

# Physiological responses of a tropical tree to elevated CO<sub>2</sub>: a century-long evaluation of *Pseudolmedia laevis* trees using stable carbon isotope values from tree rings

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## Abstract

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Since 1850 atmospheric CO<sub>2</sub> levels have been rising due to burning of fossil fuels. These rising CO<sub>2</sub> levels will influence physiology and water relations of tree species, although very little is known about these responses. A key element in these reactions is whether trees actively or passively respond to increased atmospheric CO<sub>2</sub> (C<sub>a</sub>). Stable carbon isotopes (δ<sup>13</sup>C) from tree rings are an ideal tool to investigate variations in internal CO<sub>2</sub> of leaves (C<sub>i</sub>) and it allows us to evaluate tree responses over long term. We studied these responses in a semi-deciduous tropical moist forest in Bolivia, using the shade tolerant tree species *Pseudolmedia laevis*. We collected 5 discs, measured tree ring widths and determined δ<sup>13</sup>C over 110 years. The C<sub>i</sub>/C<sub>a</sub> ratios were derived and long-term trends were evaluated. Our results show no significant trend in C<sub>i</sub>/C<sub>a</sub> ratios over the past 110 years. Besides this, we found a significant increase in basal area growth per year for all five trees ( $p < 0.001$ ), which, along with the constant C<sub>i</sub>/C<sub>a</sub> ratios, suggests increased assimilation rates and decreased stomatal conductance. Comparing our result with results from other studies we found several similarities in the reaction of trees to increased CO<sub>2</sub> levels. Where precipitation rates appear to be a factor of influence according a preformed linear regression between C<sub>i</sub>/C<sub>a</sub> ratios and rainfall levels ( $p < 0.05$ ). Our results indicate increasing assimilation rates and increased internal CO<sub>2</sub> in *Pseudolmedia laevis*, suggesting a CO<sub>2</sub> fertilization effect.

## Introduction

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Tropical forests store about 25% of the terrestrial carbon and can influence the carbon cycle by storing or releasing CO<sub>2</sub> (Clark 2004, Bonan 2008, Davidson *et al.* 2012). Since industrial revolution, the global carbon cycle is heavily influenced by the amount of CO<sub>2</sub> released from the burning of fossil fuels (Keeling *et al.* 2001). The increase in atmospheric CO<sub>2</sub>, along with other factors, like variation in solar constant (Cess 1976), causes global warming. These changes in climate are predicted to have a large impact on all ecosystems (Thompson 2010). However, what the impact is on tropical rainforest and how tropical trees in special react on increasing atmospheric CO<sub>2</sub> levels is still barely known (Clark 2004).

Dendrochronological studies in the tropics can provide knowledge about the growth responses physiological reactions of trees to increased CO<sub>2</sub> levels. This tree ring studies in the tropics are relative new since for long times it was assumed that tropical tree species do not form clear and annual tree rings (Rozendaal and Zuidema 2011). This led to a gap in tropical tree ring research. Instead of dendrochronology, relative density and growth rates from year to year measurements, were common used tools for studying growth aspects of tropical trees (Rozendaal and Zuidema 2011). In addition to tree ring research, stable isotope research has been introduced in the field of dendrochronology (Helle and Schleser 2004b, McCarroll and Loader 2004). Carbon isotopes, for instance, appear naturally in two stable, <sup>12</sup>C and <sup>13</sup>C, and one unstable, <sup>14</sup>C, form. Alternations in ratios of stable carbon isotopes ( $\delta^{13}\text{C}$ ) from tree rings are a good tool to examine long-term responses of trees to changing climate parameters as atmospheric CO<sub>2</sub> (Brienen *et al.* 2011). Analysing those long-term responses in trees could help us understanding the reactions of tropical forests to those changes in climate. The advantage of stable isotope research to initial tree ring research is that stable isotope ratios derived from tree rings are, except for the relatively short juvenile period, less influenced by tree age than tree ring widths (McCarroll and Loader 2004). Besides this, the influence of climate, on mechanisms through which stable isotopes from CO<sub>2</sub> gets sequestered in trees, is relatively better understood than the effect of climate on pathways that tree growth and ring widths influence (Loader *et al.* 2007). The mechanisms through which CO<sub>2</sub> gets sequestered consist out of two steps of isotope fractionation. Discrimination for the lighter <sup>12</sup>C form influence the ratio in which carbon isotopes ( $\delta^{13}\text{C}_{\text{tree}}$ ) are built-in in the form of photosynthetic products. The first step in discrimination occurs when CO<sub>2</sub> passes the stomata. The <sup>12</sup>CO<sub>2</sub> molecules pass stomata more easily than the heavier <sup>13</sup>CO<sub>2</sub> molecules. In addition, RuBisCO, which is the biological enzyme that binds CO<sub>2</sub> for photosynthesis, tends to prefer <sup>12</sup>CO<sub>2</sub> (Helle and Schleser 2004b, McCarroll and Loader 2004).

Each tree species could react differently to the increase in atmospheric CO<sub>2</sub> ( $C_a$ ) For interpreting the physiological long-term reactions of trees internal CO<sub>2</sub> levels ( $C_i$ ), derived from the  $\delta^{13}\text{C}_{\text{tree}}$  ratios, are a good tool (Farquhar *et al.* 1982). Saurer *et al.* (2004) divided three possible scenarios in which a tree could react on increasing CO<sub>2</sub> including changes in stomatal conductance and/or changes in assimilation rates. Those scenarios could be divided in one passive en two active responses (McCarroll *et al.*

2009). The first scenario, the passive response, adopts that trees maintain a stable  $C_a$ - $C_i$  ratio under increasing atmospheric  $CO_2$ . This means that the absolute increase in  $C_i$  is equal to that in  $C_a$  (Nock *et al.* 2011). Suggesting that no changes in photosynthesis or stomatal conductance occur (McCarroll *et al.* 2009). In  $\delta^{13}C_{tree}$  this results in a decrease that is stronger than the decrease in  $\delta^{13}C_{atmosphere}$ . The second scenario adopts that a tree keeps a stable  $C_i/C_a$  with increasing  $C_a$ . This means that the increase in  $C_i$  is proportional to that in  $C_a$ . Also considered as the first active response, implying higher levels of assimilation or/and a decrease in stomatal conductance (Medlyn *et al.* 2001, Hietz *et al.* 2005). The  $\delta^{13}C_{tree}$  will show a parallel decrease to the decrease in  $\delta^{13}C_{atmosphere}$  (Saurer *et al.* 2004). The second active response, suggest that  $C_i$  is kept stable while  $C_a$  increases over time. This implies a strong stomatal response where no increase of assimilation will occur but where transpiration rates will be strongly reduced (Brienen *et al.* 2011).

We investigated long term reactions on increased atmospheric  $CO_2$  using the annual ring forming tree species, *Pseudolmedia laevis*, from a Bolivian tropical moist forest. Using annual  $\delta^{13}C$  records and ring widths of five tree discs over >100 years the following two questions were addressed: 1. How has increased atmospheric  $CO_2$  ( $C_a$ ) influenced internal  $CO_2$  ( $C_i$ ) of *P. laevis*? 2. How are our results related to the results of other studies performed in the tropics?

## Materials and Methods

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### Study site and species

The fieldwork was conducted in a Bolivian semi-deciduous tropical forest, La Chonta. The area is 100.000 ha and situated at 15°47'S and 62°55'W in the department of Santa Cruz. The mean annual temperature is 24.5 °C and the mean annual precipitation is 1580 mm y<sup>-1</sup> ranging between 2500 mm in wet years and less than 1000 mm in dry years (Rozendaal *et al.* 2010). This makes the area a transition zone between the moist Amazonian forests and the Chiquitano dry forest (Paz-Rivera 2003). The dry season (<100mm per month) starts in May and lasts until the end of September, therefore, the dendrochronology year for trees in La Chonta starts in September until August of the subsequent year (Rozendaal *et al.* 2010). The elevation of the area is 250 meter above sea level (Fredericksen and Pariona 2002) and the forest has an open canopy with heights varying from 20 to 25 m (Paz-Rivera 2003).

