el CAPITULO 5, he evaluado la hipótesis de la fertilización de CO_2 al analizar datos de anillos de crecimiento de 1100 árboles de los tres sitios de estudio. La medida del ancho de los anillos de los árboles permitió una evaluación histórica de las tasas de crecimiento, mientras el isótopo estable de carbono ($\delta^{13}C$) en la madera de los árboles fue usado para obtener un estimado de la concentración de CO_2 en los espacios intercelulares de las hojas (C_i) y del eficiencia intrínseca del uso del agua ('intrinsic water-use efficiency'; iWUE). Para tal fin, use un método de muestreo que controla por cambios ontogenéticos (es decir cambios relacionados con el desarrollo de las plantas) en el crecimiento y los niveles de $\delta^{13}C$. Con este método, los árboles fueron comparados usando un diámetro fijo (es decir el mismo estado ontogenético). Establecí dos categorías de diámetros: 8 cm (que se refieren a árboles pequeños del sotobosque) y 27cm (que se refieren a árboles grandes del dosel). Usando un 'mixed-effect model' observé un incremento altamente significativo y exponencial del C_i en cada uno de los tres sitios, tanto en los árboles del sotobosque como del dosel. En los últimos

150 años C_i incremento en un 43% y 53% para los árboles del sotobosque y del dosel respectivamente. Aun así, la tasa de incremento en C_i fue consistentemente más baja que la tasa de CO_2 atmosférico. Esta respuesta "activa" al elevado CO_2 atmosférico resulto en un incremento significativo en la iWUE. En los últimos 150 años, la iWUE incremento en 30% en árboles del sotobosque y 35% en árboles del dosel. Un incremento a largo plazo de la iWUE indica o bien un incremento en la fotosíntesis neta y/o una disminución en la conductancia de los estomas y por lo tanto de la transpiración, ambos factores podrían haber estimulado el crecimiento de los árboles. Sin embargo, no encontré incremento en el crecimiento de los árboles en los últimos 150 años en ninguno de los sitios. Aun cuando hay varias posibles explicaciones para estos resultados, la explicación que yo considero más probable es que el crecimiento de árboles tropicales no está generalmente limitado por agua o carbono, pero por una persistente limitación en nutrientes (ej. de fosfatos) y este hecho ha prevenido a los árboles de usar CO_2 extra para acelerar su crecimiento.

En esta tesis he estudiado los determinantes ambientales y fisiológicos de cambios en el crecimiento de árboles tropicales. Encontré evidencia de la disminución en la precipitación sobre las ultimas décadas en el sitio de estudio en Camerún (CAPITULO 3), cambios en el ciclo del nitrógeno en el sitio de estudio en Tailandia (CAPITULO 4) y un cambio general en la fisiología de todas las especies de árboles estudiada (incremento iWUE; CAPITULO 5). Uno de los principales resultados de esta tesis es, sin embargo, que estos cambios no han llevado a un cambio neto en el crecimiento de los árboles en los últimos 150 años (CAPITULO 5). Este es un hallazgo importante que podría tener dos grandes implicaciones. En primer lugar, la ausencia de un estimulo en el crecimiento a largo plazo sugiere que el incremento en la iWUE es principalmente determinado por una reducción en la conductancia de los estomas, lo cual probablemente llevará a una reducción en la perdida de agua por evaporación. Si los árboles en los trópicos están reduciendo la evapotranspiración, este cambio afectará los ciclos hidrológicos, por ejemplo generando una baja humedad, altas temperaturas ambientales, y una precipitación reducida. En segundo lugar, la ausencia de un estímulo en el crecimiento en los pasados 150 años sugiere que la capacidad de los bosques tropicales como sumideros de carbono ha sido sobreestimada, como por ejemplo por los Modelos de Dinámica de la Vegetación Global (Dynamic Global Vegetation Models), los cuales asumen un efecto considerable de la fertilización del CO_2 y como tal, una gran capacidad de los bosques tropicales para mitigar el calentamiento global. Anticipo que los experimentos de Enriquecimiento en la Concentración de Aire Libre (Free Air Concentration Experiment-FACE) en los trópicos aclarará las razones para entender por que el incremento en el CO_2 no estimula las tasas de crecimiento de los árboles tropicales. Además, creo que la combinación de las medidas de anillos de crecimiento y el análisis de isótopos estables junto con la investigación en parcelas permanentes son la forma más prometedora para mejor nuestra comprensión de los cambios en los bosques tropicales.

