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Stable carbon isotopes in tree rings indicate improved water use efficiency and drought responses of a tropical dry forest tree species

Roel J. W. Brienen · Wolfgang Wanek · Peter Hietz

Abstract Understanding the responses of tropical trees to increasing $[CO_2]$ and climate change is important as tropical forests play an important role in carbon and hydrological cycles. We used stable carbon isotopes ($\delta^{13}C$) in tree rings to study the physiological responses of a tropical dry forest tree species in southern Mexico, *Mimosa acantholoba* to changes in atmospheric $[CO_2]$ and variation in climate. Based on annual records of tree ring $\delta^{13}C$, we calculated intrinsic water use efficiency (W_i) and intercellular $[CO_2]$ (c_i). Our results showed that trees responded strongly to the increase in atmospheric $[CO_2]$ over the last four decades; W_i increased dramatically by 40%, while c_i remained largely constant. The maintenance of a constant c_i indicates that photosynthetic rates are unlikely to have increased in response to higher $[CO_2]$, and that improvements in W_i are probably due to a reduction in stomatal conductance. This may have large consequences for the hydrological cycle. Inter-annual variation in c_i was strongly correlated with total annual rainfall ($r = 0.70$), and not influenced by temperature, solar radiation or cloud cover. Our results show that $\delta^{13}C$ in tree rings of tropical dry forest trees may be a powerful tool to evaluate long-term responses of trees to increasing $[CO_2]$ and to variation in climate.

Keywords Carbon dioxide · Climate change · Isotope discrimination · Mexico · Tropical dendrochronology

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Introduction

Understanding responses of tropical trees to climate change and increasing levels of atmospheric $[CO_2]$ is important as tropical forests process large amounts or carbon and water through photosynthesis and transpiration (Malhi and Grace 2000). Thus, small changes in growth rates or water use efficiencies of tropical trees affect the carbon and water cycles, and the rate of climate change itself (Betts et al. 2004; Henderson-Sellers et al. 1995). A powerful way of obtaining insight into the response of trees to climate change and $[CO_2]$ is by use of tree rings (Fritts 1976). Tree rings not only record historical growth rates, but also provide an archive of stable isotope ratios in tree ring cellulose over the lifetime of the tree (McCarroll and Loader 2004). Stable carbon isotope ratios ($\delta^{13}C$) in tree rings are the result of discrimination against the heavier $^{13}CO_2$ during carboxylation and diffusion through the stomata, which are linearly related to the ratio of intercellular and atmospheric $[CO_2]$ (c_i/c_a) (Francey and Farquhar 1982). This

ratio is driven by the demand of CO_2 for photosynthesis (A) and supply through stomatal conductance (g_s). The carbon isotope signal of plant matter relative to atmospheric $\delta^{13}CO_2$ ($\Delta^{13}C$) is therefore interpreted to reflect the balance between photosynthesis and stomatal conductance (Francey and Farquhar 1982).

Water use efficiency is the ratio of A to transpiration (E), and is a measure of the amount of water used per carbon gain. $\Delta^{13}C$ is related to the ratio of A/g_s and termed intrinsic water use efficiency (W_i), intrinsic because it assumes constant evaporative demand (Ehleringer et al. 1993; Osmond et al. 1980). By taking into account changes in the leaf-to-air water vapour pressure over time, we may adjust for losses through changes in the evaporative demand and obtain a measure of true changes in water use efficiency (A/E) (Ehleringer et al. 1993; Seibt et al. 2008). Changes in water use efficiency are very important as changes in the rate of assimilation affect the uptake of $[CO_2]$ by trees from the atmosphere, while changes in stomatal conductance and transpiration rates may have large consequences for the hydrological cycle (Betts et al. 2004; Henderson-Sellers et al. 1995).

In general, plants tend to reduce stomatal conductance (g_s) and increase their assimilation (A) in response to increased $[CO_2]$. Thus, their intrinsic water use efficiency (A/g_s) increases (Ehleringer et al. 1993; Farquhar et al. 1989), enabling them to absorb the same amount of carbon for less water loss. Such increases in W_i have been observed in short-term experiments of tree responses to elevated $[CO_2]$ (Norby et al. 1999 and references therein), and over long-time periods using records of $\delta^{13}C$ in tree rings that reflect the global increase in atmospheric $[CO_2]$ (Feng 1999; Waterhouse et al. 2004). An increase in W_i in response to increasing $[CO_2]$ since the industrial revolution has been found in nearly all temperate trees studied (Feng 1999; Saurer et al. 2004; Waterhouse et al. 2004), while for the tropics very few records of long-term W_i exist (Hietz et al. 2005; Nock et al. 2010). Studies on carbon isotope ratios over longer time scales in tropical trees are particularly important as the degree to which tropical forests have responded to increasing $[CO_2]$ and acted as carbon sinks over the last century is still a topic of heated scientific debate (Körner 2003; Lewis et al. 2009; Lloyd and Farquhar 2008). Decadal scale inventory studies show an increase in biomass of tropical forests, thought to be due to CO_2 fertilisation (Lewis et al. 2009; Lloyd and Farquhar 2008). However, others do not find such growth increases, and argue that CO_2 fertilisation does not affect the growth of tropical forest (Clark et al. 2010; Körner 2003). Predicted changes in the hydrological cycle include increases in soil humidity and runoff, and reductions in rainfall (Betts et al. 2004; Gedney et al. 2006; Henderson-Sellers

et al. 1995). Assessing the magnitude of physiological responses of trees to increasing $[CO_2]$ is important to evaluate the potential impact of increasing $[CO_2]$ on carbon and hydrological cycles of tropical forests.