From 1974 on, selective logging is taking place in La Chonta. Since 1998, this is done according a management plan from the Forest Stewardship Council at a cutting cycle of 30 years (Fredericksen and Pariona 2002, Peña-Claros *et al.* 2008). Besides the logging impact, several other types of disturbance have taken place in history of the area. Past human settlements of 300 until 400 years ago had impact on total forest growth (Paz-Rivera 2003). In addition, during dry periods, fires from surrounding agriculture fields have threatened the concession. Exact fire history is unknown but during escaped agricultural fires in 1995 and 2004, around 30% of La Chonta has been burned (Blate 2005, Peña-Claros *et al.* 2008).

Finally, CO<sub>2</sub> levels have been exponentially rising since industrial revolution, from 285.20 ppm in 1850 to 389.78 ppm in 2010 (Keeling *et al.* 2004). This potentially led to the measured temperatures increment in the Amazonian basin of 0.25°C per 10 years over the past decades (Malhi *et al.* 2008). With temperature data from Santa Cruz we found a mean increase of 0.21°C per decade from 1943 until 1989 (Climate Explorer). Human activity resulted also in increased nitrogen deposition in tropical rainforest, for the area around la Chonta this emission of nitrogen is around 100 kg/km<sup>2</sup> (Hietz *et al.* 2011). This could lead to shifts in species composition or/and to increased foliar nitrogen that leads to increased carbon gain.

*Pseudolmedia laevis* (Ruiz & Pavón) from the Moraceae family is a shade tolerant evergreen tree species (Justiniano and Nash 2002). *P. laevis* trees reach heights of 25-40 meter and diameters of up to 70 cm dbh (Justiniano and Nash 2002). *P. laevis* is abundant throughout the total tropical region of Bolivia (Figure 1) and, in La Chonta *P. laevis* is, next to *Terminalia oblonga*, *Ampelocera ruizii*, *Cariniana ianeirensis* and *Hura crepitans*, one of the common canopy species with an appearance of 58,5 trees ha<sup>-1</sup> (Justiniano and Nash 2002, Paz-Rivera 2003).

Previous research confirmed that tree rings in *P. laevis* are formed annually (Rozendaal *et al.* 2010). Each tree ring is separated from next years tree ring by a small band of parenchyma tissue.

### **Tree ring analysis**

To obtain tree discs we randomly selected 12 trees of approximately 65 cm diameter at breast height in an area of 100 ha. The selected discs were air-dried, after which they were sanded and polished (up to 400 sand grit) making the wood cells clearly visible. The ring widths, of the eight discs, which were least lobbed, were measured for several radii and visually cross-dated. With the use of COFECHA (Holmes 1983), a dendrochronology software program, we evaluated inter-tree correlation of tree ring widths for all tree discs. Since no inter-correlation between the trees was found no chronology could be formed. Therefore, as has been done in other research (Saurer *et al.* 1997, Fichtler *et al.* 2010), all series have been interpreted individually. Since basal area increment per year gives a more accurate quantification for wood production (Rubino and McCarthy 2000), ring width measurements were calculated into basal area increment per year (BAI) according equation 1 (Jump *et al.* 2006, Silva *et al.* 2009):

$$\text{BAI} = \pi(R_n^2 - R_{n-1}^2) \quad (1)$$

Where  $R$  is the tree radius and  $n$  is the year in which the tree ring was formed. We assumed that mean basal area increment per year was equal to entirely circular growth of a tree disc. However, tree discs of *P. laevis* are not entirely round since the trees have a buttress forming root system (Foster and Terborgh 1998). This increases the chance of BAI overestimations.

### **Isotope analysis**

Five discs were selected to obtain carbon isotope data from. To exclude the juvenile state of a tree (McCarroll and Loader 2004), isotope measurements were only conducted for rings formed at diameter >20 cm. Below this diameter size, isotope ratios of tree rings tend to show an age related trend probably initiated by aspects as changing hydraulic conductivity and respired air from the ground (Schleser and Jayasekera 1985, McCarroll and Loader 2004).

Wood samples of approximately 100 mg from every ring were cut using a gouge. Alfa-cellulose was extracted from raw wood samples using the Jayme-Wise method (Wieloch *et al.* 2011). For homogenizing, the extracted cellulose samples were placed in a demiwater solution where after the samples were shaken with 25 rps. After the homogenization, the cellulose samples were dried in an oven at 70 °C. Approximately 1 mg purified  $\alpha$ -cellulose was taken from each sample and placed into tin capsules. Subsequently, these samples were used to measure carbon isotope composition  $^{12}\text{C}$  and  $^{13}\text{C}$  by gas isotope ratio mass spectrometry (IRMS) at the GFZ in Potsdam ([www.gfz-potsdam.de](http://www.gfz-potsdam.de)).

### **Calculations of intrinsic CO<sub>2</sub> concentrations (C<sub>i</sub>)**

The  $\delta^{13}\text{C}_{\text{wood}}$  of every sample was calculated as follows (Francey *et al.* 1999):

$$\delta^{13}\text{C}_{\text{tree}} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 \quad (2)$$

Where  $R_{\text{sample}}$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  of the wood samples and  $R_{\text{standard}}$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  from Vienna-PDB (Coplen 1995).

The  $\delta^{13}\text{C}_{\text{tree}}$  values were used to calculate the magnitude of discrimination of plants against  $^{13}\text{C}$  ( $\Delta^{13}\text{C}$ ; equation 3):

$$\Delta^{13}\text{C} (\text{‰}) = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{tree}}) / (1 + \delta^{13}\text{C}_{\text{tree}}/1000) \quad (3)$$

In this calculation, not only the  $\delta^{13}\text{C}_{\text{tree}}$  but also the carbon isotope ratio of  $\text{CO}_2$  of the atmosphere ( $\delta^{13}\text{C}_a$ ) was used. The  $\delta^{13}\text{C}_a$  values were obtained from published data by McCarroll and Loader (2004) and included a period from 1850 till 2003. To obtain data from 2004 till 2010 the almost near linear decline of  $\delta^{13}\text{C}_a$  over the last decades has been extrapolated. The atmospheric  $\delta^{13}\text{C}$  records from the middle of the 19<sup>th</sup> century until now show a decline by about 1.9‰ (Helle and Schleser 2004a, McCarroll and Loader 2004). Since  $\text{CO}_2$  from fossil fuels contain lower isotope  $\delta^{13}\text{C}$  values than the atmospheric  $\text{CO}_2$  from before the industrial revolution. For calculating the intrinsic  $\text{CO}_2$  in C3 plants the following equation of Francey and Farquhar (1982) was used:

$$\Delta^{13}\text{C} (\text{‰}) = a + (b - a)(C_i/C_a) \quad (4)$$

Where  $a$  is the magnitude of discrimination of stomata against the heavier  $^{13}\text{CO}_2$  ( $\approx -4,4\text{‰}$ ) and  $b$  is the net discrimination from Rubisco during carboxylation ( $\approx -27\text{‰}$ ) (Farquhar et al. 1982).  $C_i$  and  $C_a$  are intrinsic and atmospheric  $\text{CO}_2$  concentrations ( $\mu\text{mol mol}^{-1}$ ). Atmospheric  $\text{CO}_2$  data for the period before 1958 was reconstructed from  $\text{CO}_2$  from ice cores, after 1958 data comes from actual measurements (Keeling *et al.* 2004).