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Short biography

Peter van der Sleen was born on August 3, 1982, in Ede, the Netherlands. He attended high school in Wageningen and during that time was also an active member of the youth society for the study of nature (NJN). After high school he went to Ecuador for six months, spending most time on hiking, bird watching and snorkeling. From 2003 to 2006 he studied Biology at the University of Utrecht. In 2006 he started his master in Plant Ecology and in the same year went to Mexico for a research internship at the Centro de Investigaciones en Ecosistemas (CIEco) of the Universidad Nacional Autónoma de México (UNAM). His research project focussed on the enrichment planting of the palm species *Chamaedorea elegans* in secondary forests in Chiapas. In 2008, he started a second research internship in collaboration with the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil. During his stay in Brazil he conducted field work in the Upper Rio Negro with a focus on the mechanism that causes the difference in floristic composition between forests on 'white sands' and 'terra firme'. He completed his master at the University of Utrecht with honours (cum laude) in 2009 and in 2010 started his PhD research. Although Peter has a strong interest in forest ecology, he is also fascinated by fishes, particularly freshwater fishes. He is currently working on a field guide to the fish genera in the Amazon basin in collaboration with many leading fish experts.

List of publications

- **Sleen, P. van der**, Soliz-Gamboa, C.C., Helle G., Pons, T.L. Anten, N.P.R. and Zuidema, P.A. 2014. Understanding causes of tree growth responses to gap formation: Δ^{13}C-values in tree rings reveal a predominant effect of light. Trees 28: 439-448.

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PhD Education Certificate

PE&RC Training and Education Statement

With the training and education activities listed below, the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
Stable isotopes in tree rings (2010-2011)

Writing of project proposal (4.5 ECTS)
Stable isotopes in tree rings (2010)

Post-graduate courses (4.7 ECTS)
Laboratory training in carbon isotope analysis; GFZ, Potsdam, Germany (2010)
Introduction to R; PE&RC (2011)
Mixed linear models; PE&RC (2013)

Laboratory training and working visits (2 ECTS)
Working visit to the carbon and oxygen laboratory; University of Leicester, UK (2013)

Invited review of (unpublished) journal manuscript (0.9 ECTS)
Journal of Ecology: wood production in relation to climate (2012)
Plant Ecology and Diversity: forest carbon dynamics (2012)
Book chapter: liana anatomy (2012)

Deficiency, refresh, brush-up courses (1.5 ECTS)
Basic statistics (2013)

Competence strengthening / skills courses (2.3 ECTS)
Career assessment; WGS (2014)
Writing grant proposals; WGS (2014)

PE&RC Annual meetings, seminars and the PE&RC weekend (11.2 ECTS)
PE&RC First year weekend (2010)
PE&RC Day (2011)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)
FEM Journal club (2010-2013)
PE&RC Discussion Group Ecological Theory and Application (2011-2013)
Local seminars on tropical ecology, tree-rings, etc. (2012-2013)
Symposium tropical ecology UvA; oral presentation (2012)

International symposia, workshops and conferences (6 ECTS)
ATBC meeting: Association for Tropical Biology and Conservation; oral presentation (2012)
TRACE meeting: Tree Rings in Archaeology, Climatology and Ecology; oral presentation (2013)
NAEM meeting: Netherlands Annual Ecology Meeting; oral presentation (2014)

Lecturing / supervision of practical's / tutorials (3 ECTS)
Introductie in de Biologie; University of Utrecht (2010)
Taller de Dendrocronología aplicada a especies tropicales; organiser (2011)
Guest lecture in course 'Tropische Ecosystemen en Klimaat'; University of Utrecht (2013)

Supervision of MSc students

- Robbert Haasnoot: The effects of different logging strategies on growth and timber yields of *Hura crepitans*
- Jeroen Buijks: The effects of different logging strategies on growth and timber yields of *Hura crepitans*
- Annemarijn Nijmeijer: Physiological responses of a tropical tree to elevated CO_2: a century long evaluation of *Pseudolmedia laevis* trees using stable carbon isotope values from tree rings
- Quirine Hakkaart: Growth sensitivity of *Cariniana ianeirensis* to local and global climatic variation and implications for its response to climate change
- Gideon Terburg: Long-term growth and $\Delta^{13}C$ trends in *Hura crepitans* do not show changes in growth, climatic and environmental drivers in a Neotropical forest concession in Eastern Bolivia

- Alejandra Romero Seas: Tasa de crecimiento radial de dos especies maderables: Yesquero blanco (*Cariniana ianeirensis*) y Serebó (*Schizolobium parahyba)* en un bosque subhúmedo tropical, provincia Guarayos, Santa Cruz, Bolivia
- Mizanur Rahman: Long-term trends in growth and physiology of a common tree species (*Chukrassia tabularis)* in a tropical moist forest of north-eastern Bangladesh

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