Stable carbon isotopes have been applied widely in temperate trees (McCarroll and Loader 2004 and references therein), whereas applications in tropical trees are scarce. Some have used carbon isotopes to detect annual cycles in ringless species (Pons and Helle 2010 and references therein), but very few studies analysed stable isotope ratios in annual rings over longer timescales (Cullen et al. 2008; Fichtler et al. 2010; Gebrekirstos et al. 2009). Here, we analysed carbon isotope ratios ($\delta^{13}C$) over the last 40 years in tree rings of *Mimosa acantholoba*, a tropical dry forest pioneer species from southern Mexico. This species forms annual rings and showed strong growth responses to inter-annual variation in rainfall (Brienen et al. 2010). Annual records of $\delta^{13}C$ in tree rings are used to calculate intrinsic water use efficiency (W_i,) and intercellular $[CO_2]$, c_i. Specific questions addressed in this study are: (1) to what degree did c_i, and thus W_i, change in response to increasing atmospheric $[CO_2]$ over the last four decades? (2) Are long-term changes in W_i reflected in long-term changes in growth rates? (3) Is inter-annual variation in c_i reflected in variation in rainfall, temperature, cloud cover, and incoming solar radiation?

Methods

Study area and climate

The study area is located on the Pacific slope of the Isthmus of Tehuantepec, close to the village of Nizanda in the state of Oaxaca, South Mexico (16°39′N, 95°00′W). The natural vegetation consists of tropical deciduous dry forest (Brienen et al. 2010). Mean annual temperature in the study region is 26°C and total annual rainfall is ∼930 mm. Rainfall is highly seasonal with a pronounced dry season from November until May (<50 mm/month), and a wet season from June until October that accounts for 90% of the annual rainfall.

Variation in annual rainfall is high, varying fivefold over the period 1968–2007, from 380–1,850 mm. The principal drivers behind this variation in rainfall are sea surface temperature anomalies in the Pacific and Atlantic Oceans. Effects of El Niño–Southern Oscillation (ENSO) on climate in the region are particularly pronounced with reduced rainfall during El Niño years and higher temperatures and solar radiation during the wet season (Brienen et al. 2010).

Study species

Mimosa acantholoba (Willd.) Poir. (Fabaceae) is a common dry-forest pioneer tree that reaches up to 7 m in height, ca. 20 cm in diameter and has a maximum age of ca. 40 years. In the study area, *M. acantholoba* is one of the first pioneers to colonize abandoned agricultural field and often forms mono-dominant stands (Lebrija-Trejos et al. 2008). *M. acantholoba* is strictly deciduous, shedding leaves at the end of the wet season (November–December) and forming new leaves usually after the first rains (May–June).

Mimosa acantholoba forms distinct annual rings characterized by a higher density of vessels and larger vessel size at the beginning of each growth zone (i.e., semi-ring porous growth zones; Brienen et al. 2009).

Isotope analysis

We selected five stem discs of trees between 28 and 40 years old. These discs were collected in February 2008 from three different forest stands that were abandoned between 40 and 64 years ago, and used previously to study secondary forest succession (Brienen et al. 2009) and climate–growth relations of this species (Brienen et al. 2010). Wood samples were isolated from exactly dated annual rings for each of the five trees. This was done manually along a small and thin section of each disc with a sharp knife. To avoid loss of material and possible cross-contamination during grinding, samples were cut into fine pieces by hand with a sharp blade. Approximately 100 mg of each sample was placed in a 2-ml sealable plastic vial and extracted first with 1 ml diglyme + 0.25 ml 10 M HCl and subsequently with 1.5 ml $NaClO_2$–acetic acid (5 g $NaClO_2$ dissolved in 500 ml of distilled water and 0.7 ml of glacial acetic acid) as detailed elsewhere (Hietz et al. 2005). The resulting cellulose was homogenized with a UP200S ultrasonic homogenizer (Hielscher Ultrasonics, Teltow, Germany) and lyophilized (Laumer et al. 2009).

About 1 mg of purified cellulose was weighed into tin capsules and carbon isotope composition ($\delta^{13}C_{cell}$) measured by gas isotope ratio mass spectrometry (IRMS). The IRMS system consisted of an elemental analyzer (EA 1110, CE Instruments) interfaced by a ConFlo II to the IRMS (Delta^{PLUS}, Finnigan MAT, Thermo Electron). Reference gas (CO_2, Air Liquide) was injected before and after each sample CO_2 peak to correct for drift. Laboratory standards were run in between samples and were calibrated against international reference materials (IAEA-CH-6, USGS-40, USGS-41, IAEA-601 and -602). The long-term standard deviation of repeated $\delta^{13}C$ measurements of the laboratory standards was 0.1‰. The carbon isotope composition ($\delta^{13}C_{cell}$) was calculated as follows:

$$\delta^{13}C_{cell} [‰ \text{ vs. } V\text{-PDB}] = \left(R_{sample}/R_{standard} - 1\right) \times 1,000, \tag{1}$$

where R represents the ratio of $^{13}C/^{12}C$ of samples and standards.

Isotope discrimination theory: calculation of c_i and W_i

The magnitude of carbon discrimination, $\Delta^{13}C$, by plants can be calculated from stable carbon isotopes in tree ring cellulose, $\delta^{13}C_{cell}$, as

$$\Delta^{13}C \text{ (‰)} = \left(\delta^{13}C_a - \delta^{13}C_{cell}\right)/\left(1 + \delta^{13}C_{cell}/1,000\right), \tag{2}$$

where $\delta^{13}C_a$ is the isotopic value of atmospheric CO_2, the input signal for the plant. Variation in $\delta^{13}C_a$ needs to be taken into account, as the atmosphere has become depleted in heavier $^{13}CO_2$ over the last two centuries due to combustion of isotopically light fossil fuels. We used published values of $\delta^{13}C_a$ for the period 1969–2003 from McCarroll and Loader (2004), and extrapolated the near-linear decline of $\delta^{13}C_a$ over the last decades to estimate the values for 2004–2007.