This model brings some constraints; first we did not correct  $\delta^{13}\text{C}_{\text{tree}}$  for the enrichment that occurs during further fractionation processes of carbohydrates from leaf to wood tissue (McCarroll and Loader 2004). Secondly, the role of mesophyll conductance and the influence of respiration on internal  $\text{CO}_2$  could be underestimated when using the linear model for isotope discrimination of Farquhar *et al.* (1982) (Loader *et al.* 2007, Seibt *et al.* 2008). General trends however, are not affected using the linear model of Farquhar *et al.* (1982). In addition, comparing our result with the outcome of other studies was not possible using the more complex model.

## Statistical analysis

### *Long-term trends in growth and $C_i/C_a$*

To investigate long-term trends in basal area growth per year,  $\delta^{13}\text{C}_{\text{wood}}$ ,  $\Delta^{13}\text{C}$ ,  $C_i$  and  $C_i/C_a$  for each individually tree over the past century, Pearson correlation, non-parametric Spearman rho and linear regressions were used. We evaluated long-term trends in  $C_i/C_a$  as well as trends in the amount of rainfall over the years using linear regression (KNMI - climate explorer).

## Results

### Long-term trends in growth

Trends in the ring widths series of the five trees were analysed using Pearson correlations. We found significant trends in diameter growth per year ( $\text{cm}/\text{yr}^{-1}$ ) in two out of the five trees (Table 1). For the basal area increment per year ( $\text{m}^2/\text{yr}^{-1}$ ) series we found significant positive long-term trends for all five trees ( $p < 0.01$ ; Table 1). Figure 1 shows the basal area growth per year for the last century. Besides the positive trend in BAI, an increase in year-to-year variation in the individual series can be observed as well.

### Long-term trends in $\delta^{13}\text{C}$ and $C_i$

Over the past 100 years four out of the five trees showed a significant decrease in  $\delta^{13}\text{C}$  (Table: 2). One of the trees (number 8135) acted differently, probably due to the more negative values at the beginning of the 20<sup>th</sup> century. Since this difference was observed in all additional tests, we excluded the data from 1900 until 1922 in further calculations. The descending trend present in the  $\delta^{13}\text{C}_{\text{tree}}$  series is similar to the decrease in  $\delta^{13}\text{C}_{\text{atmosphere}}$  (around 1.9‰; figure 2) for the corresponding period of 1900 until 2010.

Figure 3 shows the increase in  $C_i$  over time, calculated from the  $\delta^{13}\text{C}_{\text{tree}}$  values. Internal  $\text{CO}_2$  levels of all five trees increased in a similar way as atmospheric  $\text{CO}_2$  levels over the past hundred years (table 2) resulting in high correlation coefficients. However, the absolute increase in  $C_i$  is not as strong as the increase in  $C_a$  (the increase in  $C_a$  is 93 ppm, the mean total increase in  $C_i$  of the five trees is 61 ppm over the past 110 years). Although the absolute change in  $C_a$  differed from that in  $C_i$ , the relative changes in  $C_i$  and  $C_a$  were very similar, both 31%.

Hence, no significant trends were found in  $C_i/C_a$  ratios for the corresponding period (figure 4). Averages of  $C_i/C_a$  ranged between 0.62 and 0.66 with standard errors lower than 0.004. Additionally, averaging data of all five trees and calculating five year means did not give a trend either (figure 4).

Correlations between long term-trends in  $C_i$  and basal area increment gave significant positive results for four out of five trees (table: 4; figure 5).

**Table 1:** Diameter growth per year; basal area increase per year

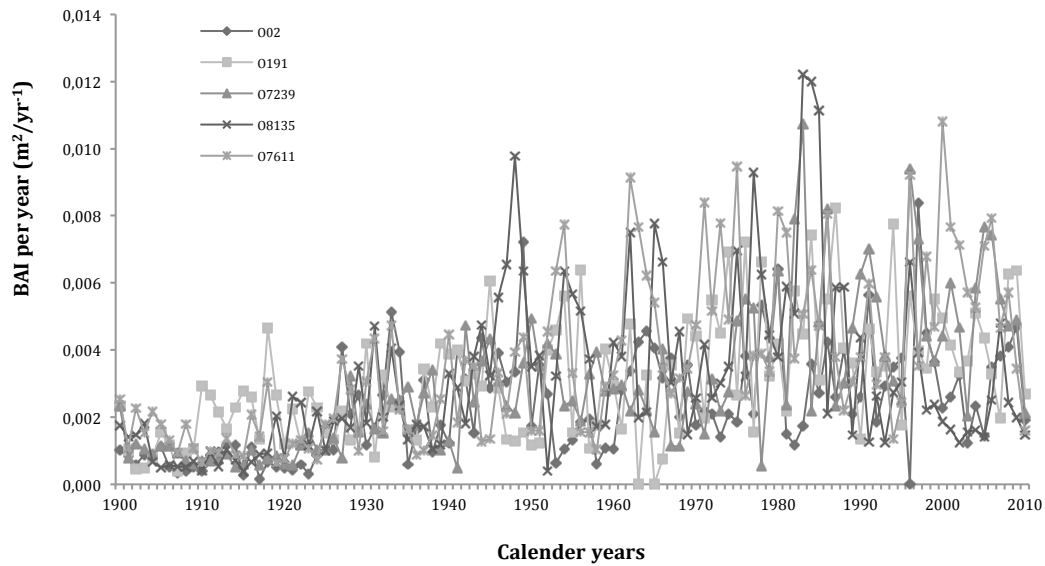
(N=111)	002	0191	07239	07611	08135
<b>Diameter growth per year (<math>\text{cm}/\text{yr}^{-1}</math>) (Pearson Correlation (Significance: 2-tailed))</b>	0.203*	0.014	0.391**	0.151	-0.017
<b>Basal area increase (<math>\text{m}^2/\text{yr}^{-1}</math>) (Spearman's rho (Significance: 2-tailed))</b>	0.582**	0.560**	0.730**	0.677**	0.446**

\*\* Correlation is significant at the 0.01 level (2-tailed); \* Correlation is significant at the 0.05 level (2-tailed).

**Table 2:**  $\delta^{13}\text{C}$  over time (Spearman's rho); Correlation  $C_i$  and BAI (Pearson); Correlation  $C_i$  and  $C_a$  (Spearman's rho)