Following Farquhar et al. (1982), carbon discrimination during CO_2 fixation of C3 plants is linearly related to the ratio of intercellular to atmospheric $[CO_2]$ (c_i/c_a) by the equation:

$$\Delta^{13}C \text{ (‰)} = a + (b - a)c_i/c_a, \tag{3}$$

where a (4.4‰) refers to the slower diffusion of $^{13}CO_2$ relative to $^{12}CO_2$, and b (27‰) is the fractionation by Rubisco against $^{13}CO_2$. By combining Eqs. 2 and 3, c_i can be calculated using c_a, obtained from direct measurements of atmospheric $[CO_2]$ from Keeling et al. (2009) (http://cdiac.ornl.gov/trends/co2/sio-mlo.html).

Intrinsic water use efficiency (W_i), is defined as the ratio of assimilation rate (A) to stomatal conductance for water vapour (g_w) (Ehleringer et al. 1993; Osmond et al. 1980),

$$W_i = A/g_w \tag{4}$$

Since $g_w = 1.6g_c$ (g_c is the conductance for CO_2), and given that the net carbon uptake by diffusion through the stomata (A) follows Fick's law,

$$A = g_c(c_a - c_i), \tag{5}$$

we can calculate W_i, using Eqs. 3, 4 and 5,

$$W_i = A/(g_c 1.6) = (c_a - c_i)/1.6$$
$$= c_a(b - \Delta^{13}C)/1.6(b - a). \tag{6}$$

Although, W_i reflects stomatal control of water use, it does not provide an actual measure of true water losses per assimilated unit of carbon as it does not account for evaporative demand. Higher water vapour pressure deficit

(vpd) across the stomata increases evaporative demand and will result in increased water losses even if g_w and W_i remained constant (Ehleringer et al. 1993; Seibt et al. 2008). The instantaneous water use efficiency is defined as the ratio of assimilation and transpiration, A/E, and thus provides a true measure of plant water losses (Ehleringer et al. 1993; Seibt et al. 2008). E can be calculated as,

$$E = \nu g_w \tag{7}$$

The term ν is the water vapour pressure deficit between leaf and atmosphere, divided by P, the atmospheric pressure,

$$\nu = (e_i - e_a)/P, \tag{8}$$

e_i and e_a are the vapour pressures inside the stomata and in the atmosphere, respectively.

Using Eq. 6, 7 and 8, we can calculate the instantaneous water use efficiency as,

$$A/E = W_i/\nu = W_i P/(e_i - e_a) \tag{9}$$

As there are no long-term relative humidity records of the study site to calculate leaf-to-air vapour pressure deficit, we used long-term air temperature and vapour pressure data (e_a) from the gridded dataset, CRUTS3.0 [University of East Anglia Climate Research Unit (2009)] to calculate vpd. We calculate e_i according to Allen et al. (1998); $e_i = 0.6108 \, \mathrm{Exp} \, (17.27 \, T_1)/(T_1 + 237.3)$, assuming saturated vapour pressure inside stomata. We also assumed leaf temperature, T_1 to be equal to air temperature, because of the small leaflet size of *Mimosa* (width = 5 mm) and high wind speed in the area (mean 9.3 m s^{-1}), resulting in very high convective energy exchange between leaf and air (Nobel 1991). We checked this assumption using detailed climate data for 2007 and 2008 and the energy balance equations of Nobel (1991), and found that T_1 rarely exceeded T_{air} by more than 0.5°C and never by more than 1°C (data not shown), even if leaves were not transpiring. We corrected for the offset between monthly vpd based on long-term gridded dataset, and vpd during daylight hours (radiation > 20 μmol m^{-2} s^{-1}) of the wet season (June–October), the period when carbon uptake can take place. To this end, we correlated daytime with gridded vpd for 2007, and used the regression to calculate long-term vpd trends over the wet season during daytime.

Data analysis

We assessed the degree of correspondence of inter-annual variation in $\delta^{13}C_{cell}$ among the five trees by calculating the mean Pearson correlation coefficient of all pair-wise combinations of trees. To study how c_i and W_i related to atmospheric [CO$_2$], climate, and growth, we calculated yearly means of c_i and W_i for the five trees. Long-term trends in yearly means of c_i and W_i over the study period

are evaluated using linear regressions. To study physiological responses of trees to inter-annual variation in climate we correlated c_i with different climate variables. We choose c_i for studying physiological response to climate, instead of W_i, as c_i was apparently unaffected by increasing atmospheric [CO$_2$], whereas W_i showed strong increases over time. To provide detailed insight into the influence of rainfall during different months on c_i, we correlated c_i with monthly rainfall, running from July of the previous year to December of the current year. We also correlated c_i with annual (from November until October) and seasonal (dry and wet season) averages of rainfall, temperature, cloud cover and solar radiation. The previous wet season was included as carbohydrates from previous years may be used for formation of tree ring cellulose in the current year (Fritts 1976; Helle and Schleser 2004). To correct for possible correlations between climate variables, we also calculated partial correlations, which allowed us to study the effect of each climate variable on c_i, while controlling for the effects of other climate variables.

Historical local climate data were obtained from several sources. Rainfall (1969–2006) and cloud cover data (1969–2003) from the nearest weather station of Ixtepec (16°33′N, 95°06′W, 14 km from research site) were obtained from the Comisión Nacional del Agua (CONAGUA). As the Ixtepec temperature records showed irregularities, we used monthly gridded temperature anomaly data (1969–2007) from GISSTEMP (Hansen et al. 1999). Average daily solar radiation data at earth surface (kWh m^{-2} day^{-1}; 1983–2005) were also obtained from a gridded dataset (NASA; http://eosweb.larc.nasa.gov).