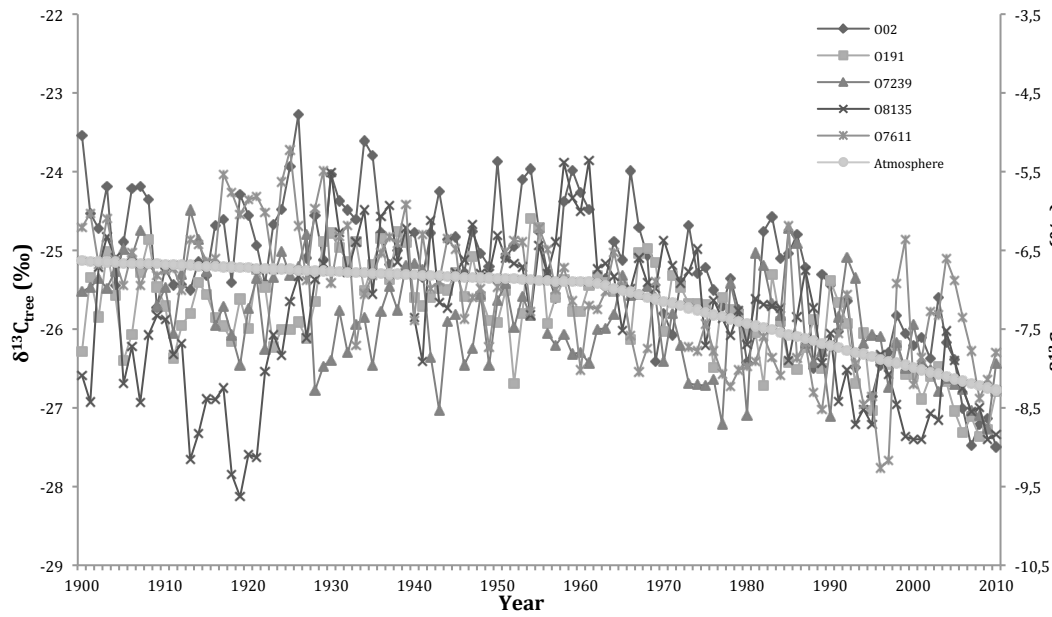
	<b>002</b>	<b>0191</b>	<b>07239</b>	<b>07611</b>	<b>08135 #</b>
<b>N</b>	109	111	109	108	88
<b><math>\delta^{13}\text{C}</math> vs. calendar year (Spearman's rho Significance (2-tailed))</b>	-0.607**	-0.467**	-0.485**	-0.685**	-0.662**
<b>Correlation <math>C_i</math> and <math>C_a</math> Spearman's rho Significance (2-tailed)</b>	0.811**	0.793**	0.825**	0.883**	0.516**
<b>Correlation <math>C_i</math> and BAI Spearman's rho Significance (2-tailed)</b>	0.514**	0.485**	0.679**	0.644**	-0.015

\*\* Correlation is significant at the 0.01 level (2-tailed); \* Correlation is significant at the 0.05 level (2-tailed). # Data from tree number 8135 starts from 1923 until 2010

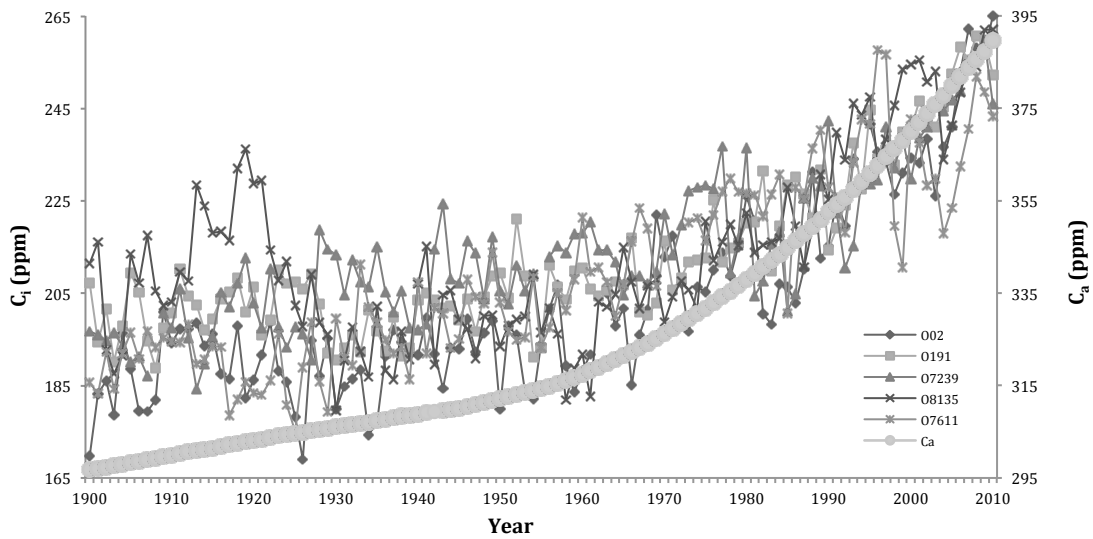


**Figure 1:** Basal area growth per year ( $\text{m}^2/\text{yr}^{-1}$ ) in five trees of *P. laevis* plotted from 1900 until 2010 for the five tree discs.

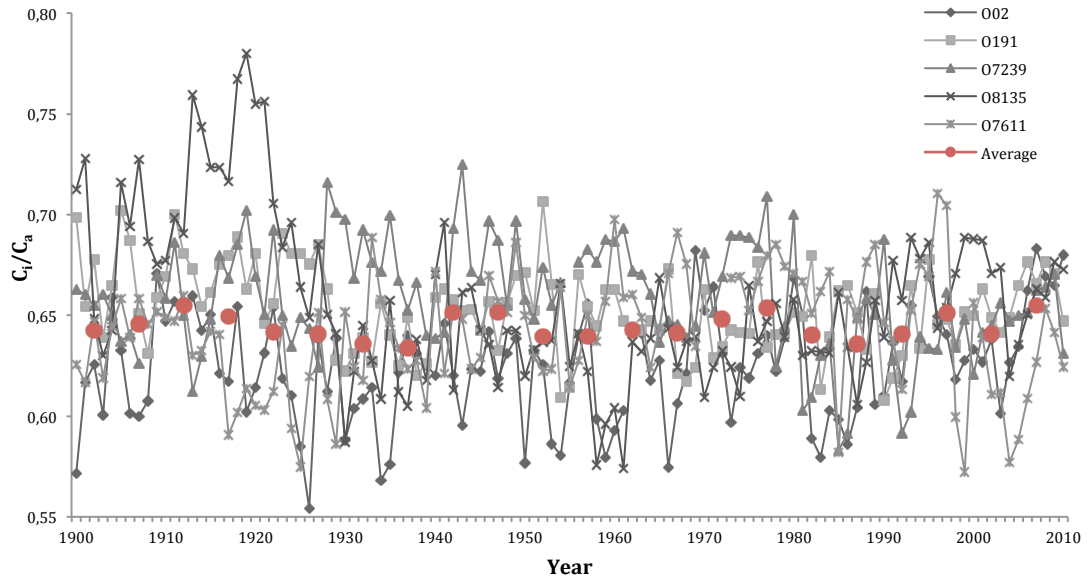




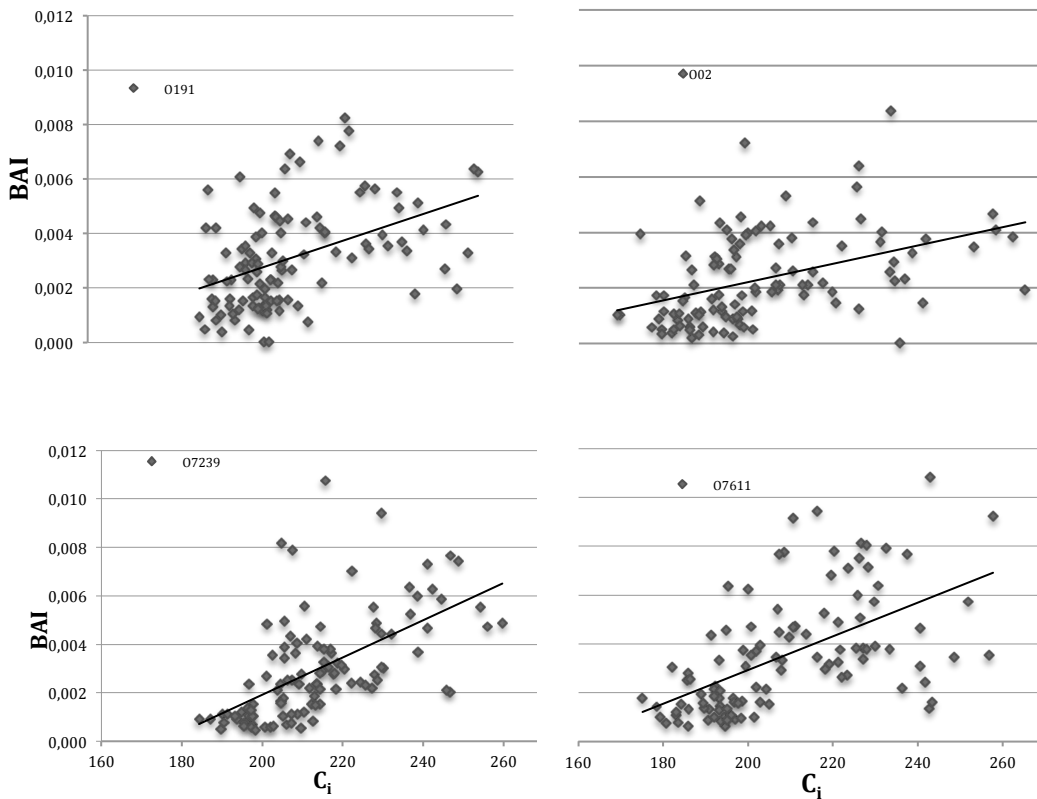
**Figure 2:**  $\delta^{13}C_{\text{tree}}$  for the five *P. laevis* trees and  $\delta^{13}C_{\text{atmosphere}}$  over time