We also correlated c_i with large-scale climate drivers. As a proxy for large-scale, inter-annual drivers of climate, we used the southern oscillation index (SOI), and sea surface temperature anomalies (SSTA) from the east and west Pacific and from the North tropical Atlantic, the principal regions that showed influences on growth of *M. acantholoba* in a previous study (Brienen et al. 2010) and affect local climate (Taylor et al. 2002). SSTA data are from the extended SSTa-dataset until 2003 of Kaplan et al. (1998). Data of the SOI, a meteorological index based on air pressure difference between Tahiti and Darwin that is often used to characterize the strength of El Niño events (Trenberth and Caron 2000), were obtained from the National Centre for Atmospheric Research (http://www.cgd.ucar.edu/cas/catalog/climind/soi.html). We define El Niño years as those with 3-monthly means of Niño3.4 SSTA exceeding +0.5 for at least 5 consecutive months (sensu National Oceanic and Atmospheric Administration, http://www.cgd.ucar.edu/cas/ENSO/enso.html).

To study how physiological tree responses affected tree growth, we correlated c_i and W_i with annual diameter growth of the five individuals included in this study. There

was no age or size-related growth trend present in our data (Brienen et al. 2010), and there was thus no need for detrending our data. Diameter growth was calculated from averaged ring width measurements along two to three radii (Brienen et al. 2010).

Results

Long-term trends in $\delta^{13}C$, c_i, and water use efficiencies

The inter-annual pattern of stable carbon isotope ratios in tree ring cellulose ($\delta^{13}C_{cell}$) between the five trees in this study was well-synchronized with a mean inter-tree correlation of 0.55 (Fig. 1a). This is of a similar magnitude as the inter-tree correlation in growth of the same five individuals ($r = 0.50$). $\delta^{13}C_{cell}$ remained rather constant between 1968 and 2007 without evident increases or decreases. Because atmospheric $\delta^{13}C$ decreased over the same period from −7.1 to −8.2‰ (Fig. 1b), $\Delta^{13}C$ decreased. Year-to-year variation in $\delta^{13}C_{cell}$ between 1968 and 2007 was relative high varying from a minimum of −28.3 to −23.0‰. The strong increases in atmospheric [CO_2] over the last decades did not result in significant increases in intercellular [CO_2] (c_i) ($P = 0.14$). Instead, c_i remained relatively constant over time, but with a large year-to-year variation (Fig. 2a). Constant c_i and increasing c_a implies that intrinsic water use efficiency (W_i) increased over time (cf. Eq. 6); we find that W_i increased from about
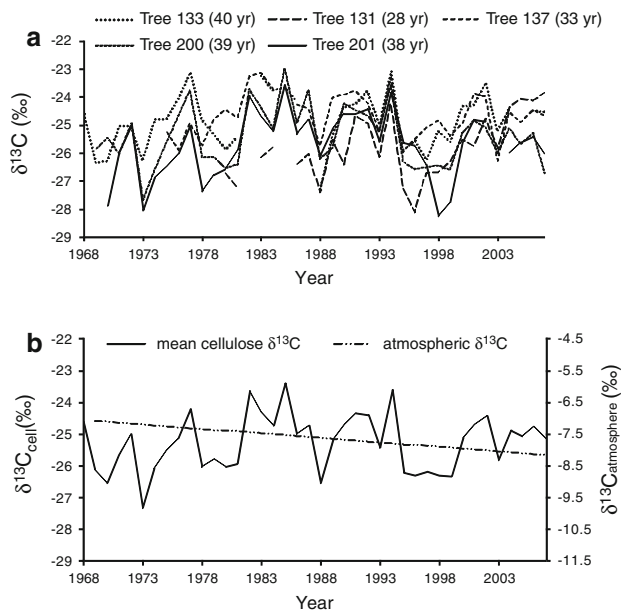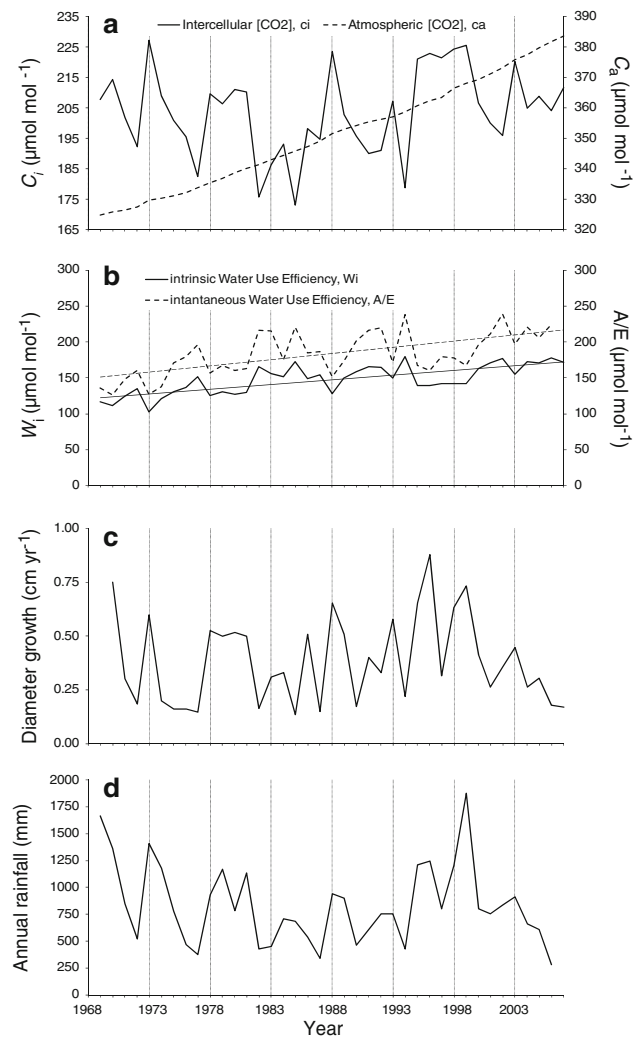