**Figure 3:** Increase in intrinsic  $CO_2$  ( $C_i$ ) the five *P. laevis* trees; the thick grey line indicates the increase in atmospheric  $CO_2$  ( $C_a$ ). The left axis represents the increase in  $C_i$  and the right axis the increase in  $C_a$



**Figure 4:** The  $C_i/C_a$  ratios of *P. laevis* over time for the five trees. The red dots indicate a mean running average of the five individuals for 5-year segments (from tree number 8135, 1900 until 1922 are excluded).



**Figure 5:** Correlation between BAI and  $C_i$  for *P. laevis* tree numbers 0191, 002, 07239 and 07611 (Correlations table 2)

## Discussion

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The aim of this research was to investigate trends in stable carbon isotope levels and tree ring widths of a tropical tree species, *P. laevis*, in a reaction to increased atmospheric CO<sub>2</sub> levels. We found increased BAI, increased internal CO<sub>2</sub> concentrations in the leaves (C<sub>i</sub>) and constant levels of C<sub>i</sub>/C<sub>a</sub> under the increased levels of C<sub>a</sub> over the last century.

### *Long-term trends in BAI and C<sub>i</sub>/C<sub>a</sub>*

We used basal area increment (BAI) to investigate growth related trends, since this gives a more accurate quantification of the amount of wood produced (Silva 2009 Jump 2006). Just as in some other tree species (Silva *et al.* 2009) we found significant BAI increase over time. Besides this result, also other evidence for increased wood production in tropical regions has been found (Phillips *et al.* 2008, Lewis *et al.* 2009, Rozendaal *et al.* 2010). Phillips *et al.* 2008 calculated that the increased carbon storage in the 20<sup>th</sup> century of intact Amazonian forests was around 0.62 t C ha<sup>-1</sup>yr<sup>-1</sup>. This increase in carbon storage could be the result of CO<sub>2</sub> fertilization although, also the availability of nitrogen and phosphorus play an important role in tree growth. Hietz *et al.* (2011) found increased nitrogen fertilization in the tropical regions influencing forest dynamics. In addition, soil phosphor content has been proposed as a strong regulator of BAI of tropical trees (Baribault 2011). In contrast, other studies did not found any evidence for increased growth rates of tropical trees (Nock *et al.* 2011, Peñuelas *et al.* 2011). In some studies a reduction in increase of BAI occurred over time, Jump (2006) proposed competition between canopy trees as a driving factor for this phenomena. However, also other factors as increased drought and temperatures can have a negative effect on the assimilation capacity of leaves, which influence growth more than increased CO<sub>2</sub> levels (Peñuelas *et al.* 2011). Peñuelas *et al.* (2011) suggested that forests are already saturated in their response to CO<sub>2</sub> due to growth limiting factors such as drought stress and low availability of phosphor and nitrogen. However, in this study only changes in tree ring width and not BAI have been studied.

Brienen *et al.* (2012) proposed possible biases in interpretation trends of tree ring width or basal area increment due to selecting only the big (fast growing) trees. Besides tree selection, BAI probably overestimates the growth of *P. laevis* since the tree discs are not round but lobbed due to the buttress forming root system of *P. laevis*. Furthermore, no chronology could be formed out of our tree ring data, although the annularity of the tree rings of *P. laevis* was already proven by Rozendaal *et al.* (2010). These difficulties forming a chronology could be due to the small number of samples (five tree discs), in other dendrochronology studies often only a small number of the measured trees end up in a chronology. Sauer *et al.* (1997) and Fichtlear *et al.* (2010) showed that individual evaluating of trees does not give a problem during investigation of trends in stable isotopes and tree ring width. The proposed overestimation in BAI will probably not influence the general trends.

Analysing trends in internal carbon derived from  $\delta^{13}\text{C}_{\text{tree}}$  we found increasing C<sub>i</sub> levels over time for all five trees. Converting the C<sub>i</sub> series into C<sub>i</sub>/C<sub>a</sub> ratios we found no significant trends overtime which can be considered as an active reaction of plants to

increased atmospheric CO<sub>2</sub> (McCarroll *et al.* 2009). An active response with maintenance of constant C<sub>i</sub>/C<sub>a</sub> ratio can be caused by a decrease in stomatal conductance, an increase in assimilation rate or a combination of both (Medlyn *et al.* 2001). It is however, not possible to determine the contribution of each of those factors. The increase in BAI suggests that increased assimilation, which results in increased biomass production, at least partially causes the active response. Similarly, Bonal *et al.* (2011) found an increase in photosynthesis instead of a decrease in stomatal conductance, in their study on the responses of leaves of two herbaria trees from French Guinea to increased CO<sub>2</sub>. Higher assimilation rates contribute to higher carbon storage and could therefore be of great importance in decreasing atmospheric CO<sub>2</sub> levels and thus in decreasing climate change.

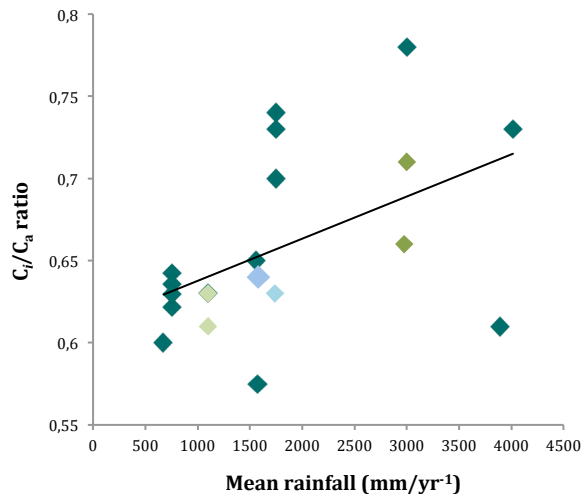
However, it is likely that along with an increase in assimilation, stomatal conductance declines. The consequences of a decrease in stomatal conductance, including a reduced transpiration rate, needs to be considered. Reduced transpiration could potentially affect environmental conditions such as hydrological budgets (Saurer *et al.* 2004). It is known that 25-50% of the precipitation in the amazon basin depends on recycled water from its own evapotranspiration (Cochrane and Barber 2009), a decrease in evapotranspiration could lead to increased drought stress (Davidson *et al.* 2012). In addition, not only the Amazonian basin but also entire Southern America and even global hydrological cycles could be affected (Fearnside 1985, Eltahir and Bras 1994, Malhi *et al.* 2008). Often changes in stomatal conductance are not considered as a threat for the hydrological cycle therefore this could come on top of already predicted decreases in precipitation of the Amazonian basin (Moore *et al.* 2007). Besides, that the hydrological system will be affected by a decrease in evapotranspiration, temperatures could also increase due to a decline in cloud cover (Malhi *et al.* 2008). Altogether, increasing temperatures and decreasing precipitation rates could lead to an increase in droughts, which could induce forest die back (Phillips *et al.* 2010).