Fig. 2 Temporal variation in **a** atmospheric (c_a) and intercellular [CO_2] (c_i), **b** intrinsic water use efficiency (W_i) and instantaneous water use efficiency (*A/E*), **c** mean diameter growth rates of five trees included in this study, and **d** annual rainfall between 1968 and 2007. c_i, W_i and *A/E* were inferred from $\delta^{13}C$ in tree ring cellulose and growth from ring widths of *Mimosa acantholoba* in a Mexican dry forest

80 to 110 μmol mol^{-1}, an increase of nearly 40% over the past four decades (Fig. 2b), while c_a increased from 323 to 384 ppm, an increase of 19%. Average increase in W_i was 0.52 μmol mol^{-1} per ppm increase in atmospheric [CO_2].

Water vapour pressure deficit increased by about 4% over the last four decades. This weak trend had little effect on instantaneous water use efficiency (*A/E*) which increased over time by 40%, parallel to W_i (Fig. 2b).

Long-term growth and correlations with c_i and W_i

There was no trend in long-term mean growth rates within the five individuals included in this study (Fig. 2c). Inter-annual variation in growth was synchronized with inter-

Fig. 1 **a** Synchronous patterns of $\delta^{13}C_{cel}$ records of five *Mimosa acantholoba* trees in a Mexican dry forest (tree ages indicated between brackets), and **b** mean $\delta^{13}C_{cell}$ and $\delta^{13}C_{atm}$ between 1968 and 2007 (data source $\delta^{13}C_{atm}$: McCarroll and Loader 2004)

Fig. 3 **a** Relationship between mean diameter growth and intercellular [CO_2], c_i, and **b** the relationship between annual rainfall and c_i of five trees of *Mimosa acantholoba* in a Mexican dry forest
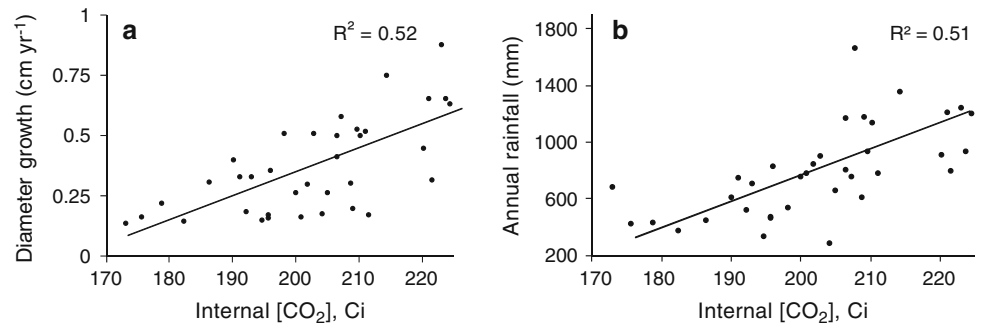

Fig. 4 Correlations between monthly rainfall and c_i of *Mimosa acantholoba* in a Mexican dry forest for 1970–2006 (*bars*). Correlations run from previous growing season (*j-1* July) to the middle of following dry season (*d* December). Significance levels are indicated above or below the bars, ***$P < 0.001$; **$P < 0.01$. The *shaded area* in the background is the mean total rainfall for each month
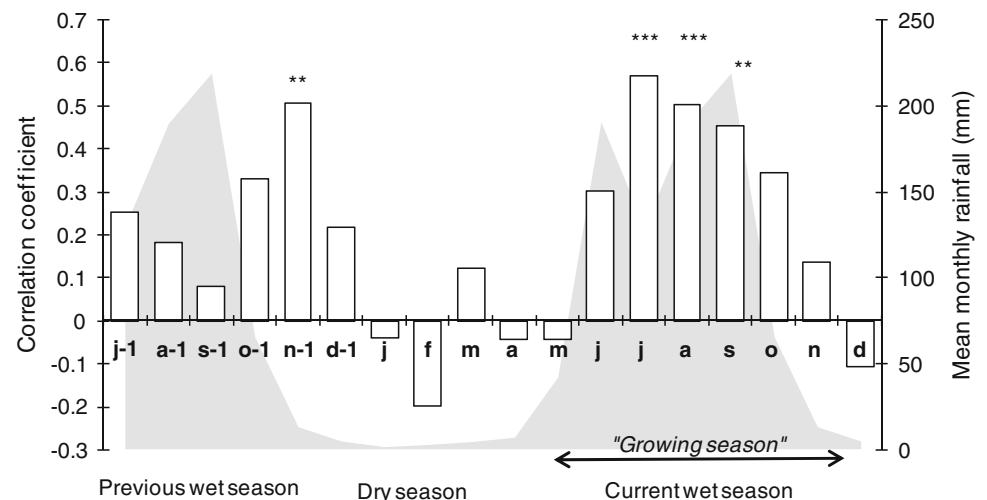
annual variation in c_i (Fig. 2a, c), with years of high mean growth having a higher c_i (Fig. 3a). Mean growth of the five trees included in this study correlated positively and strongly with mean c_i ($r = 0.72$, $P < 0.001$), and was negatively and less strongly correlated to mean W_i ($r = -0.49$, $P < 0.01$).