We summarized the outcome of similar studies that were performed in the tropics to get a broader view of the reaction of tropical tree species to increased CO<sub>2</sub> levels (table 3). Similar to our result, most studies found a stable C<sub>i</sub>/C<sub>a</sub> ratio (Hietz *et al.* 2005, Jenkins 2009, Nock *et al.* 2011, Loader *et al.* 2011). Although, in a study performed in a dry tropical forest (annual rainfall 930 mm; Brienen *et al.* 2010) a strong decrease in C<sub>i</sub>/C<sub>a</sub> has been found. In contrast, at nearly the same rainfall level (1100 mm Nock *et al.* (2011) and 930 mm Brienen *et al.* (2010)), Nock *et al.* (2011) found the opposite result. Their research revealed that C<sub>i</sub> increased parallel to C<sub>a</sub> (Nock *et al.* 2011). Considering the amount of studies, it seems that C<sub>i</sub>/C<sub>a</sub> ratios of trees in both temperate and tropical forests appear to stay constant (Saurer *et al.* 2004). In order to conclude this, more research needs to be done. Interestingly, all tropical studies with stable C<sub>i</sub>/C<sub>a</sub> ratios are in high rainfall classes while the C<sub>i</sub>/C<sub>a</sub> ratios in studies in drier areas are not stable. A decrease in C<sub>i</sub>/C<sub>a</sub> in areas with lower precipitation rates contributes to the idea that stomata of trees in drier surrounding act more water conservative (Brienen *et al.* 2011). However, since an increase in C<sub>i</sub>/C<sub>a</sub> has been found as well (Nock *et al.* 2011), this conclusion cannot be approved. Nevertheless, rainfall could be a driver of differences found in the reaction of trees to increased atmospheric CO<sub>2</sub> levels.

For examining a relation between  $C_i/C_a$  and rainfall we correlated the mean  $C_i/C_a$  of the different studies with the mean annual rainfall of the corresponding areas ( $R^2 = 0.378$   $p = 0.04$ ; figure 6). We found that an increase in rainfall leads to a higher  $C_i/C_a$  ratios, which is an indication for higher stomatal conductance. It could be that trees are less water conservative in areas with high amount of precipitation through what stomatal conductance decreases and  $C_i$  levels become higher.

Considered must be that variation in  $C_i/C_a$  could be the effect of different methods used for extracting cellulose from the whole wood samples. This can lead to differences in lignin content remaining in the cellulose samples, where higher lignin concentrations often lead to lower  $C_i/C_a$  (Hietz *et al.* 2005). Additionally, species of trees could differ in mesophyll conductance, which could alter the  $C_i/C_a$  results (Loader *et al.* 2007).

Instead of using the amounts of rainfall mentioned in every study we could have used the Palmer drought severity index (PDSI) to correlate the  $C_i/C_a$  with. Since this parameter reflects better the mixture between precipitation and temperature could be more accurate for a trees physiological behaviour than annual rainfall (Treydte *et al.* 2007).



**Figure 6:** Relation between mean  $C_i/C_a$  ratios and mean annual precipitation rates. (◆: result this research ◆: Nock *et al.* 2011 ◆: Jenkins *et al.* 2009 ◆: Hietz *et al.* 2005 ◆: Other research; Gebrekirstos *et al.* 2010, Fichtler *et al.* 2010) ( $R^2 = 0.378$ ;  $p = 0.04$ )

We conclude that most of the studied tropical tree species, including *P. laevis*, show an active response with maintenance of  $C_i/C_a$  ratios under increasing  $CO_2$  levels. This could have major effects on carbon and hydrology cycles in the tropical regions. However, more tropical tree species need to be examined for better understanding of the response of tropical forests to elevated levels of  $CO_2$ .

**Table 3:** Overview of results in changes in  $C_i/C_a$  ratios in tropic and sub tropic regions. \*Calculated from mean  $\delta^{13}C$  article \*\* Correction mentioned in article \*\*\* Correction from bulk wood to cellulose ( $\delta^{13}C$  tree -1.3‰ - (Fichtler et al. 2010)

Tree species	Country	Tree type	Mean annual rainfall (mm)	Period	$C_i/C_a$ mean values	$C_i/C_a$ overtime	Article
<i>C. odorata</i>	Brasil. Rio branco	Deciduous	3000	1850 - 1990	0.74 - 0.8 0.71**	Slightly decrease $C_i/C_a$ overtime	Hietz 2005
<i>S. macrophylla</i>	Brasil, Rio branco	Semi - deciduous	3000	1850 - 1990	0.74 - 0.8 0.78**	Slightly decrease $C_i/C_a$ overtime	Hietz 2005
<i>C. tabularis</i>	Thailand	Evergreen	1100	1960 - 2003	0.63*	Decreasing $C_i/C_a$ before 1960, after 1960 increasing $C_i/C_a$	Nock 2011
<i>M. azedarach</i>	Thailand	Deciduous	1100	1960 - 2003	0.61*	Slightly decreasing $C_i/C_a$	Nock 2011
<i>T. ciliata</i>	Thailand	Deciduous	1100	1960 - 2003	0.63*	Decreasing $C_i/C_a$ before 1960, after 1960 increasing $C_i/C_a$	Nock 2011
<i>D. micrantha</i>	Peru; Madre de Dios department	Evergreen	1740	1820 - 2006	0.60 - 0.66*	Nearly constant $C_i/C_a$	Jenkins 2009
<b><i>P. laevis</i></b>	<b>Bolivia</b>	<b>Evergreen</b>	<b>1580</b>	<b>1900 - 2010</b>	<b>0.62 - 0.66</b>	<b>Nearly constant <math>C_i/C_a</math></b>	<b>This research</b>
<i>M. acantholoba</i>	Mexico	Deciduous	930 (380 - 1850)	1968 - 2005	-	Constant $C_i$ increasing $C_a$ . Decreasing $C_i/C_a$	Brienen 2011
<i>E. zwageri</i>	Malysian Borneo Imbak	Evergreen	> 3000	1850 - 2009	-	Constant $C_i/C_a$	Loader 2011
<i>S. johorensis</i>	Malysian Borneo	Evergreen	2873	1850 - 2009	-	Constant $C_i/C_a$	Loader 2011
<i>S. superba</i>	Malysian Borneo	Evergreen	2873	1850 - 2009	-	Constant $C_i/C_a$	Loader 2011

<i>A. senegal</i>	Ethiopie	Drought deciduous	760 (550 - 900)	1973 - 2002	0.58* 0.64***	-	Gebrekirstos 2010
<i>A. seyal</i>	Ethiopie	Drought deciduous	760 (550 - 900)	1973 - 2002	0.58* 0.64***	-	Gebrekirstos 2010
<i>A. tortilis</i>	Ethiopie	Drought deciduous	760 (550 - 900)	1973 - 2002	0.56* 0.62***	-	Gebrekirstos 2010
<i>B. aegyptiaca</i>	Ethiopie	Evergreen	760 (550 - 900)	1973 - 2002	0.57* 0.63***	-	Gebrekirstos 2010
<i>T. sericera</i>	Namibia, Katima Mulilo	Obligate deciduous	675	1988 - 1998	0.57 - 0.63*	-	Fichtler 2010
<i>T. superba</i>	Cameroon Biakoa	Obligate deciduous	1570	1925 - 1938	0.56 - 0.59*	-	Fichtler 2010
<i>T. amazonia</i>	Venezuela, Dorado Tumeremo	Evergreen	1560	1978 - 1998	0.65*	-	Fichtler 2010
<i>T. amazonia</i>	Venezuela, km 98 Sierra de Lema	Evergreen	2975	1975- 1998	0.66*	-	Fichtler 2010
<i>T. amazonia</i>	Costa Rica, La Selva	Evergreen	4015	1985 - 1998	0.73*	-	Fichtler 2010
<i>T. guyanensis</i>	Venezuela, Caparo	Evergreen	1750	1959-1971 1960-1963 1965-1988	0.70*	-	Fichtler 2010
<i>C. odorata</i>	Venezuela, Caparo	Obligate deciduous	1750	1969 - 1989	0.74*	-	Fichtler 2010
<i>S. macrophylla</i>	Venezuela, Caparo	Obligate deciduous	1750	1980 - 1989	0.73*	-	Fichtler 2010
<i>T. quitalata</i>	Venezuela, Uriman	Evergreen	3890	1958 - 1980	0.61*	-	Fichtler 2010

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## References

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- Baribault, T.W. 2011, "Revisiting soil resource limitation: Resource predictors of tree growth and forest productivity change across ecological gradients", *Dissertation Abstracts International*, vol. 72, no. 05.
- Blate, G.M. 2005, "Modest trade-offs between timber management and fire susceptibility of a Bolivian semi-deciduous forest", *Ecological Applications*, vol. 15, no. 5, pp. 1649-1663.
- Bonan, G.B. 2008, "Forests and climate change: Forcings, feedbacks, and the climate benefits of forests", *Science*, vol. 320, no. 5882, pp. 1444-1449.
- Brienen, R.J.W., Wanek, W. & Hietz, P. 2011, "Stable carbon isotopes in tree rings indicate improved water use efficiency and drought responses of a tropical dry forest tree species", *Trees - Structure and Function*, vol. 25, no. 1, pp. 103-113.
- Cess, R.D. 1976, "Climate change: An appraisal of atmospheric feedback mechanisms employing zonal climatology." *Journal of Atmospheric Sciences*, vol. 33, pp. 1831-1843.
- Clark, D.A. 2004, "Sources or sinks? The responses of tropical forests to current and future climate and atmospheric composition", *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, vol. 359, no. 1443, pp. 477-491.
- Cochrane, M. & Barber, C. 2009, "Climate change, human land use and future fires in the Amazon.", *Global Change Biology*, vol. 15, no. 3, pp. 601-612.
- Coplen, T.B. 1995, "Discontinuance of SMOW and PDB [6]", *Nature*, vol. 375, no. 6529, pp. 285.
- Davidson, E.A., De Araújo, A.C., Artaxo, P., Balch, J.K., Brown, I.F., C. Bustamante, M.M., Coe, M.T., Defries, R.S., Keller, M., Longo, M., Munger, J.W., Schroeder, W., Soares-Filho, B.S., Souza Jr., C.M. & Wofsy, S.C. 2012, "The Amazon basin in transition", *Nature*, vol. 481, no. 7381, pp. 321-328.
- Eltahir, E. & Bras, R. 1994, "Precipitation recycling in the Amazon basin", *Quarterly Journal of the Royal Meteorological Society*, vol. 120, no. 518, pp. 861-880.
- Farquhar, G.D., O'Leary, M.H. & Berry, J.A. 1982, "On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves.", *Australian Journal of Plant Physiology*, vol. 9, no. 2, pp. 121-137.
- Fearnside, P.M. 1985, "Environmental change and deforestation in the Brazilian Amazon", *Change in the Amazon Basin: Man's impact on forests and rivers*, pp. 70-89.
- Fichtler, E., Helle, G. & Worbes, M. 2010, "Stable-carbon isotope time series from tropical tree rings indicate a precipitation signal", *Tree-Ring Research*, vol. 66, no. 1, pp. 35-49.
- Foster, M.S. & Terborgh, J. 1998, "Impact of a Rare Storm Event on an Amazonian Forest1", *Biotropica*, vol. 30, no. 3, pp. 470-474.
- Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M., Langenfelds, R.L., Michel, E. & Steele, L.P. 1999, "A 1000-year high precision record of  $\delta^{13}\text{C}$  in atmospheric  $\text{CO}_2$ ", *Tellus, Series B: Chemical and Physical Meteorology*, vol. 51, no. 2, pp. 170-193.

- Fredericksen, T.S. & Pariona, W. 2002, "Effect of skidder disturbance on commercial tree regeneration in logging gaps in a Bolivian tropical forest", *Forest Ecology and Management*, vol. 171, no. 3, pp. 223-230.
- Helle, G. & Schleser, G.H. 2004a, "Beyond CO<sub>2</sub>-fixation by Rubisco - An interpretation of <sup>13</sup>C/<sup>12</sup>C variations in tree rings from novel intra-seasonal studies on broad-leaf trees", *Plant, Cell and Environment*, vol. 27, no. 3, pp. 367-380.
- Helle, G. & Schleser, G.H. 2004b, "Interpreting climate proxies from tree-rings", *Climate in Historical Times: Towards a Synthesis of Holocene Proxy Data and Climate Models*, pp. 129-148.
- Hietz, P., Turner, B.L., Wanek, W., Richter, A., Nock, C.A. & Wright, S.J. 2011, "Long-term change in the nitrogen cycle of tropical forests", *Science*, vol. 334, no. 6056, pp. 664-666.
- Hietz, P., Wanek, W. & Dünisch, O. 2005, "Long-term trends in cellulose  $\delta^{13}\text{C}$  and water-use efficiency of tropical *Cedrela* and *Swietenia* from Brazil", *Tree physiology*, vol. 25, no. 6, pp. 745-752.
- Holmes, R.L. 1983, "Computer-assisted quality control in tree-ring dating and measurement", *Tree-ring bulletin*, vol. 43, no. 1, pp. 69-78.
- Jenkins, H.S. 2009, Amazon Climate Reconstruction Using Growth Rates and Stable Isotopes of Tree Ring Cellulose from the Madre de Dios Basin, *Peru*.
- Jump, A.S., Hunt, J.M. & Peñuelas, J. 2006, "Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*", *Global Change Biology*, vol. 12, no. 11, pp. 2163-2174.
- Justiniano, M.J. & Nash, D. 2002, Ecología de Especies Menos Conocidas Ojoso colorado (*Pseudolmedia laevis*), *Proyecto de Manejo Forestal Sostenible (BOLFOR)*.
- Keeling, C.D., Piper, S.C., Bacastow, R.B., Wahlen, M., Whorf, T.P., Heimann, M. & Meijer, H.A. 2001, "Exchanges of atmospheric CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> with the terrestrial biosphere and oceans from 1978 to 2000. I. Global aspects"
- Keeling, C.D., Brix, H. & Gruber, N. 2004, "Seasonal and long-term dynamics of the upper ocean carbon cycle at Station ALOHA near Hawaii", *Global Biogeochemical Cycles*, vol. 18, no. 4, pp. 1-26.
- Lewis, S.L., Lopez-Gonzalez, G., Sonké, B., Affum-Baffoe, K., Baker, T.R., Ojo, L.O., Phillips, O.L., Reitsma, J.M., White, L. & Comiskey, J.A. 2009, "Increasing carbon storage in intact African tropical forests", *Nature*, vol. 457, no. 7232, pp. 1003-1006.
- Loader, N.J., Walsh, R.P.D., Robertson, I., Bidin, K., Ong, R.C., Reynolds, G., McCarroll, D., Gagen, M. & Young, G.H.F. 2011, "Recent trends in the intrinsic water-use efficiency of ringless rainforest trees in Borneo", *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 366, no. 1582, pp. 3330-3339.
- Loader, N.J., McCarroll, D., Gagen, M., Robertson, I. & Jalkanen, R. 2007, "Extracting Climatic Information from Stable Isotopes in Tree Rings" in *Terrestrial Ecology*, ed. Todd E. Dawson and Rolf T. W. Siegwolf, Elsevier, pp. 25, 27-48.
- Malhi, Y., Roberts, J.T., Betts, R.A., Killeen, T.J., Li, W. & Nobre, C.A. 2008, "Climate change, deforestation, and the fate of the Amazon", *Science*, vol. 319, no. 5860, pp. 169.