Responses of c_i to variation in climate

Intercellular [CO_2] (c_i) was positively correlated with rainfall during July, August and September of the current rainy season and with November of the previous rainy season (Fig. 4). We also found positive correlations between c_i and cloud cover and negative correlations with temperature and solar radiation during the wet season (Table 1). Among all climate variables that were considered in this study, total annual rainfall exhibited by far the strongest influence on c_i, explaining about 50% of inter-annual variation in c_i (Fig. 3b). This is also illustrated by the strongly synchronous pattern between c_i and total annual rainfall (cf. Fig. 2a, c).

When correcting for the strong effect of wet season rainfall on c_i, using partial correlations, the effects of wet

season temperature, cloud cover and solar insolation on c_i disappeared (cf. partial correlations, Table 1). Thus, the negative correlations between c_i on one hand and temperature, cloud cover and solar radiation on the other, were completely due to correlations of these climate variables with wet season rainfall. When correcting for the influence of wet season rainfall, we found also a positive effect of dry season rainfall.

c_i also correlated with large-scale climate drivers, like Pacific and Atlantic sea surface temperature anomalies (SSTA) and the southern oscillation index (SOI) (Table 2). In the first half of the year North tropical Atlantic SSTA exhibited the strongest influence on c_i, while in the second half of the year c_i was mainly affected by west and central equatorial Pacific SSTA (Table 2). The correlation between c_i and SOI and central equatorial Pacific SSTA, two measures of the strength of the EL Niño–southern oscillation disappeared when correcting for the effect of wet season rainfall on c_i. c_i was significantly reduced during warm and dry El Niño years between 1968 and 2007 (average of 194 ppm during El Niño years vs. 207 ppm during non-El Niño years, $t = 3.55$, $P < 0.001$, $df = 38$).

Table 1 Correlations (and *partial correlations*) between c_i (intercellular $[CO_2]$) and rain, temperature, cloud cover and solar radiation averaged over the wet (June–October) or dry season (November–May)

Partial correlation	Previous wet	Dry	Current wet	Annual
Rain	0.20 (0.10)	0.20 (0.39*)	0.67*** (na)	0.70*** (na)
Mean temperature	0.29 (0.17)	0.29 (0.26)	−0.33* (−0.07)	0.02 (0.16)
Cloud cover	0.05 (0.09)	0.29 (0.20)	0.44* (0.22)	0.43* (0.24)
Solar insolation	0.03 (0.10)	0.12 (−0.27)	−0.54* (−0.31)	−0.32 (−0.40)

Partial correlations are corrected for the influence of current wet season rainfall on c_i

Values are Pearson correlation coefficients, *** $P < 0.001$; * $P < 0.05$

Table 2 Correlations (and partial correlations) between c_i (intercellular $[CO_2]$) and sea surface temperature anomalies (SSTA) in the Pacific and Atlantic Ocean and the southern oscillation index (SOI), averaged over the wet (June–October) or dry season (November–May)

Partial correlation	Previous wet	Dry	Current wet	Annual
Central equatorial Pacific SSTA[a]	0.22 (0.26)	0.06 (0.20)	−0.42** (0.06)	−0.23 (0.20)
West Pacific SSTA[b]	0.21 (−0.10)	0.19 (−0.09)	0.69*** (0.42*)	0.54** (0.19)
Southern Oscillation Index	−0.14 (−0.10)	0.03 (−0.16)	0.52** (0.28)	0.34* (0.05)
North tropical Atlantic SSTA[c]	0.41* (0.11)	0.53*** (0.33)	0.43* (0.36*)	0.52** (0.38*)

High SSTA in the Central equatorial Pacific (Niño3.4 region) and low SOI correspond to El Niño years. Partial correlations are corrected for the influence of current wet season rainfall on c_i

Values are Pearson correlation coefficients, *** $P < 0.001$; ** $P < 0.01$, * $P < 0.05$

[a] Nino 3.4 region (5°N–5°S, 120°–170°W)

[b] 0°N–20°N, 210°–240°W

[c] 5°N–10°S, 20°–50°E

Discussion

Long-term trends

Calculations of c_i and W_i based on $\delta^{13}C$ in tree ring cellulose are sensitive to several assumptions. First, we did not correct for a likely offset between wood and leaf $\delta^{13}C$. Wood $\delta^{13}C$ is usually enriched compared to leaves due to downstream fractionation of carbohydrates from leaf to stem (McCarroll and Loader 2004). This enrichment is generally not accounted for in tree ring studies and does not affect the general trends. Another simplification of our calculations is that we did not account for mesophyll conductance of CO_2 from intercellular space to the chloroplast. Seibt et al. (2008) showed that ignoring mesophyll conductance by using the linear (or reduced) form of the isotopes discrimination model of Farquhar et al. (1982) may underestimate the response of W_i and c_i to increases in $[CO_2]$, and may affect variation between species in $\delta^{13}C$. However, we had no information on mesophyll conductance for our species and using mean values of mesophyll conductance would not improve insights. We therefore preferred the use of the simpler, linear model of Farquhar et al. (1982, cf. Eq. 3), which also allowed comparison with other tree rings studies.

We observed strong increases of nearly 40% in W_i over the last four decades. Besides increases in atmospheric $[CO_2]$, other mechanisms that may have lead to the improved W_i are long-term climate trends. Two climate trends were observed; an increase in wet season temperature of 0.15°C per decade ($P < 0.01$), and an increase in cloud cover ($P < 0.001$). Increasing cloud cover probably results in lower, not higher W_i, because of lower A with lower irradiance and/or higher g_s as a reaction to lower evaporative demand. Temperature increases may indirectly result in higher W_i as higher temperatures increase vapour pressure deficits (vpd), to which plants may respond by closing their stomata (reducing g_s) (Lloyd and Farquhar 2008). However, we found only a slight increase in vpd over time, and thus trends in true (or instantaneous) water use efficiency (A/E) did not deviate from the trend in W_i (Fig. 2b).

Another mechanism that could affect trends in W_i is related to the effects of tree age or ontogeny on isotope composition in tree rings, also called the "juvenile effect" (Bert et al. 1997; Francey and Farquhar 1982; Marshall and Monserud 1996; McCarroll and Loader 2004). Although the causes behind this juvenile effect are not entirely known, one possible mechanism behind observed higher $\delta^{13}C$ in juvenile trees (or lower apparent W_i) is the use of

recycled air that is depleted in ^{13}C by young trees growing close to the forest floor (Schleser and Jayasekera 1985). This effect does not appear to extend higher than the lowest forest strata even in a dense rainforest (Buchmann et al. 1997), and it is very implausible to have affected δ^{13}C signals in our species. Another cause behind the juvenile effect, shading, can also be ruled out, as *M. acantholoba* is a pioneer species and individuals receive full sunlight throughout their entire life. A third possible cause for the juvenile effect is changes in hydraulic conductance from soil to leaves when trees are get taller (Ryan and Yoder 1997). As our trees reach maximum heights of 7 m, this is also unlikely to cause strong changes in hydraulic limitation. We therefore think it unlikely that a putative juvenile effect played a significant role in the W_i trend observed, although we could not statistically test for an age effect as we only included five trees of similar ages in this study. Even if we assumed a relatively strong age-related trend of 1‰ over the entire trajectory (cf. Duquesnay et al. 1998), we would still find an increase in W_i of 26% over the last decades. We therefore conclude that response to increasing [CO$_2$] is the dominant cause for the improved W_i observed in this study. This is in line with many other studies that showed that increases in W_i coincided with increases in atmospheric [CO$_2$] since ca. 1850 (Bert et al. 1997; Feng 1999; Hietz et al. 2005; Saurer et al. 2004; Waterhouse et al. 2004).

Reported trends in W_i in temperate trees are mostly in the range of 0.20–0.45 μmol mol^{-1} per ppm increase in atmospheric [CO$_2$] (Bert et al. 1997; Feng 1999; Saurer et al. 2004), with maximum increases of up to 0.54 (Waterhouse et al. 2004). For tropical trees, few studies on long-term trends in W_i exist so far. Hietz et al. (2005) and Nock et al. (2010) reported increases in W_i for tropical moist forest tree species from Brasil and Thailand within the range of temperate species: 0.26 and 0.34 μmol mol^{-1} per ppm. For semi-arid woodlands in Ethiopia, Gebrekirstos et al. (2009) report large differences in W_i trends in four tree species, varying from constant W_i to increases of up to 0.44 μmol mol^{-1} per ppm (based on δ^{13}C records presented in Gebrekirstos et al. (2009)). The increase in W_i observed in our study (0.52) is thus higher than in most previously reported increases. It is still uncertain what determines the observed differences in W_i responses to increases in [CO$_2$]. Substantial differences between sites within the same species (Arneth et al. 2002; Saurer et al. 2004; Waterhouse et al. 2004) indicate that differences in soil water availability, air humidity, and temperature play an important role in the physiological responses of plants to increasing atmospheric [CO$_2$].

To gain a better understanding of physiological reactions of trees to increasing [CO$_2$], it may help to use the three scenarios of gas exchange responses of Saurer et al. (2004). Under increasing c_a, we may observe; (1) a constant c_a–c_i with no improvement in W_i and no active stomatal responses, (2) a constant c_i/c_a indicating that the linkage between stomatal conductance and assimilation (Wong et al. 1979) is largely maintained under increased c_a (Medlyn et al. 2001; Sage 1994), or (3) the maintenance of a constant c_i. The most common response inferred from tree rings is maintenance of a constant c_i/c_a (Feng 1998; Hietz et al. 2005; Leavitt and Lara 1994; Nock et al. 2010; Saurer et al., 2004), but constant c_i has also been reported (Francey and Farquhar 1982; Linares et al. 2009; Waterhouse et al. 2004). Short-term experiments also show constant c_i/c_a for a variety of species, except under conditions of drought stress where c_i showed less increase (Sage 1994). This indicates that stomata may become more conservative during drought stress, and is in accordance with observations that drought-stressed plants show stronger stomatal responses to [CO$_2$] (Field et al. 1997; Medlyn et al. 2001; Wullschleger et al. 2002). A stronger stomatal response of drought-stressed trees may explain the constant c_i and relative high increases in W_i at our site, which is dry compared to other studies.

Higher W_i can result from decreasing stomatal conductance, increased assimilation or a combination of both (cf. Eq. 4). Although, it is not possible to determine the contribution of each factor by δ^{13}C alone, trends in c_i over time may give us some insight into changes of assimilation rates over time. Assuming that the relation between c_i and photosynthetic rate of leaves (*A*) did not change due to increased atmospheric [CO$_2$], we may cautiously conclude that instantaneous assimilation rates did not change as c_i did not change. However, plants raised at higher [CO$_2$] often show lower rates of photosynthesis when measured at the same c_i due to acclimation (Gunderson and Wullschleger 1994; Medlyn et al. 1999), which implies that assimilation rates may even have declined. Still, increases in assimilation rates have also been observed (Sage 1994), and we would need specific information about responses of the study species to increases in [CO$_2$] to draw definite conclusions.