- McCarroll, D., Gagen, M.H., Loader, N.J., Robertson, I., Anchukaitis, K.J., Los, S., Young, G.H.F., Jalkanen, R., Kirchhefer, A. & Waterhouse, J.S. 2009, "Correction of tree ring stable carbon isotope chronologies for changes in the carbon dioxide content of the atmosphere", *Geochimica et Cosmochimica Acta*, vol. 73, no. 6, pp. 1539-1547.
- McCarroll, D. & Loader, N.J. 2004, "Stable isotopes in tree rings", *Quaternary Science Reviews*, vol. 23, no. 7-8, pp. 771-801.
- Medlyn, B.E., Barton, C.V.M., Broadmeadow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S.B., Kellomäki, S., Laitat, E., Rey, A., Roberntz, P., Sigurdsson, B.D., Strassmeyer, J., Wang, K., Curtis, P.S. & Jarvis, P.G. 2001, "Stomatal conductance of forest species after long-term exposure to elevated CO<sub>2</sub> concentration: A synthesis", *New Phytologist*, vol. 149, no. 2, pp. 247-264.
- Moore, N., Arima, E., Walker, R. & da Silva, R.R. 2007, "Uncertainty and the changing hydroclimatology of the Amazon", *Geophys.Res.Lett.*, vol. 34, pp. L14707.
- Nock, C.A., Baker, P.J., Wanek, W., Leis, A., Grabner, M., Bunyavejchewin, S. & Hietz, P. 2011, "Long-term increases in intrinsic water-use efficiency do not lead to increased stem growth in a tropical monsoon forest in western Thailand", *Global Change Biology*, vol. 17, no. 2, pp. 1049-1063.
- Paz-Rivera, C.L. 2003, *Forest-use history and the soils and vegetation of a lowland forest in Bolivia*.
- Peña-Claros, M., Fredericksen, T.S., Alarcón, A., Blate, G.M., Choque, U., Leñaño, C., Licona, J.C., Mostacedo, B., Pariona, W., Villegas, Z. & Putz, F.E. 2008, "Beyond reduced-impact logging: Silvicultural treatments to increase growth rates of tropical trees", *Forest Ecology and Management*, vol. 256, no. 7, pp. 1458-1467.
- Peñuelas, J., Canadell, J.G. & Ogaya, R. 2011, "Increased water-use efficiency during the 20th century did not translate into enhanced tree growth", *Global Ecology and Biogeography*, .
- Phillips, O.L., Lewis, S.L., Baker, T.R., Chao, K.J. & Higuchi, N. 2008, "The changing Amazon forest", *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 363, no. 1498, pp. 1819-1827.
- Phillips, O.L., van der Heijden, G., Lewis, S.L., López-González, G., Aragão, L.E.O.C., Lloyd, J., Malhi, Y., Monteagudo, A., Almeida, S., Dávila, E.A., Amaral, I., Andelman, S., Andrade, A., Arroyo, L., Aymard, G., Baker, T.R., Blanc, L., Bonal, D., de Oliveira, T.C.A., Chao, K., Cardozo, N.D., da Costa, L., Feldpausch, T.R., Fisher, J.B., Fyllas, N.M., Freitas, M.A., Galbraith, D., Gloor, E., Higuchi, N., Honorio, E., Jiménez, E., Keeling, H., Killeen, T.J., Lovett, J.C., Meir, P., Mendoza, C., Morel, A., Vargas, P.N., Patiño, S., Peh, K.S., Cruz, A.P., Prieto, A., Quesada, C.A., Ramírez, F., Ramírez, H., Rudas, A., Salamão, R., Schwarz, M., Silva, J., Silveira, M., Ferry Slik, J.W., Sonké, B., Thomas, A.S., Stropp, J., Taplin, J.R.D., Vásquez, R. & Vilanova, E. 2010, "Drought-mortality relationships for tropical forests", *New Phytologist*, vol. 187, no. 3, pp. 631-646.
- Rozendaal, D.M.A., Brienen, R.J.W., Soliz-Gamboa, C.C. & Zuidema, P.A. 2010, "Tropical tree rings reveal preferential survival of fast-growing juveniles and increased juvenile growth rates over time", *New Phytologist*, vol. 185, no. 3, pp. 759-769.
- Rozendaal, D.M.A. & Zuidema, P.A. 2011, "Dendroecology in the tropics: A review", *Trees - Structure and Function*, vol. 25, no. 1, pp. 3-16.
- Rubino, D.L. & McCarthy, B.C. 2000, "Dendroclimatological analysis of white oak (*Quercus alba* L., Fagaceae) from an old-growth forest of southeastern Ohio, USA", *Journal of the Torrey Botanical Society*, , pp. 240-250.

- Saurer, M., Borella, S., Schweingruber, F. & Siegwolf, R. 1997, "Stable carbon isotopes in tree rings of beech: Climatic versus site-related influences", *Trees - Structure and Function*, vol. 11, no. 5, pp. 291-297.
- Saurer, M., Siegwolf, R.T.W. & Schweingruber, F.H. 2004, "Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years", *Global Change Biology*, vol. 10, no. 12, pp. 2109-2120.
- Schleser, G.H. & Jayasekera, R. 1985, " $\delta^{13}\text{C}$ -variations of leaves in forests as an indication of reassimilated  $\text{CO}_2$  from the soil", *Oecologia*, vol. 65, no. 4, pp. 536-542.
- Seibt, U., Rajabi, A., Griffiths, H. & Berry, J.A. 2008, "Carbon isotopes and water use efficiency: sense and sensitivity", *Oecologia*, vol. 155, no. 3, pp. 441-454.
- Silva, L.C.R., Anand, M., Oliveira, J.M. & Pillar, V.D. 2009, "Past century changes in *Araucaria angustifolia* (Bertol.) Kuntze water use efficiency and growth in forest and grassland ecosystems of southern Brazil: Implications for forest expansion", *Global Change Biology*, vol. 15, no. 10, pp. 2387-2396.
- Thompson, L.G. 2010, "Climate change: The evidence and our options", *Behavior Analyst*, vol. 33, no. 2, pp. 153-170.
- Treydte, K., Frank, D., Esper, J., Andreu, L., Bednarz, Z., Berninger, F., Boettger, T., D'Alessandro, C.M., Etien, N., Filot, M., Grabner, M., Guillemin, M.T., Gutierrez, E., Haupt, M., Helle, G., Hiltunen, E., Jungner, H., Kalela-Brundin, M., Krapiec, M., Leuenberger, M., Loader, N.J., Masson-Delmotte, V., Pazdur, A., Pawelczyk, S., Pierre, M., Planells, O., Pukiene, R., Reynolds-Henne, C.E., Rinne, K.T., Saracino, A., Saurer, M., Sonninen, E., Stievenard, M., Switsur, V.R., Szczepanek, M., Szychowska-Krapiec, E., Todaro, L., Waterhouse, J.S., Weigl, M. & Schleser, G.H. 2007, "Signal strength and climate calibration of a European tree-ring isotope network", *Geophysical Research Letters*, vol. 34, no. 24.
- Wieloch, T., Helle, G., Heinrich, I., Voigt, M. & Schyma, P. 2011 "A novel device for batch-wise isolation of  $\alpha$ -cellulose from small-amount wholewood samples", *Dendrochronologia*, .