Potentially, one could use trends in diameter growth rates to evaluate whether assimilation rates changed over time. The constant diameter growth (cf. Fig. 2c) actually implies that biomass gains increased over time (as basal area increment increased), but it is difficult to separate ontogenetic effects from responses to increased [CO$_2$] as most tree species increase in growth rates during ontogeny.

Finally, it is not clear how increases in W_i manifest itself on the ecosystem. Assuming assimilation stayed constant we can estimate that stomatal conductance (g_s) declined by ca. 30% (cf. Eq. 4). If this decrease in g_s did result in reduced transpiration losses (*E*), this can have substantial consequences for the ecosystem and its hydrological cycle.

Potentially there is a delayed water stress and the duration of assimilation may have increased either on a daily basis or by extension of the growing season length if soil water moistures increased (Henderson-Sellers et al. 1995). However, feedback mechanisms could exist. For example, lower stomatal conductance reduces leaf cooling effects and increases leaf temperature (Idso et al. 1993). This may lead to increases in leaf-to-air vapour pressure deficit and in turn increase in transpiration losses, but this is unlikely in a species with open canopies and small leaves where leaf and air temperatures are closely coupled. Reductions in transpiration losses may lead to a dryer atmosphere as well as increased soil moisture and runoff (Gedney et al. 2006) and changes in precipitation, which could significantly influence regional climate (Henderson-Sellers et al. 1995). For example, reduced stomatal conductance accounted for about one-fifth of the predicted rainfall decreases in the Amazon and is predicted to accelerate the rate of warming using global dynamic vegetation models (Betts et al. 2004). In contrast to large continental areas such as the Amazon, rainfall in Central America is largely of oceanic origin and not from regional transpiration (Taylor et al. 2002), thus reductions in stomatal conductance are unlikely to affect regional rainfall.

Clearly, more insight into physiological responses of trees to increasing [CO_2] is required, especially for tropical forests as they will play a crucial role in the future evolution of climate change (Malhi and Grace 2000). To understand the conditions under which either c_i or c_i/c_a tends to remain constant, and their implications for carbon cycling rates and true water use efficiencies, more studies on tree ring $\delta^{13}C$ trends across major environmental gradients in the tropics, including dry and wet sites, would be helpful. Given the recent advances made on tropical tree ring studies (Brienen et al. 2009; Schöngart et al. 2004; Worbes 2002), tropical forests need no longer be excluded from tree ring studies including long-term annually resolved carbon isotope series. Results from such studies may be used to test DGVM's which predict large-scale die-off of the Amazon rainforest (Betts et al. 2004), but remain highly simplistic due to lack of data and understanding of key processes.

Physiological responses to inter-annual variation in climate

Interpreting the long-term reactions to rising CO_2 levels may be helped by understanding short-term reactions to factors other than [CO_2]. Of all local climate variables, total annual rainfall is the dominant controlling factor of c_i, and neither temperature, nor cloud cover or solar radiation influenced c_i after controlling for the effect of rainfall on c_i. The difference between simple and partial correlations of c_i

with climate variables shows that simple correlations can be misleading because of correlations between different climate variables. For example, El Niño years in the study region are not only drier, but also hotter and receive more solar radiation (Brienen et al. 2010). This emphasizes the importance of taking all local climate variables into account (cf. McCarroll and Loader 2004). A strong influence of rainfall on c_i (and thereby tree ring $\delta^{13}C$) was also found in other studies of dry sites in the tropics (Fichtler et al. 2010; Gebrekirstos et al. 2009) or temperate regions (McCarroll and Loader 2004), and after correcting for changes in atmospheric [CO_2], $\delta^{13}C$ is mostly interpreted as a drought signal (McCarroll and Loader 2004). The relation between c_i and rainfall is well understood for dry sites where moisture stress is limiting and can be explained by stomatal responses to soil moisture and relative humidity; during dry years, stomatal aperture decreases to prevent excessive water losses, leading to reduced influx of CO_2 into the intercellular space, and thus a lower c_i and lower ^{13}C-discrimination (Farquhar and Sharkey 1982). Where drought stress is uncommon, the dominant factor controlling $\delta^{13}C$ may be the photosynthetic rate as affected by irradiance and temperature (McCarroll and Loader 2004). However, temperature and irradiance may also indirectly influence stomatal behaviour, and thereby the carbon isotope signal, through their effects on the water vapour pressure deficit across stomata (Lloyd and Farquhar 2008; Seibt et al. 2008).

A main reason to analyse tree ring isotopes and indeed tree rings in general is to use correlations with past climate for climate reconstructions (McCarroll and Loader 2004). The reasonably high between-tree correlations and the correlations with rainfall are in the same order of magnitude as Gebrekirstos et al. (2009) reported for Ethiopia and show that the use of $\delta^{13}C$ records is promising for tropical dry regions, although in our case limited by the short life-span of *Mimosa acantholoba*. Of particular interest is the high correlation with ENSO indices (SOI, SSTA-pacific), as palaeoclimatic proxies of ENSO from the tropics are scarce (Brienen et al. 2010; Schöngart et al. 2004). Our study shows that trees in Central America dry forests may be promising in this respect, although the signal of $\delta^{13}C$ in tree rings was slightly lower than the signal in ring width in this species (Brienen et al. 2010). A multi-proxy approach combining ring width and isotope measurements (including water isotopes) may improve interpretation of climate signals and strengthen the overall palaeoclimatic potential compared to the use of one single proxy (McCarroll and Loader 2004).

Our results showed that $\delta^{13}C$ in tree rings is a promising tool to evaluate long-term responses of tropical trees to increasing [CO_2] and to variation in climate. Extending such studies to a larger number of tropical tree species with

annual rings and to sites differing in rainfall, could improve our understanding of the responses of tropical forests to predicted changes in climate and atmospheric [CO_2].

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